Biology Term Paper

Induced Pluripotent Stem Cells

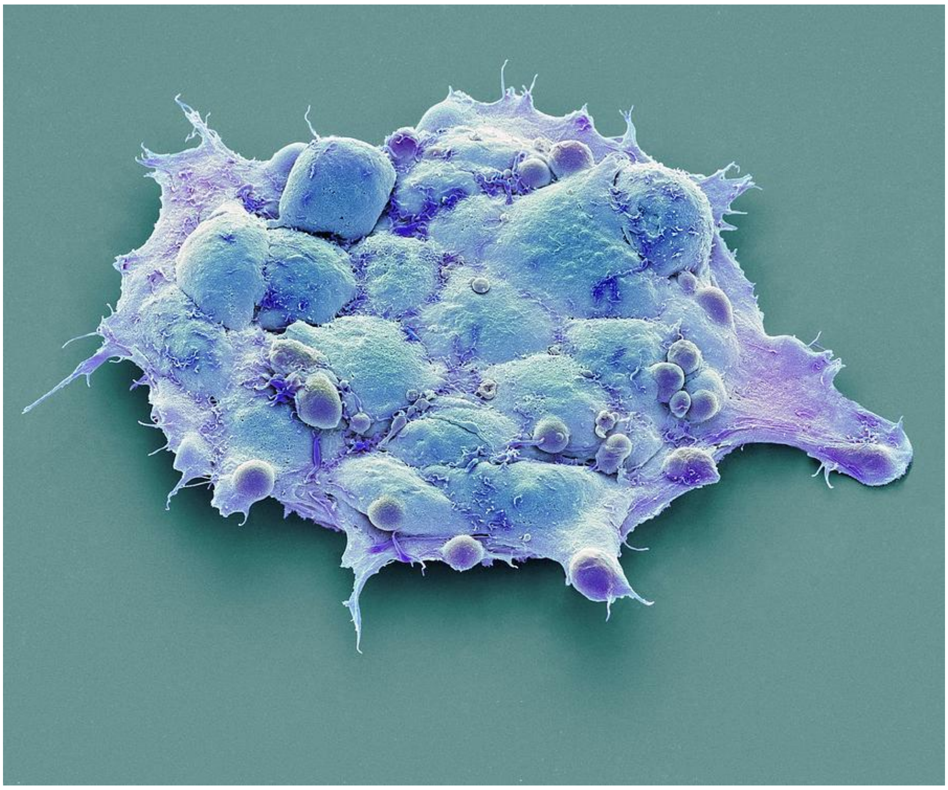

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Table of contents

Introduction

What makes the chosen topic especially interesting and acute?

Humanity has always pondered on the idea of infinite life. Yet, mankind has never been that close to solving this complex puzzle. Some medical approaches that were once far beyond our imagination are now commonly used and many more are being discovered on a daily basis. The technology of pluripotent stem cells, also called terminally differentiated somatic cells brings us forward in our thriving for everlasting life. By using the induced pluripotent stem cell technology, we can deal with terminal illnesses or severe injuries like cancer or massive skin burns. Since the stem cells can be exo-, endo and mesodermal, almost any type of disorder can be treated. On a larger scale we could achieve, if not infinite life, then at least an immensely higher life expectancy, which would be a crucial step in our development as a species.

Thus, we are convinced that the induced pluripotent stem cell technology has a lot to offer and will be expanding rapidly in the next years. One thing is certain: there is yet a lot to be unveiled, which will soon result in a great leap for future medicine.

Motivation

Our motivation is rather simple: We, as contributing members of society and future healthcare/science professionals want to be a part of high-end technology research and development, and the best time for that is now.

In our work, we will dive deep into the stem cell technology and give explanations to various questions.

Brief scientific history

Figure 2: Shinya Yamanaka

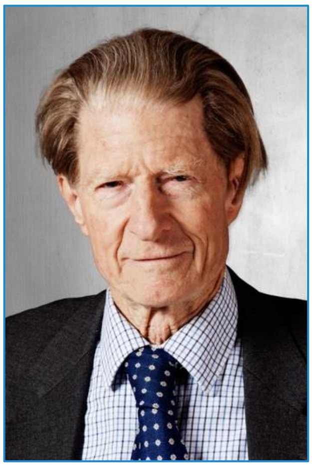


Figure 3: John Gurdon

Without further ado, let us start with a brief scientific history of the technology. Firstly: how does one acquire stem cells in the first place? Until the 1980s it was only possible to derive stem cells from embryos, which one could only describe as wrong and inhumane. Thus, 2006 was the year when things were about to change drastically. Shinya Yamanaka, a Japanese scientist, and John Gurdon, a British biologist, discovered induced pluripotent stem cells, isolating them from mouse skin cells. A year later, in 2007, the same was achieved with human skin cells, which ultimately proved that mature somatic cells can be transformed back into stem cells. This scientific breakthrough brought them worldwide recognition and a Nobel Prize in 2012. In our work, we will look thoroughly into the technology and answer common questions.

Technique

Definition and different variants of pluripotent stem cells

One might ask themselves at first: what are pluripotent stem cells? **Pluripotent stem cells** are cells, that can renew lost or damaged tissue by rapidly dividing and forming the needed type. The cell characteristic of being able to change its structure, differentiating into the types of cells needed, is called **pluripotency**.

There are 3 types of cells that the human body consists of:

- Ectoderm (Make up the skin and nervous system)
- Endoderm (Form most of the human organs)
- Mesoderm (Is responsible for bone, cartilage, muscles and connective tissue)

Pluripotent stem cells can form any of these under specific circumstances, which potentially makes them an ultimate solution to a vast variety of diseases.

- Induced pluripotent cell (iPS cells)
- *Conventional* embryonic stem cell (ES cells) — derived from embryos
- Embryonic stem cells made by somatic cell nuclear transfer (ntES cells)
- Embryonic stem cells from unfertilised eggs (parthenogenesis embryonic stem cells, or pES cells)

Nowadays, embryonic stem cells are known to be the preferred ones to work with due to their ability to form any type of somatic cell without needing to go through the transition process, due to being a stem cell in the first place. In this case the cells cannot be called induced because no transition from a somatic to a stem cell occurs. There are also certain ethical concerns about the usage of such cells (Couples that undergo an in vitro fertilization process sometimes decide to donate embryonic cells). Still, the ethical aspect is discussed a lot and common ground hasn't been yet reached, making ES cells rather rare. This makes iPS cells, compared to ES cells, a better candidate. Most importantly though, iPS cell therapy cannot result in the cells being rejected since those are the cells of the same patient unlike the ES cells.

Description and explanation of the technique

For creating and using induced pluripotent stem cells, we must go through a long process. Starting with a tissue biopsy, mostly of skin tissue or hair, because the process of isolating them is the least invasive. Then these somatic cells are activated and reprogrammed in the lab to form induced pluripotent stem cells. They can now be used to grow different tissue types or even whole organs. To do that, the iPS cells must receive signals from somatic cells or artificially produced signals by scientists, telling them what type of cells they need to become. After being fully differentiated, they can be used for transplantation or other purposes.

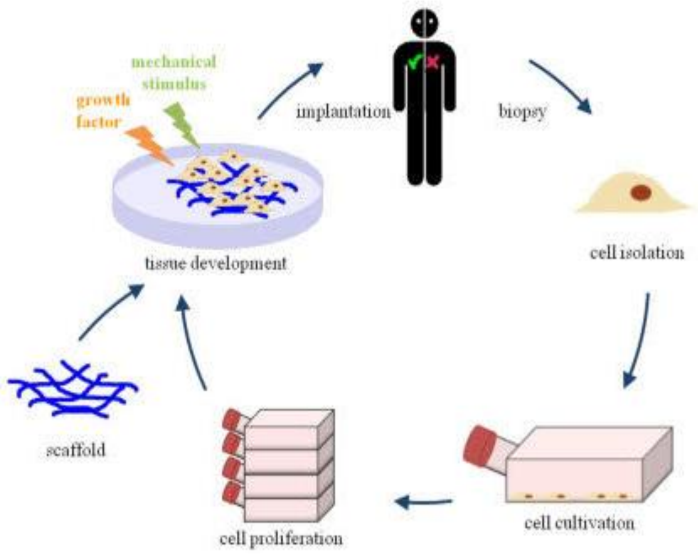


Figure 4: Cycle of iPS cells

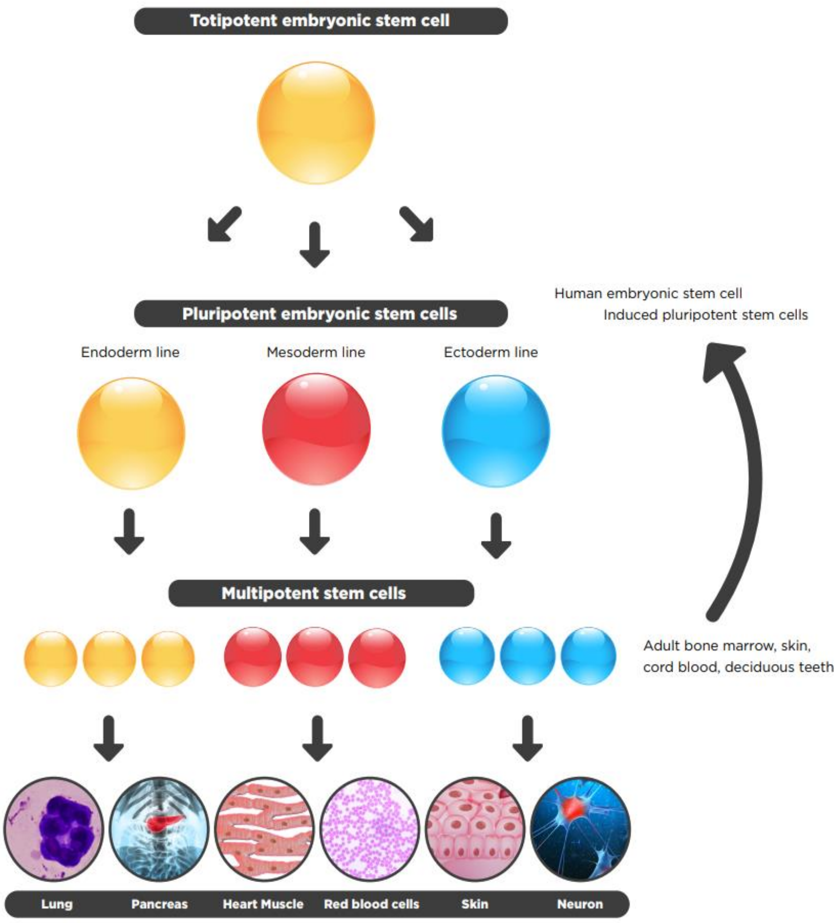


Figure 5: Types of stem cells

• As already mentioned, we do not take totipotent embryo-derived stem cells into consideration, as they cannot be induced, but can form a whole organism.

• Pluripotent stem cells can be of three kinds. As illustrated, there is an Endoderm, Mesoderm and Ectoderm line. They later form the multipotent stem cells, which are their subtypes and aren't as versatile as pluripotent stem cells.

• Eventually they turn to regular somatic sells. They differ in accordance with the line. Because of that, we do not usually pick a skin cell to differentiate it and make it a lung cell, as skin belongs to the ectoderm line, whereas lung cells to the endoderm one, which would make the process harder.

A wonderful example of the iPS cell technology being used is called **cell therapy**. The goal of it is to repair the patient's damaged tissue by multiplying an exact cell type of the person being treated instead of using a transplant from another individual. To achieve that, we need to turn a somatic cell into a pluripotent stem cell, which is described as **differentiation.**

So, let's break the process down into steps:

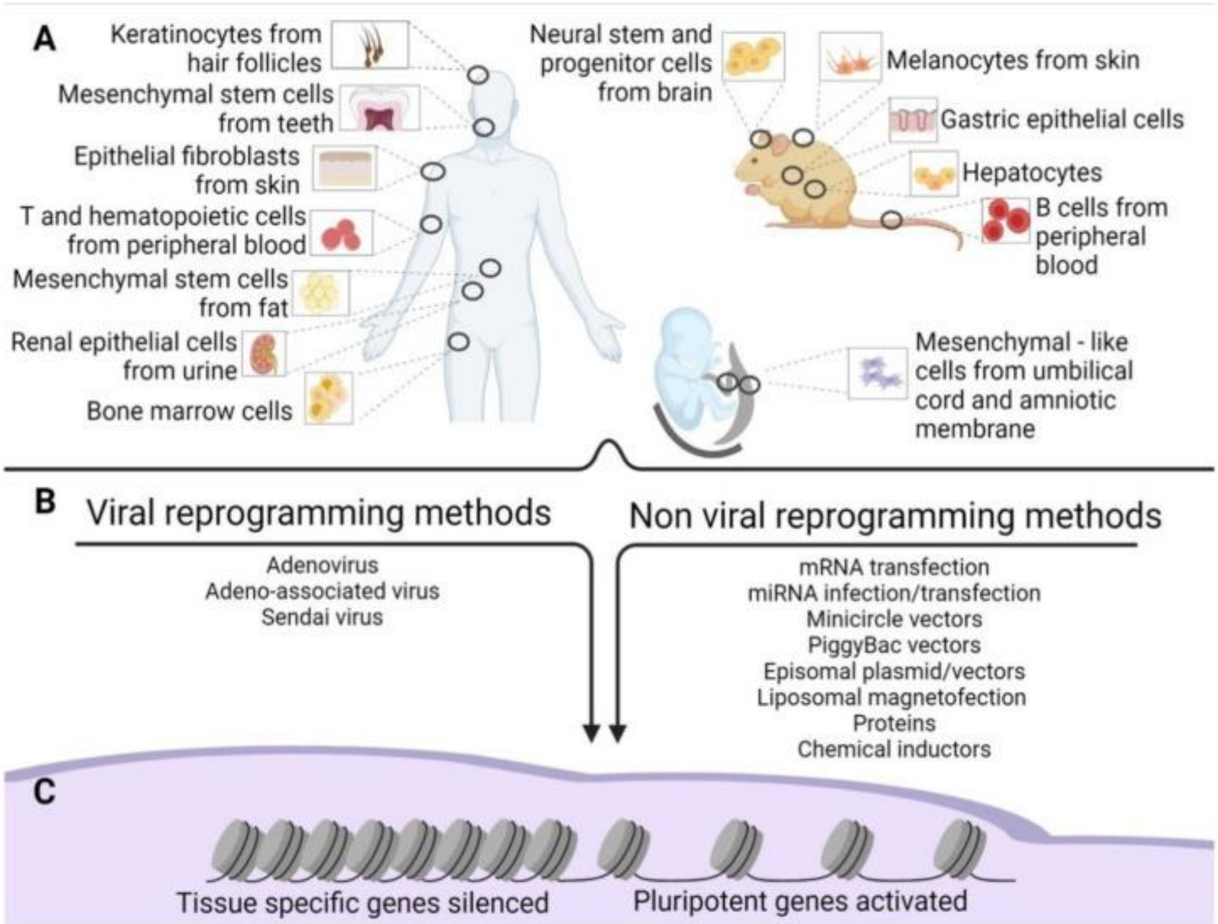
1. Biopsy
2. Cell activation and reprogramming
3. Cell multiplication and cultivation

1) Starting with the biopsy. Although it sounds self-explanatory, there is something to be considered. As mentioned previously, there are 3 linages: Endoderm, Mesoderm and Ectoderm. They play a crucial role in making the somatic cell we chose. We do not convert a skin cell to a lung cell or for example a blood cell to a neuron cell. Theoretically we could do that, but it would involve much more challenges we don't necessarily need to face.

• Being the easiest ones to obtain, skin, blood and hair are the ones of choice to undergo differentiation.

2) After the biopsy comes the next step, by far the most important one. One must ensure that all needed genes get absorbed by the cells with the least possible outer influence. As a result, we will end up having fully reprogrammed iPSCs to our disposal.

The following image (Figure 6) shows us human and mouse cells that can potentially get reprogrammed, the reprogramming methods and the activation step.



A: Here are the exact cell types that we choose to reprogram. Speaking of “Hair cells” we mean keratinocytes and by skin cells nothing else but epithelial fibroblasts. That’s because the ones shown are the most studied and verified cell types suitable for reprogramming.

B: There are numerous reprogramming methods suitable for specific cell types and genes that are supposed to be integrated.

C: Activation of the genes that are significant to the process and silencing of insignificant ones.

Having isolated the target cells, we move on to reprogramming and activation. The process involves four transcription factors called OSKM, which are: Oct4, Sox2, KLF4, and MYC; Depending on the cells of origin and the methods of reprogramming, gene cocktails other than OSKM, such as p53 shRNA, Lin28, L-Myc, SV40LT, Nanog, Glis1 and others have been used in different reprogramming mixes. New differentiation kits are developed daily, improving the efficiency of reprogramming.

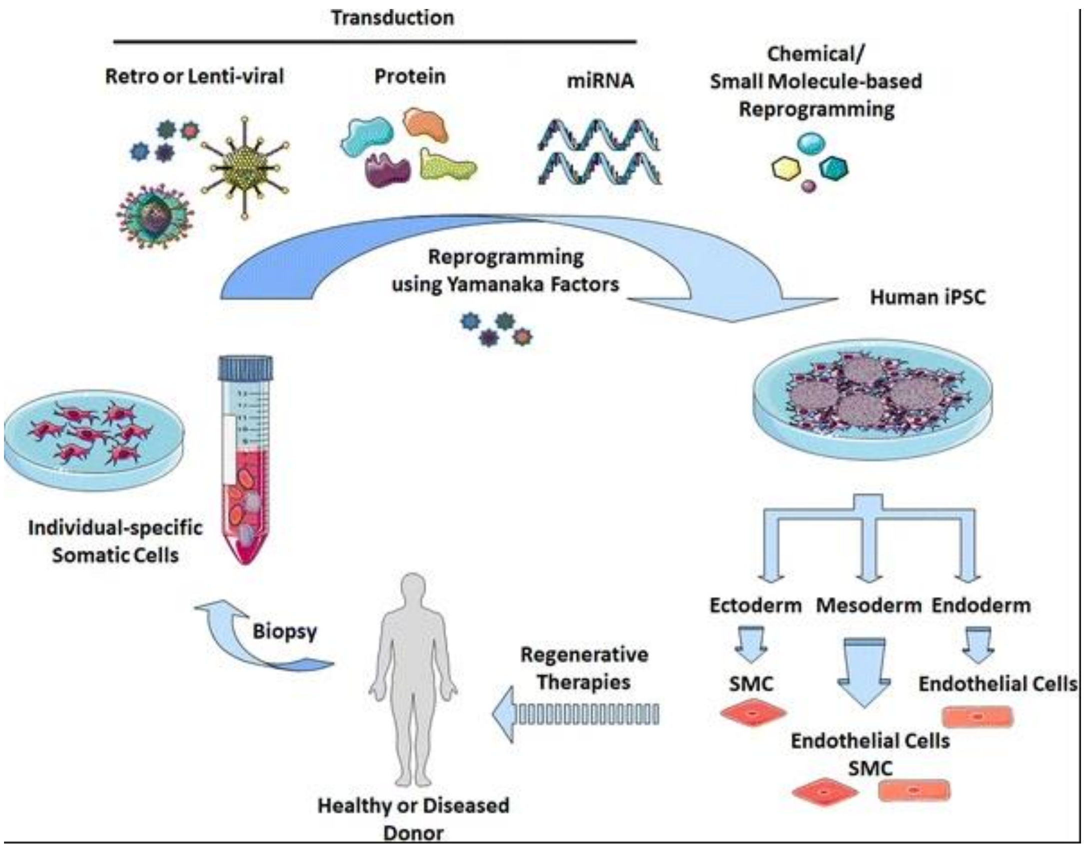
The ways of integrating these genes into the targeted cells differ depending on the initial cell type and the genes we need to integrate. In some cases, numerous viral vectors are needed to inject the right gene sequence into the host genome. This also increases the probability of mutations taking place and thus the efficiency of the whole process decreasing. We must make sure that the iPS cell production is mutation-free because if there are any, mutated iPS cells being able to multiply quickly, can cause trouble. In such cases, cancerous cells can be formed and can potentially harm the patient.

The following image describes the overall process of differentiation. In this certain case a process of transduction is being used to integrate the genes into initial cells for them to be differentiated.

• Transduction involves a viral vector inserting the targeted proteins/genes/miRNA needed for differentiation.

• Nevertheless, plasmid vectors are also commonly used to integrate the factors from the differentiation kit. This process being called “Translation”.

• Chemical substances can be also included and serve different roles depending on the differentiation kit’s specifications.



As a result of successful differentiation, we acquire iPS cells.

Lastly, using cooperative differentiation protocols like the ones mentioned before and adding growth factor supplements, we finally derive and multiply the desired somatic cells from iPSCs. The way such cells can be kept will be shown in the photos taken in the lab. In the case depicted on the upper image those cells are SMC (Smooth muscle cells) and endothelial cells. They make up blood vessels, allow normal blood circulation and play an important role in different physiological processes of the human organism. They can now be cultivated and later applied.

What is special about the technique?

The core idea behind reprogramming cells back into pluripotent stem cells is incredibly unique in the field of human medicine as we know it. Also, in comparison to the conventional stem cell methods, induced pluripotent stem cells can be produced out of any type of cell and not just the exact same as needed. This is a big advantage if you need brain or nervous tissue, which is not easily accessible.

Cases where the technique can be applied

The technique can be used for many different purposes.
A big part is the use in transplantations. In case of a largescale burn skin can be easily transplanted. By producing a living piece of skin out of the persons own cells, the risk of rejection is eliminated, and it is possible to maintain the initial functions, like hair follicle growth. Another possibility is to make older cells constantly renew themselves which can theoretically result in longer life spans.

Therapeutical approaches

As stated before, there are several types of treatment that are possible but only few of these techniques have been used in an actual treatment (by now mostly skin and cartilage transplants), as more complex approaches are still in development. Many of the feasible treatments are hoped to be possible in the future once stem cell therapy becomes more consistent and less time consuming as well as expensive.

Stem cells in nature

Stem cells are essential to our growth and early development as humans, so pluripotent stem cells, here embryotic stem cells (ESC), naturally form in the human embryo. These naturally forming pluripotent embryotic stem cells can be extracted and used, though the ethnicity behind such extraction is often questionable.

Additionally, stem cells can also be found in many parts of the adult body, such as skin, muscle, or blood. These stem cells however aren't pluripotent and thus cannot grow into the three different cell lines but are themselves used to make induced pluripotent stem cells.

Pros and cons

As in many other topics regarding gene technology there are many benefits and risks. This approach does have many benefits even though it is not fully developed. It solves the rejection problems that come with transplanting organs and allows new possibilities in the field of stem cell research because they are ethically better acceptable than for example embryonic stem cells. It opens the way for many new possible treatments, contributing to the development of human medicine.

However, there are also disadvantages to this new technology, the first point being, that it is a relatively new and therefore an unexplored area of research. As a result of that and because of the long and complex procedure of creating the pluripotent stem cells, it is extremely expensive and time consuming. Growing new stem cells can take way longer than usual transplantation, making it useless in situations, where a patient needs to be treated urgently. Another problem with iPS cells is that because they once were somatic cells, they could either possibly switch to their original type of cell or there can be problems with not differentiating them correctly, both causing mutations which can lead to cancer.

Alternative ways of treatment

Nowadays tissue from other body parts or from another person are used for transplantations which can cause many complications that could be avoided with the new technique. In terms of stem cell treatments there are already possibilities to grow some organs in vitro with help of differentiated stem cells. At the same time with induced pluripotent stem cells, it would be possible to grow less accessible and more complex nervous or brain tissue. In this regard, stem cell treatment can handle unique injuries or conditions, meaning there often is no alternative way of treatment, which is why it holds so much promise in human regenerative medicine.

Interview

Questions answered by a professional

For this interview we visited the university of Basel to meet the young scientist, Evelia Plantier. She responded to the following questions orally and in writing and showed us the lab she is working in. Her research project is to grow bone marrow of mice and humans by using induced pluripotent stem cells.

Q: What are the properties of pluripotent cells, how does one determine if it is a stem cell?
A: A stem cell is defined by its ability to renew and differentiate cells into several types (can be totipotent, pluripotent or multipotent). Pluripotent cells can give rise to the 3 germ layers (all cell types).

Q: Can any cell undergo stem cell conversion, and does it make a difference which cell one chooses to convert? If so, how does that affect the outcome?
A: Mainly skin or blood cells are used for the easy access, but technically any somatic cells can be turned into iPSCs. The difference during differentiation depending on the cell source can be that some cells will keep some features from their origin, but by culturing the cells you can diminish these differences.

Q: How do you isolate stem cells from a mouse or a person (for example from bone marrow)? What are the differences between those two types?

Q: How do you keep the cell culture?
A: Having them in the proper medium is essential to cultivate them as pluripotent stem cells are super sensitive explain protocol. Multipotent stem cells are easier to grow in vitro.

Q: How would one describe a reprogrammed cell's features and distinguish it from other types of reprogrammed cells?
A: There are a couple of difference between ESC and iPSC:

- *Ethical concerns*
- *No rejection after transplantation*
- *Less qualitative, can take defaults or mutations from the somatic cells from which they originate*
- *Difference at epigenetic level*

Q: How can you activate a cell in such a way, that it shows the ability to get reprogrammed?
A: The process involves using a differentiation kit, where all the needed factors are to be found. Depending on the initial cell types and the methods of reprogramming, certain gene mixes have been used, yet many are still in development.

Q: What are the examples of successful and unsuccessful pluripotent stem cell treatments?
A: These are still quite new methods. The reprogramming was only discovered in 2006, less than 20 years ago. There haven't been many treatments and you never inject or treat with pluripotent stem cells directly, so than minimalizes the risk. Understanding basic molecular mechanisms of human development and molecular aspects of degenerative diseases is important. Even though it is not fully researched yet, the potential for personalized cell replacement therapies and regenerative medicine is big.
Stem cell transplants, also known as bone marrow transplants, have been applied. In stem cell transplants, stem cells replace cells damaged by chemotherapy, diseases or serve as a way for the donor's immune system to fight some types of cancer and blood-related diseases, such as

leukaemia, lymphoma, neuroblastoma and multiple myeloma. These transplants use adult stem cells or umbilical cord blood but not pluripotent stem cells.

Q: Are there any differences in the therapeutical approaches considering the initial cell type of the “transformed” pluripotent stem cell? Which types of cells are preferred for creating human stem cells?
A: Mainly cells obtained by non-invasive methods so peripheral blood cells and keratinocytes from hair are used. For human stem cells: Cells of the same donor as the receiver cause no problem of engraftment.

Q: Can a treatment cause harm or result in mutations, thus cancer cells being formed? Are there similarities between cancer cells and pluripotent stem cells?
A: Yes, this is a risk if we are using differentiated stem cells. With every step of renewing a cell or growing one in vitro, mutations can occur that sometimes cannot be repaired. An accumulation of these mutations is the beginning of cancer. One similarity is the unlimited cell proliferation that occurs both in pluripotent stem cells and cancer cells. This means that the cells multiply rapidly and are hard to control.

Q: What is yet to be discovered about the properties of the pluripotent stem cells?
A: To date, the molecular mechanisms that underlie the derivation and maintenance of iPSCs are not yet wholly understood. Most of the studies on the transcriptional and epigenetic circumstances driving pluripotency and reprogramming have been performed on mice. Due to the strong cross-species similarities, many of these results have been translated to humans. All process to get to a specific cell type or organ in vitro from a pluripotent cell are under research. What is yet to come is being able to recreate organs with the help of pluripotent stem cells.

Photos of the lab visited

• On the right you can see the conditions under which pluripotent stem cells are kept in the laboratory of the Basel University (bodily temperature of 37° Celsius). The cells are constantly observed to make sure that they are developing properly.

Figure 8: iPSC incubator

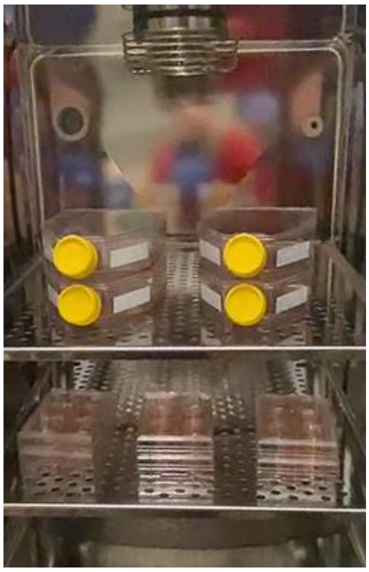


Figure 9: iPSC incubator inside

• Here we observe mouse and human bone marrow derived induced pluripotent stem cells. The difference can be seen in the density and length of the cells. Human cells are clearly longer and form a more organised tissue, not having much spare space in between them.

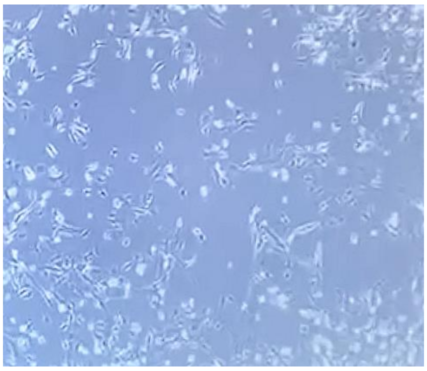


Figure 10: Mouse iPSCs

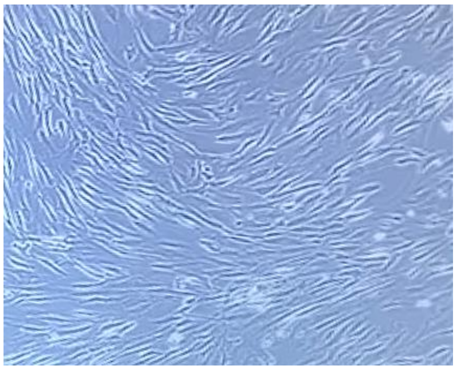


Figure 11: Human iPSCs

• The following image on the right gives us a better understanding of what a lab working environment looks like. The scientists working with iPSCs at the Basel University lab have high-end research technologies to their disposal.

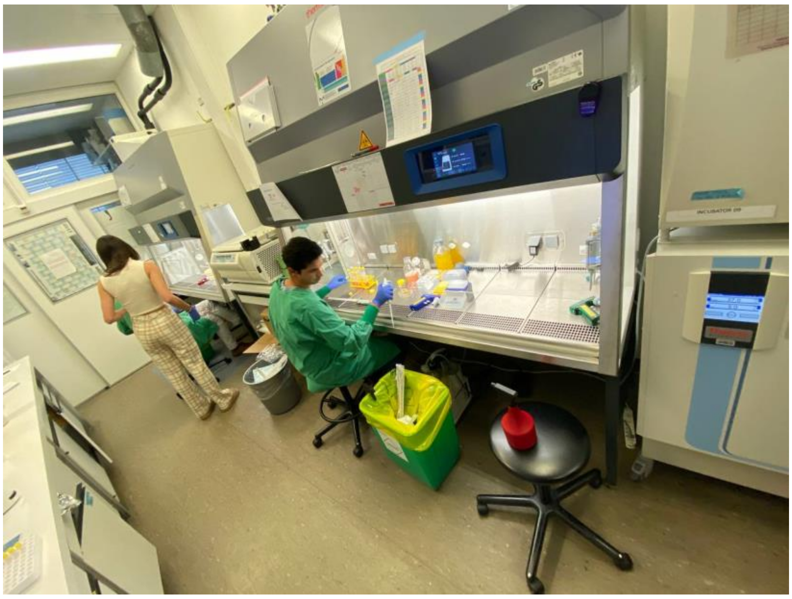


Figure 12: lab work progress

Figure 13: Substance storage

• The image on the left shows how substances crucial for the research are stored under special conditions. There is one more room where the stem cells that are being grown for patients are stored. The entry requires a high level of access with several sterilizing procedures to ensure that the iPSCs are kept without outer influence.

Overview and Conclusion

Short summary

Induced pluripotent stem cells is a new and promising technique. It can be used for a variety of medical treatments. New organs can be created by reprogramming somatic cells into pluripotent stem cells, that are then differentiated. It can also be used to renew living cells.

Opportunities and threats

It is a big opportunity as it can be used for transplantations causing no rejection and being fully sensing tissue. However, as it is not yet totally researched, it holds many known and unknown negative factors. One being the possibility of causing mutations resulting in cancer. All in all, the positive side overweighs.

Possible development of the technology in the future

With time, scientist should be able to come so far in development, that this technology can be put into practice. With more research, they should even be able to minimalize the risk of causing cancer. Probably induced pluripotent stem cells can even be used to cure cancer or other illnesses.

What can we achieve on a larger scale?

If this method was widely used, it would be a big step in transplant medicine. It could also be the basis for many new technologies using stem cells.

Conclusion

Induced pluripotent stem cells is a revolutionary technique in the field of regenerative medicine and currently holds high promise to cure new diseases and injuries fast and easy like never before, as well as new diseases that can't be treated yet. It is hoped to create alternative ways of treatment and eliminate the problems that come with the techniques we use today, such as organ transplantation.

Development is still ongoing, which is what makes this technique exciting in the world of human medicine but also interesting to follow, as we believe that it will be a breakthrough in medicine as we know it.

Sources and Acknowledgements

Acknowledgements:

We would like to say special thanks to Evelia Plantier for giving us a lab-tour and for explaining the protocols with the technologies involved. It really helped us with understanding the topic.

Sources:

Allsopp, Timothy E, Andreas Ebneth, and Alfredo Cabrera-Socorro. 'Deploying Human Pluripotent Stem Cells to Treat Central Nervous System Disorders: Facts, Challenges and Realising the Potential'. *Stem Cell Research* 41 (1 December 2019): 101581. https://doi.org/10.1016/j.scr.2019.101581.

Tocris Bioscience. 'Cell Biology'. https://www.tocris.com/cell-biology/stem-cell-differentiation.

Demirci, Selami, Alexis Leonard, and John F. Tisdale. 'Hematopoietic Stem Cells from Pluripotent Stem Cells: Clinical Potential, Challenges, and Future Perspectives'. *Stem Cells Translational Medicine* 9, no. 12 (29 July 2020): 1549–57. https://doi.org/10.1002/sctm.20-0247.

Ebrahimi, Mohsen, Mehdi Forouzesh, Setareh Raoufi, Mohammad Ramazii, Farhoodeh Ghaedrahmati, and Maryam Farzaneh. 'Differentiation of Human Induced Pluripotent Stem Cells into Erythroid Cells'. *Stem Cell Research & Therapy* 11, no. 1 (16 November 2020): 483. https://doi.org/10.1186/s13287-020-01998-9.

'Pluripotent Stem Cell Research | Stem Cell Program Research | Boston Children's Hospital'. https://www.childrenshospital.org/research/programs/stem-cell-program-research/stem-cell-research/pluripotent-stem-cell-research.

Tan, Yu-Ting, Lin Ye, Fei Xie, Ashley I. Beyer, Marcus O. Muench, Jiaming Wang, Zhu Chen, Han Liu, Sai-Juan Chen, and Yuet Wai Kan. 'Respecifying Human IPSC-Derived Blood Cells into Highly Engraftable Hematopoietic Stem and Progenitor Cells with a Single Factor'. *Proceedings of the National Academy of Sciences* 115, no. 9 (27 February 2018): 2180–85. https://doi.org/10.1073/pnas.1718446115.

Wang, Aline Yen Ling, and Charles Yuen Yung Loh. 'Episomal Induced Pluripotent Stem Cells: Functional and Potential Therapeutic Applications'. *Cell Transplantation* 28, no. 1 Suppl (December 2019): 112S-131S. https://doi.org/10.1177/0963689719886534.

Health Jade. 'You Searched for Pluripotent Stem Cells - Page 3 of 4' https://healthjade.net/search/pluripotent+stem+cells/.

Bhartiya, Deepa, Nibedita Lenka, Deepa Bhartiya, and Nibedita Lenka. Pluripotent Stem Cells, 2013. https://doi.org/10.5772/45917.

"Schematic Diagram of the Time Line for Generating Blood Vessel Organoids from HPSCs. | Nature Protocols." Accessed February 17, 2023. https://www.nature.com/articles/s41596-019-0213-z/figures/1.

Moradi, Sharif, Hamid Mahdizadeh, Tomo Šarić, Johnny Kim, Javad Harati, Hosein Shahsavarani, Boris Greber, and Joseph B. Moore. "Research and Therapy with Induced Pluripotent Stem Cells (IPSCs): Social, Legal, and Ethical Considerations." *Stem Cell Research & Therapy* 10, no. 1 (November 21, 2019): 341. https://doi.org/10.1186/s13287-019-1455-y.

“Autologous Induced Pluripotent Stem Cell-Based Cell Therapies: Promise, Progress, and Challenges - PubMed.” Accessed February 17, 2023. https://pubmed.ncbi.nlm.nih.gov/33725407/.

Figures:

Figure 1:

Gschmeissner, Steve. ‘Pluripotent Stem Cell’. https://images.fineartamerica.com/images-medium-large-5/2-pluripotent-stem-cells-steve-gschmeissner.jpg.

Figure 2:

NobelPrize.org. ‘The Nobel Prize in Physiology or Medicine 2012’. https://www.nobelprize.org/prizes/medicine/2012/yamanaka/facts/.

Figure 3:

NobelPrize.org. ‘The Nobel Prize in Physiology or Medicine 2012’. https://www.nobelprize.org/prizes/medicine/2012/gurdon/facts/.

Figure 4:

ResearchGate. ‘The Tissue Engineering Paradigm. The Tissue Engineering Paradigm...’ https://www.researchgate.net/figure/The-tissue-engineering-paradigm-The-tissue-engineering-paradigm-consists-of-isolation_fig3_227341144.

Figure 5:

Team, Health Jade. ‘Totipotent Cells Defined, Totipotent vs Pluripotent vs Multipotent Stem Cells’. *Health Jade* (blog), 6 June 2019. https://healthjade.net/totipotent/.

Figure 6:

Scesa, Giuseppe, Raffaella Adami, and Daniele Bottai. ‘IPSC Preparation and Epigenetic Memory: Does the Tissue Origin Matter?’ *Cells* 10, no. 6 (11 June 2021): 1470. https://doi.org/10.3390/cells10061470.

Figure 7:

Klein, Diana. ‘IPSCs-Based Generation of Vascular Cells: Reprogramming Approaches and Applications’. *Cellular and Molecular Life Sciences* 75, no. 8 (1 April 2018): 1411–33. https://doi.org/10.1007/s00018-017-2730-7.

Figure 8-13:

Ivanov, Oleg. ‘Photos of the lab visited’.