Building Cells From Scratch: possibilities and perspectives

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S3186091
14/06/2019
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Abstract

Understanding the entirety of the living cell is one of the main challenges in cell biology. A very interesting way to do this, is to build an artificial cell from the bottom-up; starting with smaller particles and constructing a cell with them. In this review some of the latest developments as well as traditional methods in synthetic biology will we discussed and compared. Three integral parts of the cell are used as a guideline, namely the plasma membrane, cell organelles, and the cytoskeleton. It was found that many different ways can be used for artificial cell biology, but most rely on partly natural, partly synthetic systems. The creation of a completely functioning artificial eukaryotic cell is not yet realized, but may be achieved in the future.

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Introduction

Life usually form from other life, but originated from inorganics at least once. With this in mind, biologists all over the world have been looking for ways to replicate this origin in what is so called 'artificial cells'. An artificial, or synthetic cell, is a mimic of the basic building block for all organisms on earth. The artificial cell is the subject of studies in many different fields of biology, and will give insights into the origin of life. First it was thought to just be a concept on paper, but in the past few decades, this concept seems to come closer to being reality. The ultimate goal is to make a completely artificial, living, eukaryotic cell that has functioning metabolism and can self-reproduce (Xu, Hu, & Chen, 2016).

The first step in the direction of an artificially made cell was by Thomas Chang in 1964, who managed to construct microcapsules in which proteins retain their biological functions. The encapsulation of the proteins meant that they were protected from the environment, but the capsule being semi-permeable, still allowed for exchange of small molecules between the inside and outside of the cell (Chang, 1964). Later, the technique for artificial capsules improved and directed its use to controlled production of pharmaceutical compounds and with this, a broader interest in synthetic biology grew (Prakash, 2007). Many developments have been made ever since, and some of them will be discussed in this review.

At first sight, it may seem trivial to make an artificial cell, but when looking closer at the possibilities, the applications seem endless, such as in research in the field of molecular cell biology. Spatiotemporal separation and interaction between separate parts of the cell, are not understood in detail and are necessary for artificial cell construction. This has been a central challenge in molecular cell biology for years, and the construction of artificial cells can be of great importance to the field (Liu, 2019). Not only will the construction of artificial cells give insight into the origin of life on earth, it can also lead to breakthroughs in regenerative medicine, stem cell therapy and nanobiotechnology. Such as the construction of artificial Islets of Langerhans as a treatment method for diabetes patients, or helping with enzyme or hormonal deficiencies (Prakash, 2007).

To make a living cell means to understand what it is, and what are the building blocks needed to make one. Therefore, it is necessary to know the requirements for life itself, and what it means for something to be alive. Although a widely discussed topic, there is an overall consensus on what life is, with three main points; it has i) a semi-permeable enclosure, that provides protection and exchanges with the environment ii) macromolecules that carry genetic information, such as DNA and RNA and iii) a self-maintaining and renewing metabolic system (Chang, 2007). If it is possible to completely, synthetically create a single cell that has all these characteristics, then we could say that an artificial cell has been made. However, technologies so far have not been able to do this so far. Yet, there have been many attempts and many of them took a step in the direction of a complete artificial cell.

There are different ways to make something from scratch. In synthetic biology, two different ways of assembly of cells are known; the top-down method and the bottom-up method, see figure 1. The top-down approach involves a simplified or unmodified living cell as starting point. From there on, the cell is modified with different molecules, such as genes for different signals, or morphologies. This approach is similar to bioengineering a cell, but involves more complexity in its starting material. One example of a successful top-down experiment is a living bacterium that had only genetic material that had been chemically synthesized, yet could proliferate and maintain its health with no problem (Gibson et al., 2010). The bottom-up approach takes non-living particles as a base and couples them together to make an artificial cell, making its starting point simpler, but moving towards more complexity (Schwille & Sundmacher, 2014). Both methods are valid options in synthetic biology, however, in this review the focus will lie on the bottom-up approach.

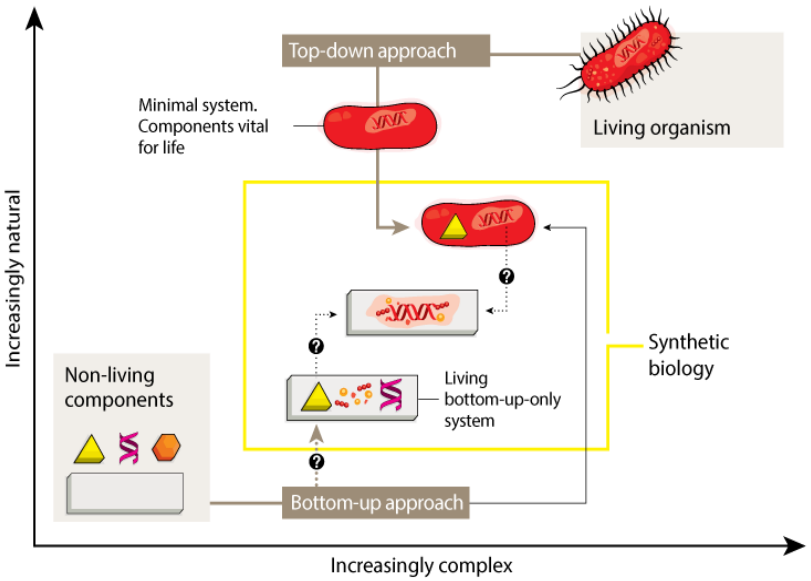

Figure 1: The two approaches in synthetic biology; the top-approach in which the cell is used as a base for engineering, and the bottom-up approach in which non-living particles are the first things in construction of the cell (Schwille & Sundmacher, 2014).

There are also different types of artificial cells. Xu and colleagues. describe typical and non-typical artificial cells. The difference herein lies with what they are used for. Typical artificial cells are described as cells that are most like actual cells, in that they exhibit characteristic of cells such as metabolism, evolution and self-replication. The typical artificial cell is a cell in the sense of a complete cell, not just parts of it. Non-typical artificial cells are simply smaller parts that are mimicked in a convenient way to study specific aspects of the cell, such as artificial membranes, or separate organelles. The non-typical artificial cell can be used as a tool to be specifically engineered for a single purpose. Most developments in making a complete artificial cell come from artificially made parts of the cell. In a way, typical and non-typical cells are both used in the quest towards the complete, living artificial cell (Xu et al., 2016)

In this review, we will look at the different aspects of the cell that need to be constructed to make a working artificial eukaryotic cell, and compare the techniques that have been used up until now to achieve this goal in this particular field of biology. The main focus will be with three different parts of living eukaryotic cells, namely the membrane, cell organelles and a cytoskeleton, and the techniques involving the construction of these.

Compartmentalization in artificial vesicles using various methods

One of the most important parts of the cell, responsible for maintaining inner and outer balance of the cell, is its plasma membrane. The membrane protects the cell from harm, while maintaining a flexible exterior that fulfills several tasks such as transport and contact with neighboring cells. To construct a membrane is not the most difficult part of creating an artificial cell and was done in 1992 by Bachman and colleagues. In their study, they found a way to generate micelle structures using lipids. The reactions taking place would feed and grow the micelles, thus creating self-assembling structures (Bachmann, Luisi, & Lang, 1992). This later led to more studies involving self-assembling semi-permeable membrane structures, and gave insight into what might have been a naturally occurring phenomenon in the creation of the first cells (Stano, Souza, de, Kuruma, Carrara, & Luisi, 2013). This further led to more developments in artificial membrane technologies, sometimes based on the construction of lipid bilayers, but some assembled membranes from inorganic compounds. These (artificial) liposomes usually are called small, large or giant unilamellar vesicles (SUV, LUV and GUV). They are used for a variety of membrane studies and in synthetic biology. It was found that exposing a vesicle with double a membrane to certain solvents sometimes results in spontaneous formation of a single membrane. This method is called gentle hydration, but cannot be used with all combinations of phospholipids and is quite tedious (Reeves & Dowben, 1969). Thus, different methods were developed to produce GUVs.

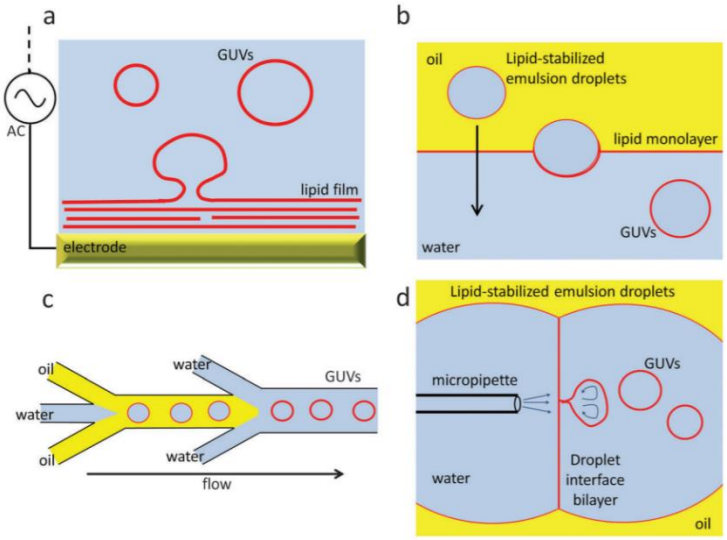

Figure 2: Four different ways of creating GUVs. A) Electroformation of vesicles, using a lipid film or gel and exposing it to an electric field. B) Using transferring a water-oil-droplets across different phases to construct a lipid bilayer. C) Microfluidics system based on emulsion. D) Microfluidic jetting, using a micropipette to grow droplets from an interface bilayer (Beales, Ciani, & Cleasby, 2015).

Water-in-oil droplets

Using water droplets in oil can be a convenient way of creating self-assembling vesicles, and has been used in several studies regarding artificial cells (Yoon, Tanaka, Sekiguchi, & Shoji, 2018). In synthetic biology, some advances were made regarding the assembling methods of phospholipids to form layers around droplets using phase differences, see figure 2b. One way of water-in-oil droplets was achieved, not using conventional phospholipids, but inorganic silica nanoparticles. These particles were formed in oil from aqueous droplets with the silica particles around them, and were then transferred to an aqueous phase, resulting in isolated vesicles. The way the vesicles are made is not with self-assembling particles, but rather on the basis of an inorganic membrane, and thus, as the authors state, can be controlled very well, according to the study. This would be beneficial for a variety of studies, since the environment in and outside of the particles can be made custom to fit the goal of the study. The authors were also able to modify the surface of these vesicles. The study showed that the vesicles, although not constructed from a lipid bilayer, could still sustain biomolecules in them that retained their function (Li, Green, Anderson, Binks, & Mann, 2011). Furthermore, for several years now, these vesicles have been used in a large variety of experiments that require high-throughput creation of microcompartments that vary between one µm and two hundred µm (Schaerli & Hollfelder, 2009). The overall consensus of using oil-in-water droplets is that they are not too

difficult to make, can be controlled in size quite precisely and are a very convenient method of constructing a compartment in which biological reactions can take place. Advanced machines are usually needed for these techniques however, and can be very expensive as well. Moreover, a downside to these vesicles is that oil left in the membrane can pose problems (Weinberger et al., 2013). Furthermore, they do not completely imitate biophysical properties that are found in plasma membranes (Weiss et al., 2018).

Electroformation of GUVs

Another way to make vesicles is with the use of hydrogels or lipid films to produce a double membrane from lipids. Nowadays, electroformation is a common practice to make GUVs, see figure 2a. This method uses the stress that the vesicles have to undergo under electrical field and is preferred because it is much faster than gentle hydration. It was shown that these vesicles can sustain membrane proteins in them and also have a relatively long lifetime. Moreover, using this method, the size of the droplets can be controlled (Okumura & Sugiyama, 2011). Electroformation used to be only possible in inorganic solvents, because a high salt concentration would readily destroy the vesicles under voltage. Experimenting with different buffers has made it possible to acquire GUVs in physiological conditions, forming from (proteo)liposomes (Pott, Bouvrais, & Méléard, 2008). However, electroformation is still highly dependent on the type of buffer that is used, and while developments have been made regarding this, it is still quite difficult. Weinberger and colleagues propose a mechanism using polyvinyl alcohol (PVA) gels as a way to grow only unilamellar vesicles in a shorter time. They did succeed in this, and found that the did not contaminate the lipids, and still could support proteins inside (Weinberger et al., 2013).

Microfluidics

To make vesicles as mentioned above, often a lot of money, time and work is involved, especially when making them in bulk and all of preferably the same size. Microfluidic technologies, chip based technology on which you can control small droplets, may offer a solution to this. Droplet microfluidics are generally promising in synthetic biology, but also in artificial cell research (Gach, Iwai, Kim, Hillson Acde, & Singh, 2017). There are two most common ways of making the vesicles; microfluidic double emulsion, and microfluidic jetting. Microfluidic jetting involves the use of a micropipette inside a water phase that is surrounded by oil, see figure 2d. The droplet is separated into parts by a droplet interface bilayer (DIB), and the pipette jets water into the membrane, creating GUVs (Beales, Ciani, & Cleasby, 2015). To make the droplets using the double emulsion method, it is necessary to stretch and fragmentate a liquid, see figure 4c. This is usually done by hydrodynamic shear force, giving droplets of approximately the same size. It has even been done by light actuation (Diguet, Li, Queyriaux, Chen, & Baigl, 2011). The droplets are on a chip, see figure 3, and can be manipulated in several ways, making use of electric currents or capillary forces (Gach et al., 2016). One interesting application of microfluidics technologies is the creation of vesosomes; liposomal structures with smaller vesicles inside them. The use of microfluidics provides a fast way to produce many vesosomes of the same size. Inside the smaller vesicles, there is possibility to reconstitute different proteins in or on the separate membranes. This gives way to perhaps one or more distinguished organelles, such as a nucleus.(Deng, Yelleswarapu, Zheng, & Huck, 2017).

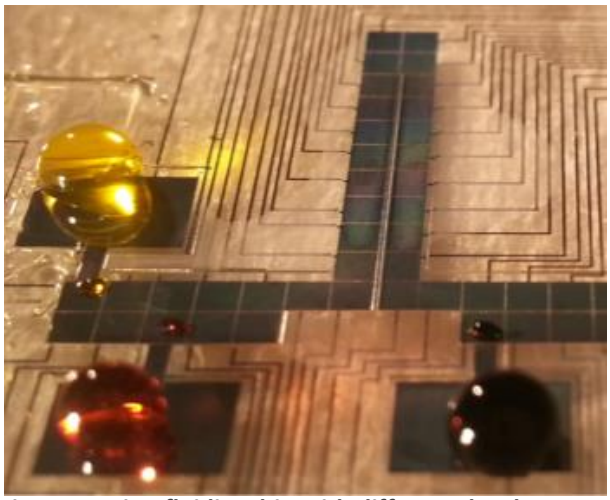

Figure 3: microfluidics chip with different droplets . The different lanes can bring droplets together and the source droplets can be replenished (Gach et al., 2016).

Encapsulation and picoinjection of proteins into vesicles

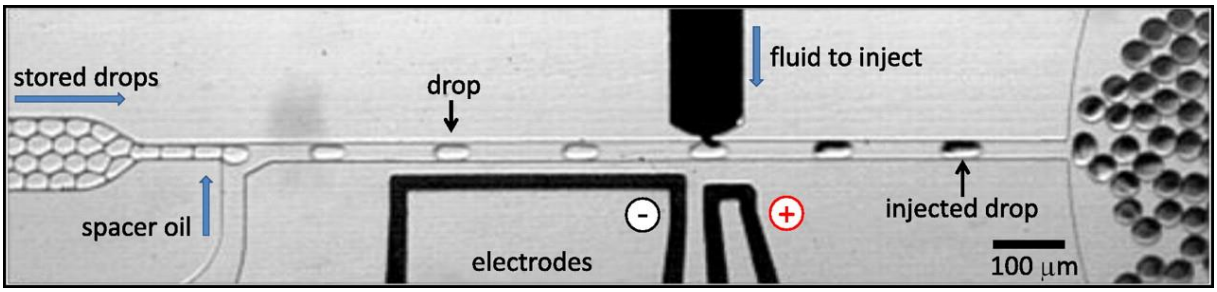

Figure 4: Microscopic image of picoinjector in a microfluidics device. As the droplets move through the oil phase, it gets injected with a fluid. The injector is controlled by an electric field (Abate et al., 2010)**.**

The use of microfluidics is often paired with encapsulation techniques and picoinjections. These are necessary to bring proteins and other molecules of interest into the generated drops. Picoinjection, as the name suggest, is the injection of – usually – fluids, into droplets or liposomes. Because a picoinjector is controlled with an electric field, the volumes released when making contact with a droplet are very precise, see figure 4. Multiple injectors can be used to create multistep reactions inside the droplets (Abate, Hung, Mary, Agresti, & Weitz, 2010).

Encapsulation of proteins can be done on a microchip, during the formation of liposomes. The surface of the chip contains proteins or particles of interest, and the liposomes are formed around them inside microscopic wells. This is done by applying an electric field inside the lipid containing solution, so that the liposomes are formed in the right place and with the right size. Because the particles of interest are displayed at the surface of the chip, the solution outside the formed liposome will not contain the particles, thus creating a different environment outside the cell (Osaki, Kamiya, Kawano, Sasaki, & Takeuchi, 2012)

There are some challenges with GUVs regarding their actual use in artificial biology, such as pH sensitivity and instability in highly ionic environments (Weiss et al., 2018). One overarching main problem with artificial liposomes, can be that they can collapse quite easily in the wrong circumstances (Kurokawa et al., 2017). This is the reason that Weiss and colleagues proposed a new mechanism, combining beneficial properties of GUVs and water-in-oil droplets to make droplet-stabilized GUVs (dsGUVs). The vesicles were generated from GUVs, and were observed to be functional in biophysical properties (Weiss et al., 2018).

Cell organelles

A characteristic of eukaryotic cells is the presence of internal membrane compartments that host several separated biological processes in the form of cell organelles. The first challenge in constructing organelles is the complex morphology inside the cell that encapsulates all these different environments. It has been found that certain proteins are responsible for certain types of curvature in cellular membranes, such as Epsin and AP180 (Busch et al., 2015). Another proposed mechanism is protein-protein crowding, or a high concentration of proteins that causes curvature simply because of the density (Stachowiak et al., 2012). Knowing this might help with creating organelles within an artificial cell, such as a nuclear envelope or protein translation machinery. These structure have intricate membrane morphologies in cells as we know them, but there might be option in synthetic biology to work around this.

DNA as building blocks

An interesting, very promising tool for making cell organelles may be so called ‘scaffolded DNA origami’, first designed by Paul Rothemund in 2006. Rothemund found a way to use the intrinsic assembling characteristic of strands of DNA to make several shapes. He used a single strand of DNA to make a

scaffold with a general shape, and then cross-linked the shape with additional pieces of DNA to make a whole, stable structure, see figure 5 (Rothemund, 2006). Several different applications were found for the DNA origami, such as three-dimensional structures on nano- and microscale (Arora & de Silva, 2018). One of such applications, which is especially important for synthetic biology, is the use of DNA origami coats to reproduce a curvature that is similar to the curvature of the cell membrane (Franquelim, Khmelinskaia, Sobczak, Dietz, & Schwille, 2018). Another outstanding application of DNA origami is the creation of nanopores. Functioning porins were made, inserted in a lipid bilayer and tested using an ionic current (Göpfrich et al., 2016). Using this nanopore *in* vivo is most likely not entirely possible yet, since there is no selectivity in what goes through it and was does not. Nonetheless, the use of DNA origami may provide sufficient building blocks for creation of artificial cells.

Another few interesting developments have been made with the use of DNA as a building block. A step forward in stimuli sensitivity (essential in living organisms) has been done with artificial membrane coating. Exposing this coat to certain pH or molecules, such as ATP, break the structure to release the molecules that are inside (Liao et al., 2017). A transport molecule that looks like a mousetrap, made from DNA, has been constructed as well. It can carry different molecules and can be locked and unlocked with an aptamer key that is also made from DNA. This has not yet been tested within a physiological environment yet, but might be useful in the future (Ding, Seeman, Zhang, & Winfree, 2006). Furthermore, an ensnaring cascade has been made, and tested in liposomes (Löffler et al., 2017), as well as DNA-construct regulated polymerization (Meng et al., 2016), and a myriad of other biologically functioning artificial molecules (Göpfrich, Platzman, & Spatz, 2018). All in all, it is safe to say that DNA origami will be a tremendous tool in synthetic biology as it is very versatile and can be made relatively easy and fast.

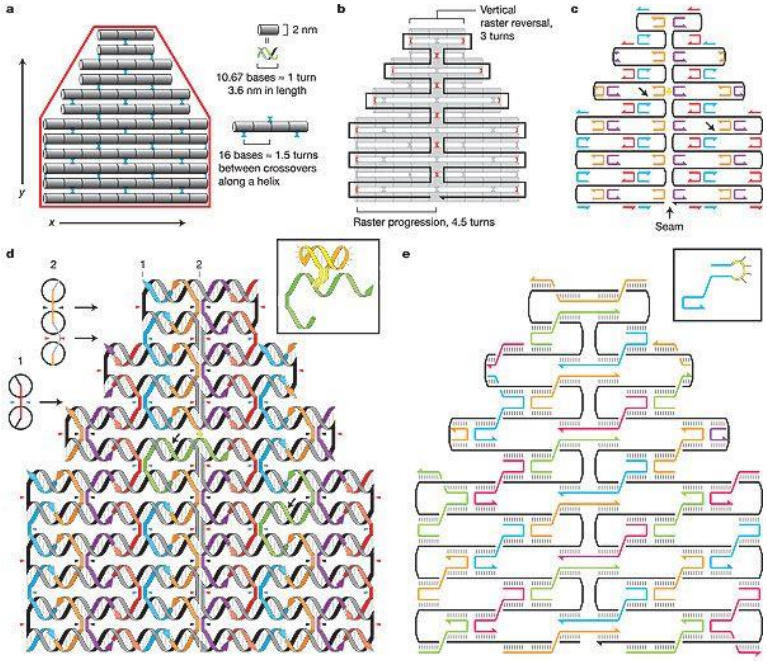

Figure 5: Example of shape made from scaffolded DNA origami. The DNA origami is comprised of a scaffold (black) running through the whole shape, with cross-overs (colored) that link the molecule together at the bends and seam (Rothemund, 2006).

Energy generation

Mitochondria, as they are the powerhouse of the cell, are an essential part of eukaryotic cells, providing energy in the form of ATP (Siekevitz, 1957). Making a cell without ATP seems like an unthinkable concept in artificial biology, so a mimic for either mitochondria or parts of the mitochondria need to be constructed in order for the cell to sustain itself. Already some ways of artificial ATP production have been explored in the last decades. The use of bacteriorhodopsin in combination with the F_0F_1 complex in artificial vesicles has proven fruitful and showed that the ATP synthesis system can be used in artificial membranes. Creating such construction may be useful as artificial mitochondria inside an artificial cell (Choi & Montemagno, 2005; Pitard, Richard, Dunarach, Girault, & Rigaiud, 1996). One step further was taken in the advancement of a synthetic cell, that is also energy independent, very recently; taking the developments of artificial ATP synthesis by using bacteriorhodopsin and combining it with ATP synthase inside an artificial membrane. The result was a cell system inside a GUV, where protein synthesis was possible with photosynthesized ATP. The activity of this system was tested using a protein synthesis

system, and was deemed useful and active (Berhanu, Ueda, & Kuruma, 2019). Using ATP synthesis for sustaining the artificial thus seems to be possible.

Translation organelle

Cell free translation inside a test-tube has already been made possible in the past. The so called 'protein synthesis using recombinant elements-system' or 'PURE-system' uses purified translation proteins and combines them with all the necessary elements for protein translation. This system allows for high activity and highly pure proteins to be synthesized and purified (Shimizu et al., 2001). This is however not encapsulated inside a vesicle, thus might not be ideal for the creation of an artificial cell.

One of the biggest advancements in artificial cells so far, might have been done by Reinkemeier and colleagues in the beginning of 2019. Their research involved a breakthrough involving artificial organelles, made using noncanonical amino acids. The organelle that was made, could translate the artificial mRNA into proteins. The organelle was made without a membrane encapsulating it, instead using phase and spatial separation inside the cell. Phase separation is an already existing mechanism that cells use to separate biological processes within the cell (Boeynaems et al., 2018). Reinkemeier and colleagues used this as inspiration to separate their organelle from the rest of the host cell, yet still have the mRNA gain access to the organelle. Using custom made RNA allows for site-specific protein engineering to be a possibility for future uses, outside of synthetic biology as well (Reinkemeier, Girona, & Lemke, 2019).

Cytoskeleton

The cytoskeleton is an important factor in cell deformation, adhesion and shaping, working closely together with the plasma membrane. Using oil droplets or artificial liposomes, the workings of actin filaments were studied in detail. Since the droplets were fluid, the deformations and motility caused by the actin network were examined. It was found that the actin filaments could influence movements and even induce the creation of vesicles solely by polymerization (Simon, Caorsi, Campillo, & Sykes, 2018). The plasma membrane is partly responsible for actin filaments bundling. Thus, the membrane alone is of great importance to stability and formation of cytoskeleton as well (Liu et al., 2008). Finding a way to incorporate a cytoskeleton in an artificial cell is still essential .

For cell motility, the cell membrane and cytoskeleton closely cooperate with each other. Essential to this are integrins, a group of cell adhesion molecules that reside inside the plasma membrane and form contacts between the cytoskeleton and the extracellular matrix, with the help of a protein called talin. This group of proteins and their mechanisms have been involved in studies regarding artificial cells. They were integrated into GUVs, small unilamellar vesicles and membrane structures. It was found that actin and integrins together are responsible for adhesion, and that the mechanism works well in GUVs (Brüggemann, Frohnmayer, & Spatz, 2014; Streicher et al., 2009). The encapsulation of the cytoskeleton proteins into membrane structures is an important step in artificial cell technologies. However, it is quite a difficult technology that knows some struggles. Many different ways have been used in the past to encapsulate filament proteins into vesicles. Tsai et al. (2011) have found an easier and quicker way to do this, which was originally used for studying the cytoskeleton, but provides an interesting perspective in the field of artificial biology. (Tsai, Stuhrmann, & Koenderink, 2011)

As mentioned before, the emergence of scaffolded DNA origami has been an important development in synthetic biology. The scaffolds can give stability and strength to the plasma membrane as well, as was done by Kurokawa and colleagues in 2017. This group used positively charged phospholipids in such a way that they attracted the negatively charged scaffold. The properties of the DNA scaffold match greatly with what a quite fragile liposome membrane needs in terms of stability and flexibility. A further benefit of using DNA is that it can mimic biophysical properties of cytoskeletons quite well (Kurokawa et al., 2017). While many developments have been made by reconstituting cytoskeletal proteins such as actin filaments

into liposomes or GUVs, it appears only very few developments were made using artificial building blocks for the cytoskeleton. Actual motility of artificial cells has not been achieved yet either, but may be possible in the future, for example with the use of already existing proteins and ATP (Göpfrich et al., 2018)

Discussion

Many developments have been made regarding the creation of artificial cells in the past few decades. With the emergence of technologies on a smaller scale such as microfluidics and picoinjections it has been made possible to create plasma membrane mimics and liposomal structures that can hold active proteins. The use of GUVs is very convenient for GUVs take up less time than other methods of vesicle construction. They do not completely mimic a biophysical environment and some complications can occur, such as breaking of the membrane or collapse of the whole vesicle. A suggestion would be to use dsGUVs to combat this. However, the use of vesosomes would seem more ideal in that several compartments can be made inside a larger compartment, and a nucleus could be constructed in one of the smaller particles. This is not necessary when using the translation organelle that Reinkemeier and colleagues named in 2019. For stability inside the cell DNA origami could be used, but then the cell will not be able to move, so something like an integrin mimic as well as actin filaments and microtubuli need to be present too. The use of already existing proteins in artificial compartments would be useful for further research on the cytoskeleton and its functions and abilities. This would contribute to non-typical artificial cell development, but not necessarily to a typical artificial cell. For motility, most likely ATP is necessary. To use a mimic of ATP would be extremely difficult, so a mechanism of ATP synthase is essential. As shown, this has already been achieved in GUVs.

All of these different aspects of cell biology have been focused on separately in different studies. Isolating different parts of the cell and assembling them seems like not truly creating a typical artificial cell. A suggestion would be to focus on a way to artificially translate DNA and RNA structures to make proteins that will be active in the environment of an artificial vesicle. This seems like a good basis for building the rest of a cell interior around it; a true bottom-up approach. Moreover, creating separate parts is of course necessary to make sure all functions of the cell are realized, but combining loose parts together might not always be possible. The vesosome method is, for example, not compatible with the translation organelle of Reinkemeier and colleagues, and nanopores consisting of DNA origami might interfere if the cytoskeleton of the cell were to be comprised of the same material.

Creation of a non-typical artificial cell has already been done in several ways, since the requirements for this are far more feasible than for a typical artificial cell. The benefit of having non-typical artificial cells is that they can be tailor-made to fit a certain purpose. Testing or manufacturing pharmaceutical compounds can be done far more easily in a cell mimic. Also, using non-typical synthetic cells is very convenient for studying various aspects of the cell, thus helping advancements in molecular cell biology. In the end, building non-typical artificial cells is essential to making a finished typical artificial cell.

Up until now, mostly individual parts of the cell are constructed in vesicles or partly natural cells. This may lead to believe that creating a 100% synthetic, living, eukaryotic cell is either nearly impossible or in the far future. However, since technology still advances every year, and new techniques are discovered and new ways are found to work with the smallest building blocks, I think it may be possible to create an artificial cell at some point in time. After all, it has been done before by mother nature herself.

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