Artificial cells is now not an unrealistic goal -A state-of-the-art-review

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Abstract:
The development of artificial cells is the research field which has been progressive in the past and in the present expanding rapidly. Artificial cell it is a cell-like system consist of a lipid-bilayer which separates its inner aqueous environment to the outer aqueous environment. In this field there is a emphasizes on the construction of models which are cell-like created by synthesizing a genome and installing it into a recipient cytoplasm. It has a biomimetic structure. We are going to summarize a few vital aspects of the artificial cells which will be based construction, advantage and applications and the enzymatic reactions. Our content will be much relatable to the current or the present scenario of artificial cells.

Keywords: Hemoperfusion; Enzyme therapy; Cell therapy; Gene therapy; Artificial cells; Synthetic biology; Biophysics; Synthetic cells; Liposomes

1. Introduction

In the recent past, we have witnessed a rapid growth of interest in the field commonly known as synthetic biology and to be more specific the main concern remains the construction of cell-like bodies [1-2].
"Substitutes for natural cells" is the ultimate goal for developing artificial cells. Artificial cells can be defined cell-like structures; biological cell imitators which can be engineered materials perform specific functions, mimic important properties of cells, such as shapes, morphology etc. It is a'Biological or chemical structures which consist of a set of reacting molecules and whose structure is just similar to a cell and similar in functions too, are called as "artificial cells" or "synthetic cells" or "protocells".' Artificial cell was designed to understand a particular process in a cell, for example protein synthesis. It also helps in designing any biochemical reaction which is either difficult to understand in a complex cellular environment like functioning of membrane proteins.
Hybrid design is mixing of non- lipid compartments in the midst of non-biological components [3-4]. Here, in this review paper we will be referring to cell-resembling systems also recognized as synthetic cells (SCs) which are put together from genetic materials such as DNA, RNA, and ribosomes, etc. within liposomes. In the areas of formation and role, the synthetic cells will be alike to that of biological cells. High potential is involved in the making of these artificial cells for fundamental and applied science and so our interest is towards this topic that is "semi-synthetic" approach [5-7].
The establishment or the making up of living SCs is a demanding goal which requires stepwise process to get it achieved [8]. As of now the constructed SCs resemble the living cells at most externally and have structure like a cell and also perform life-like operations. It is tough to achieve the chemical manufacture of all synthetic cells. Present-day SCs are found to be resembling to self-determinant bioreactors, reduced to micrometer size or in simple words, they are more likely said to be machine-like than the organism-like [9].
This can't be considered as a gloomy scenario because the present SCs which are under the trial are of great use in many aspects and it also helps us gain an extensive study of scientific technological insight. The synthetic cells are beneficial replicas of earliest blocks, the parents of earliest biological cells. While looking at the similarity with machines in terms of programmability, modularity, orthogonality, etc. the synthetic cells are good research materials. The existing technology will surely contribute to the advancement of SCs.
Now it is of utmost importance that this field now experiences growth with the help of automation and conceptual framework. It is very certain to say that research on artificial cells is a wide and speedily growing field.

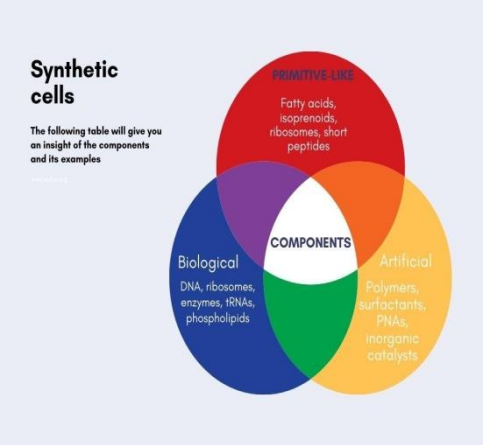

Figure 1. The figure illustrates variety of artificial cell visualization depending upon depending on the experimental scope

Research on Artificial cells has been gradually increasing in the past 20 years, as they lead to a variety of new opportunities.

This study paper answers the following curiosity of students on –

1. Understanding the basic building block of 'Artificial cell' [AC].
2. Cultivation or synthesis of 'Synthetic cell' or 'Artificial cell'.
3. Advance research in phenomenally growing areas of the advanced life system and other non-medical areas.
4. Limitations in evolution of artificial cells.

Background of origin -What are artificial cells?

Cells were first discovered by Robert Hooke, in 1665 [11]. According to the cell theory, cells are stated as the most essential structural and functional unit of all living organisms [11].

An artificial cell is an idea or product discovered referring to the structure & attributes of a 'natural cell' which is a nuclear truth of any physical body or substance. The idea for artificial cells was first spelt out by 'Chang' in 1957 [12]. The innovative idea was to create a phospholipid vesicle with DNA[10]. Artificial cells can undergo configuration and have unlimited varieties. Artificial cells are now of nano to molecular dimensions [12].

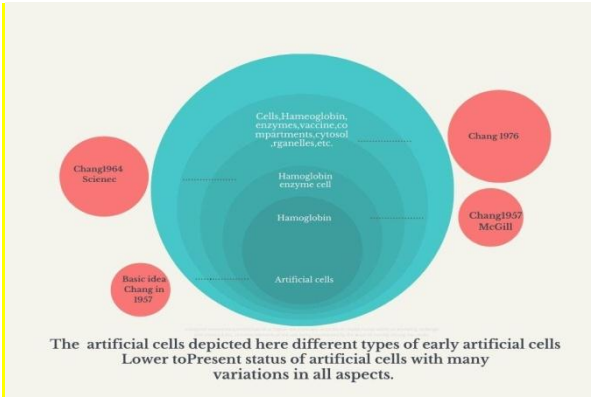

Figure 2.The figure illustrates the diverse types of artificial cells.

Why do we need research on Artificial cells(AC)?

While we are advancing in bio-technology, we are trying to achieve alternate form & structure to support human life and try to overcome weaknesses of humans. Hence, presently we are researching deeper areas like cellular engineering, protein engineering, biotechnology, enzyme engineering, medicine and biosensors [11].

The reason for such researches is to understand and improvise the -

- **Self-healing**: AC can help in Self-healing. Capability of cellular structure to re-form on its own, in other words Self-healing.
- **Grouping**: AC can help in grouping. Despite being very small and can take any form capability to group and act like one.
- **Regeneration**: AC can help in regeneration It can adapt features, characteristics and can evolve them over a period.

- **Decaying process**: If maintained in a condition it defeats the decaying process, be ever young and keep going with the energy, form and strength.
- **Application of artificial cells in non-medical areas:** To discover other vast application of artificial cells in non-medical areas like AI, robotics etc which can create machines which will work like a human in space research, Hazardous or chemical research areas where human can't enter due to our physical limitation

Construction of Artificial cells:

There are two methods to construct an artificial cell –
Top-down method, in which an artificial cell is formed by manipulating its genes which are non-essential and are either removed from the cell or replaced. Top-down method focuses on creating 'minimal' cells by reducing the genes in the cell and the cell has only necessary genes to perform essential functions. 'Minimal' cell was first inspired by a parasitic bacterium 'Mycoplasma genitalium', which had just 517 genes and the bacterium was discovered by Venter and colleagues in 1995[11].
Bottom-up approach, is more challenging than top-down approach as it creates a cell from scratch with the help of nonliving material.The cells designed from bottom-up approach can be considered as more simpler and controllable to perform desired functions[11].

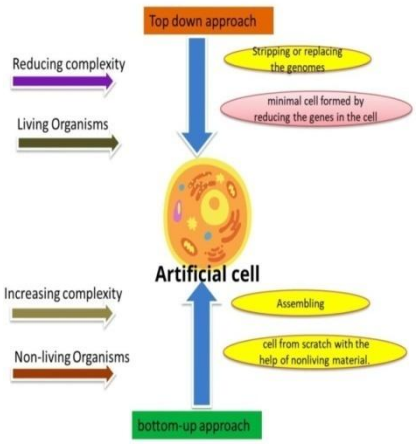

Figure 3. Approaches and design for construction of Artificial cells

Major consideration in constructing Artificial Cell-

1. To synthesize a cell that act as a link between its three most basic and essential components: the DNA information, cell membranes and metabolism [10].
2. The artificial cell which is built would have the organization comparable to a bacterium[10].
3. ATP (adenosine triphosphate) and GTP (Guanosine Triphosphate) are the source of energy in the first stages of development[10].
4. The artificial cell would have a physical boundary made up of phospholipid bilayer. Membrane proteins carry out the exchange of material and information has been shared in and out of the cell [10].
5. Even though there have been a few examples and experiments of using microfluidic device describe the limitations of the artificial cell, to allow exchange of material and information in and out of the cell.[13]

Models of Artificial cells:
To create a successful alive artificial cell, firstly we should understand the basic amenities required for life. There is a model, known as the chemoton model, introduced by TiborGanti, which describe the smallest life [14]. So, in this model the basic 5 points are-(i) boundary system made up of chemicals, (ii)information system up of chemicals,
iii)a self-replicate metabolism (iv) for survival of the species growth as well as reproduction is important (v)in a dynamic environment, adaptively is the need for life's survival.

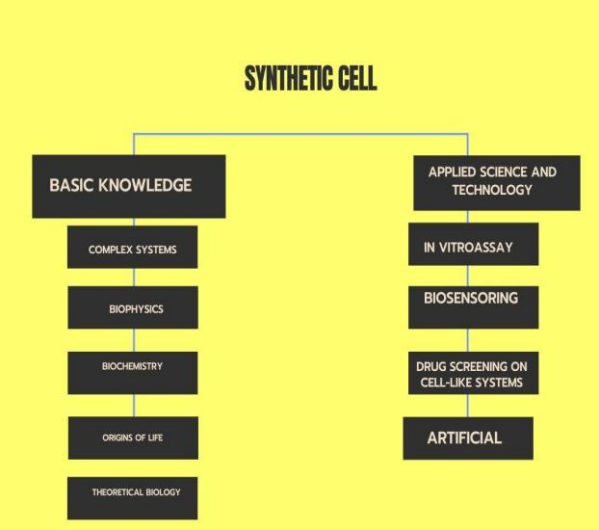

Figure 4-Synthetic cells are made by enclosing the chemical substances inside the artificial compartments.

On seeing the deficiency in cell functions, Chang attempted to synthesize some artificial structures for its substitute widely known as artificial cells[15-19]. The living substances which are contained in the synthetic cells need to be preserved and prevented from coming into contact with the external materials such as leukocytes, antibodies or tryptic enzymes. The particles smaller in size than protein can travel rapidly through the semi- permeable membrane and equilibirise. Hemoperfusion is the first successful development of synthetic cells for clinical purpose [20]. The experimentation has been done and some clinical trials have been done on modified hemoglobin for the substitution of the blood; in various therapies like cell, enzyme and gene therapy,are the synthetic cells are used and these are a few of the recent developments [21]. For nephrologists, the benefit of SCs which contain naturally brought off cells in uremia is an area of great interest.

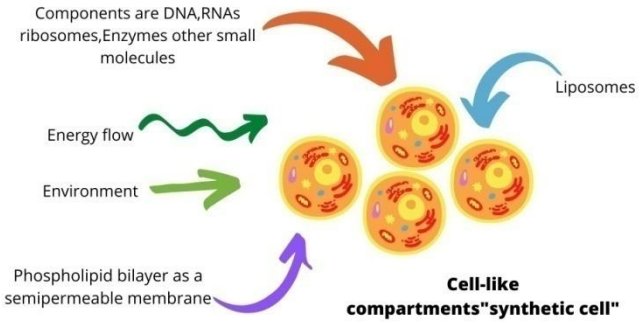

Figure 5.The figure illustrate the semi-synthetic cells using biochemical components and liposomes

Quantization is there in the biological world as the tiniest of the organism are made up of cells and all the cells are synthesized from the pre-existing cells. Virchow in 1858 had pointed out this basic feature in his cellular pathology book [22]. The point to be noted is that not only the organisms are made up of cells but also the cell originates from a cell.

In 1665, Robert Hooke had observed the cellular organization in a cork material [23] and proposed the name as "cell". Later a more systematic study was introduced by Schleiden [24]. In 1839, the cell model was finally presented by Schwann [25].

Hooke in 1665 had observed the cellular organization in a cork material.

Cells observed through a slice of cork with a microscope in 1665 by Robert Hooke. He observed a series of holes and he named it as "cells". In the fig. observe on the left, the cells are divided into "long ways" and show "diaphragms" as described by Hooke. A branch of the cork oak tree is shown below. Reprinted from ref. [23].

Present way in SCs Research:
Many researchers have been found interesting corporal and synthetic features of protein creation and accomplishment of reactions inside liposomes. In common view, the kind of intra- liposome reactions are

understood nicely. The recently discovered trends can act like a booster in the progress of this research field. The topics included in the "currents directions" are:

- the operationalization of an artificial cell membrane
- the vesosome building
- the group viewpoint
- chemical information exchange

Part of this write up has been briefly written in a convention paper [26].

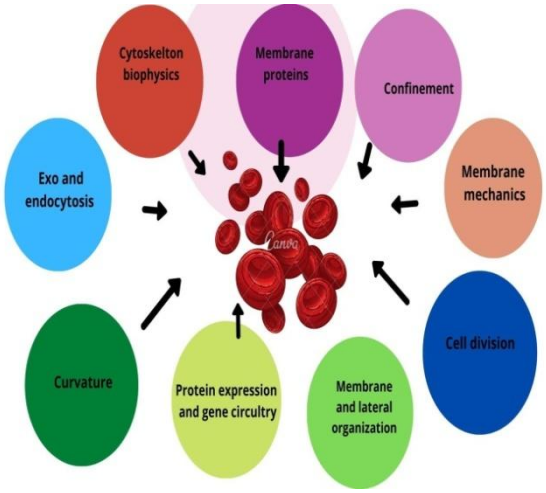

Figure 6 Schematic summarization of the cellular parts as well as biological occurrence of the artificial cell.

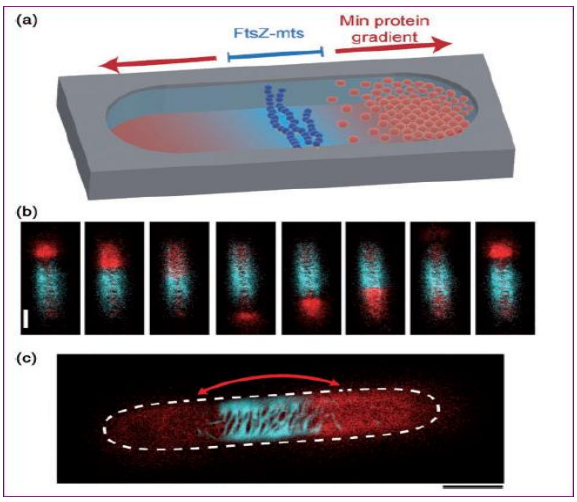

Figure 7 Model represents the cell-like compartment with Min protein oscillation and FtsZ accumulation. (a) Illustration of pole-to-pole Min proteins oscillation and accumulation of FtsZ-mts in the center (b)The figure represents the Time course and (c) and superimposed image of Min protein and FtsZ-mts

In a current study, cell –free synthesis of MreB filaments (a homogeneous of action)done by Maeda et al. inside lipid vesicles[68]. The MreB filaments can fruitfully assemble themselves into micron-sized stiff bundles which can stick and distort the vesicle surface. Biomimetic cells as models is used to understand the process of cytokinesis. The process of cell division can be understood by several constituent parts. First identification of clear spatial location where membrane fission can occur which is possible by accumulation of accessory proteins [69].

An in vitro system was invented by the Schwile group consisting of the system of bacterial which enabled the formation of an internal defined protein gradient [70].

In cells, the cell-division machines are positioned at defined locations, where the cell-division can take place, through oscillations pole-to-pole. The reconstruction was done in the cell–mimetic lipid-coated droplets which have been proved to be a simple platform to investigate further in cell-division proteins [71-72].

3. Artificial cell mimics act as a basic model and help in studying cell biology

The fastest growing area of research is artificial cell that present biological cells. Artificial cell is a platform that leads to various applications such as engineered micro machines and assume chemical synthesis [27-32].Vehicle formed on artificial cells that express the essential features of bio-molecules, cellular reactions and also chain reaction by polymerase [33-34]. Simple imitate of real biological cells are related to application. There are fundamental questions that relate to cell, molecular, and system biology etc. The application that focuses on mini review is deal with the 'building understanding and approach. The discipline that falls under the field of artificial biological science deals with the machines, design of biological modules dealt with by engineering principles. On the application of these cell models there were extensive reviews. Especially minimal cells [35-37]. This review explain the usefulness of using models of artificial cell which will be helpful to examine and address biological questions. This includes investigation that is linked to cellular membranes, the consequences of molecular crowding and process of various cellular structural components. This investigation is covered is shown in fig.1

3.1 The advantage of artificial cell:

Eduard Buchner's revealed enzyme cell-free fermentation and explainedthat not always chemicalconversion in biological systems requires living cells. The modern biological application is bottom-up synthetic biology [38-39].

The molecular composition of cells is diversewith RNA, DNA,and lipids metabolites,and all of them continue living in a variety of chemical states. there is order of Approximately 21,000 of protein coding genes are present in human DNA. Approximately $1x10^6$ different types of proteins are produced.Each protein species and biomolecules work together with other they do not remain in isolation. Therefore inside the cellintricate web of interactions signaling pathways continue living together. In this way molecular parameter of a cell is huge and disentangling information is present which is associated with each component and it is present in a quantitative manner. There is an important prospective for redundancy within intensely related chemical networks as there are a number of other ways that relate one part of a network with the other network. Bulk self-assembly as well as emulsion based methods can generate artificial cells [38-39]. Which could permitted syntheticcells to increase content of internal volumes and connectivity with in different compartments and molecular structure [40-42].

3.2 Cell membrane mimics:

The plasma membrane or cell membrane is the most important part of a cell. As it is responsible for the protection of cells and acts as a barrier to bacteria. Therefore, it has been the most developed cellular component used as a model which is mimicked. The second reason it quite easy to synthesize lipids and assemble themselves into membranous structures. Most frequently plasma membrane mimics take the form of lipids vesicles some examples are Nano discs, black lipid membranes and lipid bilayers[43].There are various feature of model membranes for the systematic study on biological procedures and lipid composition[44-46].

Droplet microfluidic technologies greatly enhances the degree of control in vesicle construction. Such as, the routine of fabrication of asymmetric vesicles is enabled. [47-48]. The universal feature of biological membrane is membrane asymmetry. Membrane mimics are important to investigate the phenomena of lateral membrane organization and segregation, which is also known as lipids rafts [49].

3.3 Exploring the structural components of the cell:

The Artificial cell models is most frequently used to understand the structural components and associated machineries. The beneficial area of research in cell mimics is cytoskeleton biophysics. Cell mimics were used to enumerate the forces which are involved in action polymerization, exerted in different membrane regions, and also membrane-bound.The local membrane curvature decides the retractive or propulsive forces and strength of binding amid membranes and the actin gel[50]. Mechanical forces generation, morphology changes based on cytoskeleton extensive studies could be done utilizing cell mimics[51].

Cells are the most superior micro reactors that shape the basis of a lifetime. It is impressive that Scientists are developing analogies to artificial and natural processes using the method below. The end point here is to Combine man-made cells that demonstrate function and adaptation as they are found in nature, not only they

provide information about the basic processes in natural cells, but also prepare the way for the new use of these non-cell types. In this review paper, we highlight our recent work with others in building non-artificial cells. Firstly, let's introduce the characteristics that characterize live Bacteria to follow, as we will discuss the forms imitated in artificial cells.

4.1 Synthetic Compartments

Every living system requires semi-variable limits and to support conversions in the environment. In fact, for the most part synthetic cell permeability has critical components transforming environmental nutrients and waste. The use of semi-natural materials carefully regulates the cellular process variable containing lipid membrane channels and receptors vector glass, among other things. Over the years, wide synthetic compartments is developed through multiple cells control your permeability,of the shell.Factors influencing permeability, includes the functional nature,building blocks the components of the membrane, and whether the membrane is the rich in the presence of pores and domain formation channels in heterogeneous membranes.Currently a large number of references are developed for generate nano- and microcompartments, the small initial reactions may vary from installation then conventional products. These contain lipids, Polymeric vesicles (liposomes and polymers) , hybrids, colloidosomes ,capsids etc [52-53].

4.2 Multi Compartmentalized Vesicles

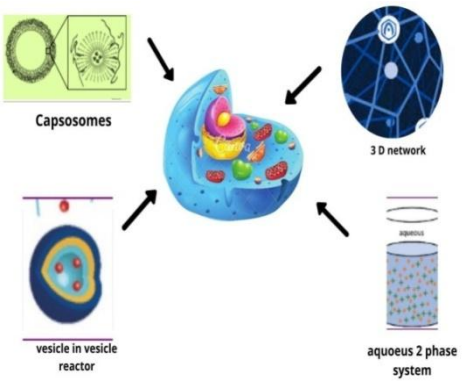

Figure 8. The figure illustrate the multi-compartmentalized vesicles, encouraged by the architecture of a eukaryotic cell

Multi Compartmentalized systems have been developed using liposome cover by cover Capsules (capsosomes),3D network, liposomes-in-liposomes (vesosomes) and also by two phase systems etc. The illustration architecture has been captured in Figure-9.These works are widely studied and reviewed in so many places Now just highlighting recently developed realistic geometry [52].

3.4 Cell-free Unnatural Enzyme Synthesis:

Dealing with natural enzymes requires utmost care and suitable environment as they are extremely fragile. So in the contemporary world researchers are looking for the unnatural incorporating unnatural amino acids. Enzyme activity or stability is greatly enhanced incorporation of UAA in vivo. One of the examples are mentioned for describing artificial enzyme used UAA p-aminophenylalanine multi-drug transcriptional regulator from Lactococcus lactis, LmrR by Drienovská et al.into the loose binding pocket.

Photo-controllable activating enzymes can also be studied by the help of this technology. Photocaged UAA incorporation to proteins enzymes facilitate researchers to specificallycontrol their biological activity all the way through photochemical reactions[64-67]

Conclusion and Overview:

Artificial cells prepared by bottom up construction are in demands if we see the past decade literature. Gene circuits are developed displaying multifaceted behavior, enhanced control over their division with self-reproducing compartments are another milestones achieved in developing artificial cells. Now a day's artificial cells are developed which are more adaptive with the environment. In the future prospects scientist are looking for more advance fully autonomous artificial cells.

The future prospects of any cell depend on the effective metabolism that is successful enough to assist the biometric process carry out inside the compartment. Strategies for the development of the cell should be such that it provide free access for the duplication of the membrane parts as well as genetic information. Mutual compatibility is utmost important for integrating advanced functional elements inside the cell. The bottom up

approach are developed for building up of SCs which will not only enhances our knowledge for the development of physical and chemical processes in living organization but also provide foundation for the use of the biomedicine, cytobiology, smart science, automatic micro-reactions etc. Challenges faced during synthesizing Artificial Cells are :

1. In 1858. Virchow observed the most basic and essential feature of a cell, that a cell can originate only from another cell [54].

2. A self-replicating 'Artificial Cell' is an enduring and essential goal.

3. Practical and theoretical problems- referring to Creation of advances triggers, new stimulation, such as [54].

    a. Student related to organization of the cell (intricacy, life-resemblance),

    b. Materials required (primal-like, contemporary biochemical's, entirely synthetic),

    c. What is the aim (basic or applied science).

4. As quoted in The chemoton model for chemical research of a living, designed by Tibor Gánti, is mostly used to explain the least life. As seen in his design, a substance consists of [54].

    a. A chemical boundary system for the cell,

    b. The chemistry behind the information system

    c. A self-duplicate metabolism which can resemble with the living cell.

    d. Moreover, growth and reproduction are the vital part for the survival of the species.

    e. Lastly compatibility with the changing environment for life's survival.

Our future prospects is to put all these features in a solo artificial system. The advance research in field of the artificial cell evolution are:

Artificial cell has now spreading its wings in the field of drug delivery, nanomedicine, enzyme/gene study and therapy, cancer treatment, cell/stem cell treatment, development of nanoparticles, regenerative medicine, nanobiotechnology, nanotechnology and related fields .The research progress in field of synthetic cells is gradually increasing, though some questions still has to be answered in order to continue our endeavour to achieve advance goals of life science and applied science considering complexities in present & future life as we envisage today –

i. How will SCs be in touch with the environment?

ii. How will their inability to survive for a sufficient period of time increase?

iii. How will the functionality of artificial cells increase? Evolution is essential to keep improving the research on artificial cells. As quoted "As engineers, we can't build a perfect synthetic cell. We have to build a self-correcting system that becomes better as it goes"[54-56].

Future prospects and outlook:

Several researcher and scientist have developed a cell-mimic units such as (a) erythrocyte membrane-resembles nanoparticles for drug delivery [57] (b) nanosponges to eliminate toxins within the living [58], (c) nanoparticles coated membrane-cancer cell for enhance immune response and drug delivery for anticancer (d) nanoparticles coated bacterial membrane- for formulating antibacterial vaccine and (e) nanoparticles used for cloaking platelet for improving drug flow and protect against platelet-remain pathogens [59].Cancer cell is used for the targeted delivery stem cell nanoghosts and leukocyte-like vectors also plays a important role into the tumor cell[60-63].

There have been many researches and talks about 'Life-Likeness' of artificial cells even though having a 'fully-living' artificial cell is still implausible but as it keeps us progressing in the field of SCs, it will give pivotal & multi-dimensional benefits through its new & vast applications There is still requirement of filling the wide gap to achieve from artificial cells to biological cells. Many issues needs to be taken into accounts such are (i) How to develop artificial cell so that it can easily communicated within them and outside environment. (ii) How to prepare artificial cell networks so that all the organelle work together. (Iii) How to improve self replication and

propagation in the cell (iv) how to feed themselves for their growth and propagation. Dealing with all these aspects require lots of energy understanding which challenge our technologies. Totally living artificial cells is our ultimate goal which will new rare of hope and provide solution to various applications biotechnology, drugs medicine, diagnosis and industry etc.

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