ARTIFICIAL CELLS: THE BIOROBOTS

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INTRODUCTION

The ability of living cells to replicate, assimilate and react in an environment makes them unique. Each living cell type has its own unique habitat, biochemical processes, replicative mechanisms, and adaptive and survival methods. This uniqueness can be altered with human intervention through genetic engineering or specific cell culturing methods in a controlled environment. Even if we are capable of such technologies, we are unable to regulate or alter the free will of a cell with 100% efficacy. The cell must possess abilities to grow, adapt, survive, and replicate with respect to its environment either alone, or as a tissue. The abilities of cells depend solely on its type of differentiation during the developmental phase of an organism that is from pluripotent cells in the embryo to a differentiated cell in an infant. These factors can be specified *in vitro* by specific growth hormones and growth cues via stimulants.

But can we create a cell or tissue to carry out a task designed by us? We can, with these two options. First, is to create a whole artificial and programmable cell, or the second option to use the differentiated stem cells and programming them based on programmable cues. Then the question arises why we should create artificial cells. The need for such cells is to mimic the natural cells without risking an inflammatory response and achieve a task to solve biological issues.

ARTIFICIAL CELLS

The artificial cell is synthesised based on the actual molecular composition of a natural cell. These components include carbohydrate units, proteins, and lipid structures. Also, they require specific organelle based biochemical reactions to carry out essential activities in a certain controlled environment. Current advancements in this field of research have produced artificial cells or its components to work individually or in complex with undifferentiated cells. Following are the details of such achievements:

1. Artificial cell nuclei

A research team from Osaka University successfully synthesised a nucleus inside a mouse embryo cell using microinjection technique. They discovered that inserting a double stranded DNA of suggested base pair length conjugated with a 3µm bead can initiate the formation of nucleosome and nuclear membrane with the help of additional factor RCC1 inside the embryo cell. They validated the formation of histone complex in the inserted DNA-bead via immunofluorescence. The formation of double layered membrane, and nucleoporin pore complex formation were confirmed with electron microscope and fluorescence imaging technique. But transmissibility across the nuclear pore complex was low or negligible. The study will help investigate the cell formation mechanisms, and how cell nuclei can be reconstructed for functionality acquisition. In the future, if the functional artificial cell nucleus will nuclear transport activity can be constructed by combining other factors with DNA, the mechanisms behind cell nuclei formation and functionality acquisition can be further understood. The current knowledge will help.

2. Synthetic Cells

Synthetic cells are minimally functional cells made of lipid bi-layer membrane carrying specific biomarkers. When produced in batch they generate precisely uniform and consistent custom constructs to be used with any optical, fluorescence, and biochemical features. They are better than conventional diagnostic units in terms of expense, variability, supply, and accessibility of live cells.

They have better shelf life when compared with live cells, and thus easing global shipping constraints. The application list of synthetic cells includes diagnostic instrument calibration, routine, and rapid diagnostics for existing, new, or rare diseases, and development of novel cell therapies.

3. Self-polymerising cargo cells

Zhan and co-workers created cargo cells formed from DNA based cytoskeletons operating in cell-sized microfluidic compartments. The cargo cells possess essential functions such as compartmentalisation, ATP-triggered self-assembly, and reversible assembly. These cytoskeleton units are formed with conjugation of DNA filaments of predetermined sequence and actin filaments, assembled in a microfluidic chamber in optimised buffer. To test the cargo transport ability of these systems they designed DNA filaments with RNA overhangs. They synthesised small unilamellar vesicles (SUV) to act as organic cargo, with gold nanoparticles to track inorganic particle transport. RNase H powered the SUV movement across the DNA filaments via the RNA-DNA hybridisation. The movement and cargo transfer were further confirmed in transmission electron microscopy and confocal microscopy confirmed the binding and transport of the SUV along the DNA filament. At present the limitations of this system include intracellular transport, communication, and organisation like a multifunctional living cell. But this is a start point towards achieving a multifunctional artificial cargo cell.

4. Self-dividing artificial cells

The self-dividing artificial cells were created by scientists at the Max Planck Institute of Colloids and Interfaces, Potsdam, and at the Max Planck Institute for Polymer Research, Mainz. They tested and validated the novel mechanism of constriction force on cell membrane in a systematic manner. They designed bilayer membranes where the outer membrane had varying concentration of protein than the inner membrane. The asymmetry between the two layers generated spontaneous curvature that determines the shape of the artificial cells. When the closed membrane neck is formed, the spontaneous curvature forms a local constriction force on the membrane leading to the division of the cell. Optimising the protein concentration of bilayer membrane will create uniform batch of self-dividing artificial cells. The amount of required protein is so low that we can add specific markers to the membrane surface based on specific applications.

5. Xenobots

These cells are formed from the Xenopus laevis embryo, and thus termed as xenobots. The cells isolated from the animal most region of the Xenopus blastula embryo was microinjected with messenger RNA for the gene expression regulation studies in vitro. Douglas et. al. developed several test attempts and analysed behavioural patterns, movement state transition, wound healing, motion tracking and carried out simulation study for collective behaviour of the xenobots. These tests revealed that, xenobots have varying motions, pile collecting ability, ability to self-repair and retain molecular memory. The computational simulation on varying shape of xenobot cell shape, revealed that they can be further improved for varying applications with different cell type combinations (such as a combination of myosin cells and normal eukaryotic cells).

6. Multicompartmentalized Artificial cells

These are multicompartmentalized vesicles that mimic a eukaryotic cell. These artificial cells are generated based on a work by Bayley and team, 2011. The interconnected vesicle networks are

formed via contact between multiple lipid monolayer-covered water-in-oil droplets, which form droplet interface bilayers (DIBs). These individual compartments can hold individual enzymatic reactions and substrates and share among themselves under specified conditions. These artificial cells can be further improved and made into a cluster of cells with soft body to mimic a tissue. These units can be used as a patch over a repairing tissue to synergise the repair mechanism or can target cancer tissue.

FUNCTIONING OF ARTIFICIAL CELLS

To benefit and achieve full potential of the artificial cell, it must mimic the qualities of a live cell. These qualities include the ability to grow, divide, sense, adapt, communicate and motility. Researchers have focussed to introduce such traits into artificial cells, and they have achieved the same.

1. Growth

The membrane of artificial cells may wear off with time. This can be solved by adding more membrane units either externally or internally. One approach adopted by researchers is to auto-generate membrane by encapsulating a catalyst into a membrane or lumen, where it produces an amphiphilic molecule for auto-integration into the artificial cell membrane. This method results in dilution of the catalyst following several cycles of growth. A solution was designed by Devaraj et. al., by creating a catalyst that can both generate new membrane components and undergo autocatalysis. This system could achieve 15 cycles of near-complete conversion of lipid precursors generating many new vesicles.

One more criterion for growth is to repair and rejuvenate the reaction centres/vesicles to act as bioreactors. Till now, only minor development has been done in this area. Research by Sugawara et.al., has shown a minor approach in this regard where self-reproduction of vesicles is coupled to internal DNA amplification.

2. Division

Division of a artificial cell can be regulated and is much easier than the stringent conditions within a natural cell. Researchers have tried cell divisions by methods like, external mechanical shearing, membrane growth, protein loading to membranes, volume reduction, or phase separation. More spatiotemporal control over artificial cell division has been achieved with regulatory proteins FtsZ, FtsA and Min proteins from E. coli. These proteins produce a contractile Z-ring that helps in cell pinch off and division in a artificial cell. The challenges still faced in this regard is the equal distribution of biomolecules following division and adequate cell cycle checkpoints.

3. Adaptivity

Environment sensing in artificial cells can be achieved by tagging the membrane with proteins carrying pH modifying enzymes like alcohol dehydrogenase (ADH). Addition of substrates like acetone or alcohol will change pH inside the cell. Using a substrate antagonist will reverse the effect. Similarly, covalent addition of targeted proteins to lipid membranes with spatiotemporal control or changing the lipid composition by exchanging head and tail groups with reactive proteins. Similar modifications can help change the morphology of artificial cells based on light, temperature, or pH variations.

4. Communication

In any living cell the coordinated actions are carried out through chemical mediated communications. Such communications can be mimicked in artificial cells via selective reaction products and their interactions. The first artificial cell to test the same used formaldehyde and boric acid to produce sugar derivatives which in turn activated bioluminescence activity in the bacterium *Vibrio harveyi*. Mann and group developed hydrogen peroxide producing

colloidosome which reacted with the outer shell of secondary colloidosome to alter its enclosed enzymatic reaction. Despite such studies the reciprocal interaction among artificial cells remains unexplored. Achieving reciprocal communication scheme between artificial cells can help achieve tissue level organization.

5. Motility

Living cells respond to its chemical environment for motility, also known as chemotaxis. Artificial compartments can be made motile using molecules that respond to magnetic, acoustic, or electric field, light, or a chemical fuel. Vesicles with sugar, or hydrogen peroxide catalysing enzymes have shown motility in specialised environment containing that substrate. These movements are caused by a combination of diffusiophoresis and oxygen bubble formation following the enzymatic reaction. Such vesicles can move across the gradients of the enzyme substrate and their movements can be regulated with several feed forward loops independent of fuel concentration.

ARTIFICIAL INTELLIGENCE (AI) AND ARTIFICIAL CELLS

Integrating the power of data computing into studying life forms can generate idea-based simulations and insights into predicting the possible nature of a cell. Such algorithms can be developed in order to understand the replicative, adaptive, environment sensing and motile feature of artificial cells. These algorithms will train on the in vitro data based on natural cells or artificial vesicle-based cells. Also, the chemical reactivity, functioning and stability of artificial cells can be further improved based on the insights provided by such algorithms.

Kriegman and group were successful in developing an evolutionary algorithm combined with a physics simulator to find insights into conditions that could enhance the self-replicative cue in an artificial cell (xenobot). They also studied the effect of cell body shape to enhance pile

aggregation. The results were replicated in vitro, with results similar to the simulation. Several generations of training the algorithm are required to achieve such insights. Generating and collecting in vitro data and applying it into an algorithm is less time consuming than testing same conditions in vitro. As in vitro data accumulates computational models integrating multimodal data becomes possible. Multimodal deep learning will enhance learned features for individual task over multiple modalities. Generation based learning will further improve the predictive abilities of these models and will further aid in optimising artificial cells for specific tasks.

CONCLUSION

Creating artificial cells from the ground up is starting to pick up speed. Researchers have focussed on mimicking the natural features of a cell into artificial cells. These features include growth, division, environment sensing, adaptability, communication, and motility. Prominent advances include the construction of gene circuits with increasingly complex behaviour and vesicles with self-reproducing compartments, environment sensing, and adaptive abilities.

The future developments will provide the life-like systems the ability to self-sustain with biomimetic metabolic reactions. These activities should have several check point systems to control growth, rate of division, and motility. These systems when developed will function as biorobots and can act in swarm model to achieve naturally impossible tasks. Such a life-like system will have huge potential from industries such as food and beverages, pharmaceutical and medical.

Initial step towards swarm-biorobots would be the integration of compatible functional elements within a static multicompartmentalized vesicle platform. Second step is the integration of enzymatic or non-enzymatic features to enhance communication between artificial cells similar to a bacterial colony or multicellular organism. Adaptive ability can be further enhanced with the help of AI based insights while understanding the principles of genetic evolution.

Finally, the construction of artificial cells with the integration of AI will improve our understanding of biochemical functioning in a living system and aid further developing biomedicine and environmental science.

Reference

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