IPS-Cells

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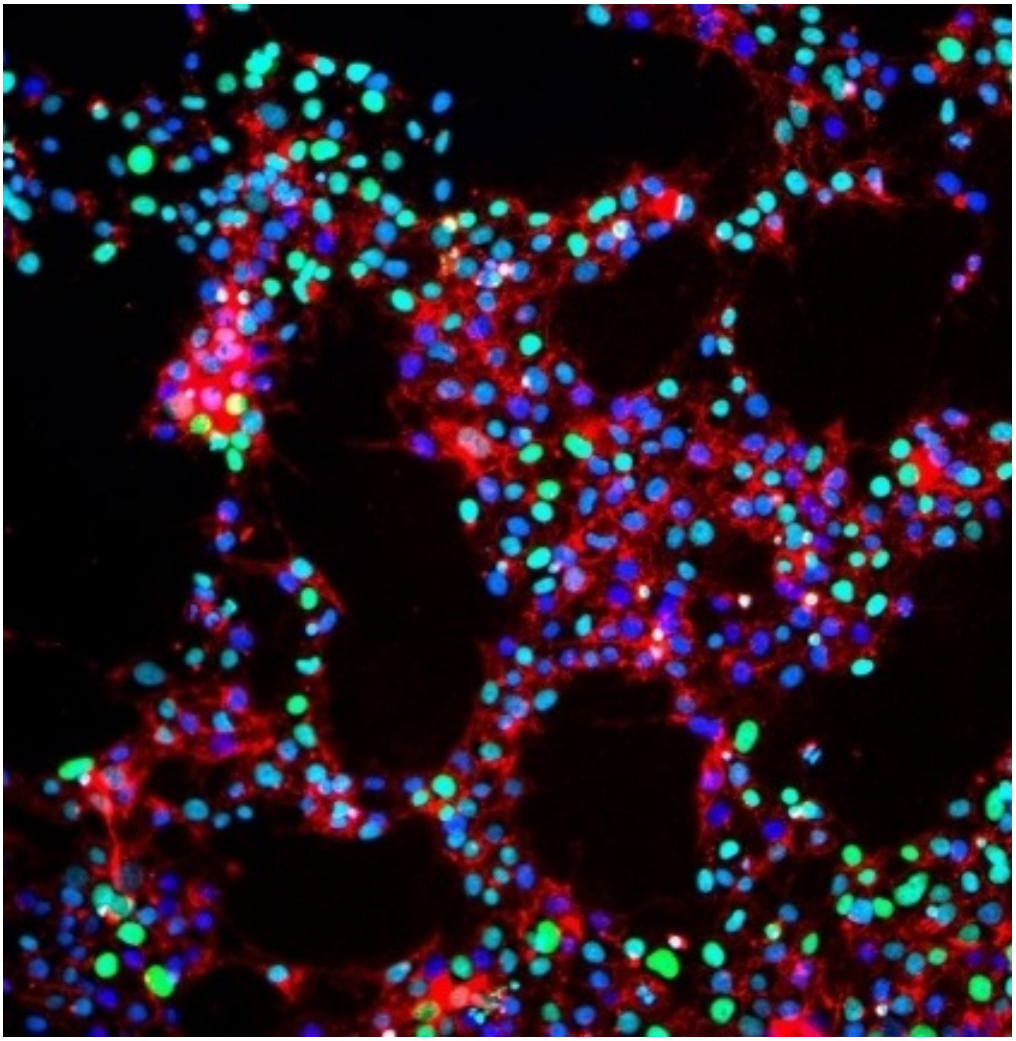
Biology immersion, Class 4e

April 27, 2018



Induced Pluripotent Stem Cells (iPSC) under the microsope.
https://www.ucsf.edu/sites/default/files/styles/2014_wysiwyg_full/public/fields/field_insert_file/news/ips-cells.jpg?itok=0RvL9RO8

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1 What is our motivation to work on the topic we chose?

We find it very interesting that it is even possible to reprogram cells to express a specific characteristic. And that these can then be very helpful to patients by specifically changing the cells in a beneficial way which supports the patient to recover, is amazing. There is also a high medicinal potential in this subject. And what makes iPSC (induced pluripotent stem cells) different from its peers is that it involves less ethnic problems than in embryonic cells. Also, because iPSC cells can be reprogrammed to become any kind of cell, they may one day provide an unlimited supply of replacement cells and tissues for diseases and illnesses. Also, when they are taken from a patient's own cells, the body is more likely to accept them. iPSC is very helpful and of great value to the world being one of the fastest developing subjects in biologie.

1.1 What is especially interesting?

Using iPS cells it is possible to regenerate tissue. It was possible to help a monkey with a damaged spinal cord by promoting regeneration using derived neural progenitor cells, which were made with ips-cells. Also it may be possible to find a cure or to progress greatly in curing the disease Alzheimer. The simulation of diseases in organelles is also very interesting and beneficial to finding the best treatments for a variety of diseases and disorders. In type 1 diabetes, the cells in charge of producing the hormone insulin are destroyed. Researchers made a significant breakthrough in developing a potential cure for type 1 diabetes when they found a way to turn iPS cells into insulin-producing cells and transplant them into the abdomens of diabetic mice. [1] In theory, insulin-producing cells could one day be generated from a diabetic patient's skin cells.The possibilities of iPS research are immense and these are just a few examples which demonstrate the potential and importance of iPS research to the world.

2 Questions

These are the questions we had in mind while writing this paper:
Can a cell be changed in a specific way which largely benefits the physical performance of a human?
What advantages does this technology yield ?
Are there any drawbacks? And if so how severe?
How far has research got ? What is still in development ?
Where its boundaries? How far can we go? How far do we want to go ?(Both technically and ethically)

3 Introduction

3.1 Context, recent events

Induced Pluripotent stem cells, are cells which haven't differentiated(haven't decided what to be) yet and are reprogrammed from somatic(adult) cells. Therefore they can become any cell type.
Ips cells is a very broadly applied, very important resource and researching topic in stem cell technology. It enables researchers to use ips instead of embryonic stem cells. This way there are not as many ethical problems with stem cell production, because no organism has to be harmed/killed.

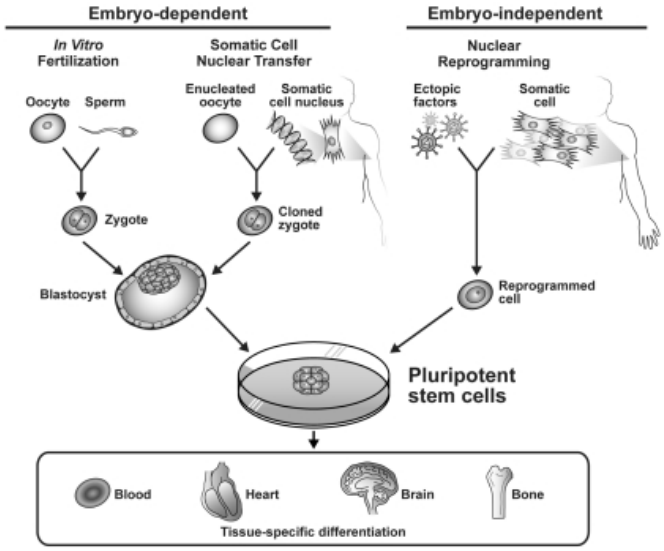

Figure 1: The difference between embryonic and induced pluripotent stem cells `https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3127559/bin/634.fig.jpg`

3.2 Recent scientific history

The possibility to produce Ips cells, first called embryonic stem cell like cells, was discovered simultaneously in 2006 by Shinya Yamanaka and by Sir John B. Gurdon. 2012 they both got The Nobel Prize in Physiology or Medicine for their discovery. As such it's a very new research topic. [2]

3.3 Where is it used

It's used for the stem cells therapy disease model(simulate a disease in a dish) production [3] and just generally to replace embryonic stem cells. At

the moment ips cell production is the best and ethically most correct method to produce specific stem cells. The other use is for regenerative medicine.

4 Description of engineering technique

4.1 The choice of Donor cells

Before we can start reprogramming a cell we need some cells to begin with. In theory we could take any cells, but some are more appropriate than others. One of the decision factors for example is the efficiency and speed of the reprogramming. A paper gave the comparison that the reprogramming of mouse embryonic fibroblasts takes about 8-12 days whereas the human foreskin fibroblast takes 20-25 days. Scientists are still researching for more efficient cell types. The Human primary keratinocytes(the cells of which the epidermis consists to of 90%) are able to be reprogrammed a 100 times faster.

The cell type does not only affect the speed, it also has an influence on the quality of the ips-cells. iPS-cells can have a higher or lower tendency to form tumors.
(Currently 80% of the published papers have used human fibroblasts) [4]

4.2 Finding the correct way of reprogramming

After we have chosen our cell type that we will reprogram we have to find its reprogramming factors(Yes, different cells are reprogrammed differently). Some of the main aspects are several genes.

4.3 The process itself

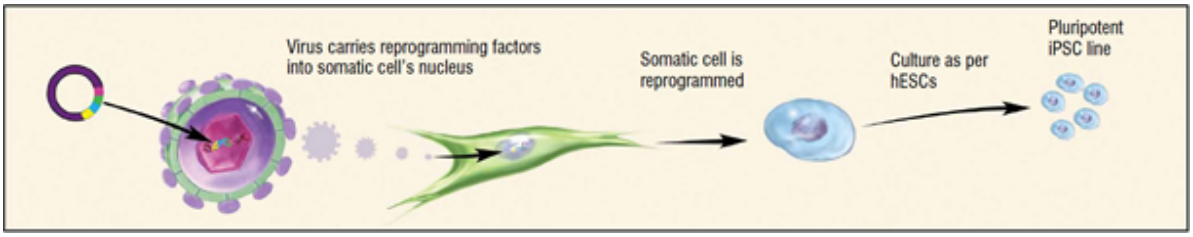

Figure 2: Somatic cells becoming with every division more like stem cells
`https://stemcells.nih.gov/sites/all/themes/stemcells_theme/stemcell_includes/10.1_chapter10regenerative.jpg`

So far we have a cell and the required transcription factors for reprogramming. To get it into our cell we use viruses. These viruses contain genes that create transcription factors. In the cell itself these factors are

transcribed. (The scientists who developed iPSC used Oct4, Klf4, Sox2, and c-Myc (OKSM).) [5] [6] The cell now thinks that it is in an embryonic environment and becomes more and more like an embryonic stem cell with further reproduction until it is almost indistinguishable from a real one. Afterwards we pick one single cell from these induced pluripotent cells and put them in a petri dish. The real challenge is now to prevent differentiation, as many factors support/initialize differentiation.
Currently there is also a technique using microRNA, which is still in development.

There are even sets in the internet being sold for making ips-cells:
`https://www.thermofisher.com/order/catalog/product/A34546`
Here is a picture so visualize how iPS Cells are made and used:

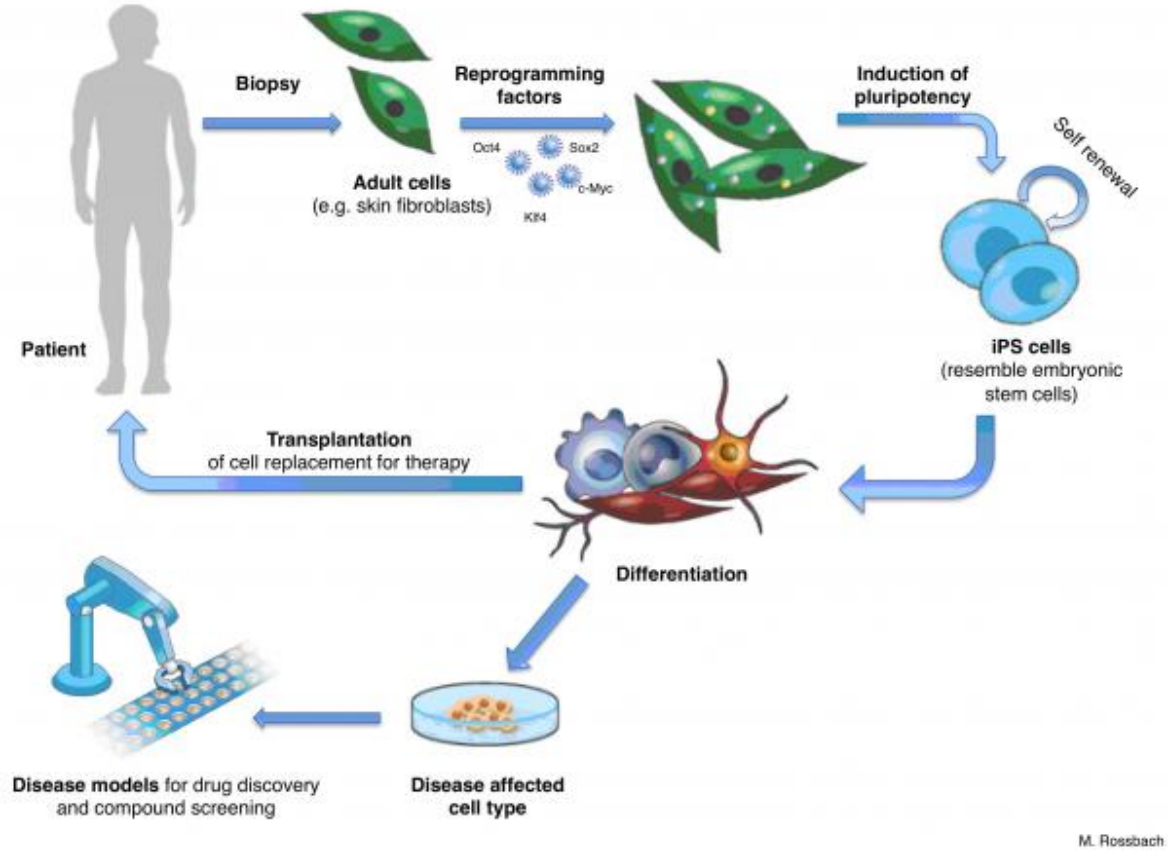

Figure 3: Visualization of the use and production of iPSC
`http://4.bp.blogspot.com/-1nx9IVpCfEM/T8GTwNCd7fI/AAAAAAAAI0g/_LmDiam5JOg/s1600/Applications+of+iPSCs.jpg`

5 Interview with Dr. Christoph Patsch

We led an interview with Dr. Patsch. We found him through the stem cell center of competence and met him at Roche.

Figure 4: Dr. Christoph Patsch (r)

What is your job? I am leading a team of scientists at Roche. We are working at generating disease models to discover and test early Drugs. As the leader of the team I have to think of the next steps, prepare presentations, make publications and coordinate the team. I also inform and engage people. For example I reach out to people and ask if they would be willing to work on a certain project. (e.g. external collaboration partners).

How did you get to your job? I studied molecular biology in Cologne. Then at some point in my studies I did an Internship at the Institute of reconstructive neurobiology in Bonn. It was the first Institute in Germany that was allowed to use embryonic stem cells (at this time IPSC were not discovered yet). There I got really fascinated by the potential of stem cells and this excitement lasted. In 2006 I did my PhD in molecular biomedicine and after that I went to Basel (to Roche) to do my postdoc. For two years, I have been leading the stem cell team. We have scientists, technicians, researchers, postdocs and PhD-students on the team, and we all work together.

Do you produce ips cells by yourself at Roche? Now we have about 300 IPSC-lines in house. Most of them come from external collaboration partners. One of them is the Harvard stem cell institute. They Identify the patients, collect samples of cells (e.g. skin- or blood- samples), reprogram them and finally send them to us. We've also got very few, which we made by ourselves. And one that is not coming from a human. Its derived from monkeys. The great thing now is that, when you have monkey IPS cells, you can nicely compare the two different species in vitro (Latin for in the glass: in the petri dish or test tube). Some of the medication might work on a human but might not work on the monkey and the other way around. And this is where you can find differences.

What do you mean with IPSC-lines? When you get a sample from a donor you add a cocktail of viruses and the cells start proliferating. If you are doing the reprogramming by yourself it's a very random approach. You

have the virus affecting some cells while others not. This leads to a very heterogeneous cell colony. You take one specific cell with the pipette out of the dish and put it into another dish. By doing so, you make sure you have a clone. When this one cell divides and becomes a new colony, this is called a line. Having a line also means that you can keep them forever, because they divide indefinitely.

What are you using IPS cells for? You are probably aware that there are many ways to use ips. One for example is stem cell therapy. But here at Roche, we try to mimic diseases in the petri dish to study it or find disease modelling compounds to develop drugs. To develop these disease models we need ips cells.

I read that the differentiation of IPS cells is the most difficult part is that true and how are you doing that? Stem cells essentially want to differentiate. You need to keep them actively in the pluripotent state by controlling them, using different environments, mediums and other factors. You could compare them to uneducated kids who want to learn everything, but you have to educate them so they are able to develop a specific knowledge. Then you add specific differentiation factors to direct differentiation. Such factors can be the right environment, the medium, a certain matrix, morphogens and other factors. These are naturally highly regulated by the placenta. In this whole process we try to mimic embryonic development in a very short timeframe. And the other part is getting the right cells by selection methodologies. This can be done by finding antibodies that bind to surface markers of specific cells. By adding small parts of iron (through parts of iron you can make them magnetic) or fluorescent material to the antibody you can mark these specific cells. This is important to derive a pure population with which we can work afterwards. Through such processes you can basically make all the 250 different cell types of our body.

What are the most used differentiation methods to produce IPS cells? There are Integration methods (virus integrates into genome), such as putting antiviruses into the host which produce transcription Factors and therefore also change the DNA sequence. But there are also integration free methods such as sendai viruses which persist into the cytoplasm just long enough to reprogram the cells. This is the easiest and most used method, but there is also the possibility to use proteins changed RNA or small molecules.

What are the specific advantages of IPS cells? IPS cells have more potential than adult cells, which means they can differentiate into any cell type whereas the adult cells are much more limited. Other than that, you

can derive patient specific cells and you can derive samples of cell types that usually no one would want to donate. This could be per example neurons.

What's the difference between ips cells and embryonic stem cells and do we still use embryonic stem cells? Embryonic stem cells were the starting point. IPSC are developed artificially and were oriented to be embryonic stem cells as the golden standard. That's why we needed to compare the functions of the embryonic stem cell like cells to the real ones. Today we only need embryonic stem cells to check if some effects are real or only artificial. We're near to the possibility to do everything embryonic stem cells can with IPSC. Were even able to clone mice per example.

Do you think that in far future, it will be possible to regenerate human body's completely with IPSC (further development) and therefore maybe even making them live forever? I think for the regenerating approaches for sure. It already works to regenerate the brain and the eyes partly by replacing cells by new IPSC derived cells. I met met a person that was blind in the first place and now can see where the light comes and see day and night. Developing an organ is much more difficult, because there are many different cell types involved. But I think it will be possible at some point. To go further, cloning as such would be very unethical. Also, it holds lots of uncertainties. Because the reprogramming is not a hundred percent perfect, there can occur lots of mutations. There surely will be some diseases which we can, if not cure then at least slow down by adding cells, small organoids (part of an organ). Let's go step by step.

Do you think it's good that the lifespan of humans increases more and more? Also, in terms of evolution/demographics? I think we already tricked evolution with all the modern medicine we have. I would not sit here and talk to you if there was no modern medicine. I had infections, surgery's as most of us did and probably I would have died or been crippled. On the other side, evolution gave us the brain, so it's probably part of the evolution. However, I do not know if we are mentally prepared to live forever. I do not think so, I think we would go nuts. I do not want to die, for sure, but I think when you reach a certain age it would be fine to have lived a certain life and had the pleasure of making the experience. At some point maybe it's ok. But, if you want to increase the lifespan you definitely also need to increase the quality of life, otherwise it's not the way we want to live. We need to focus on making life quality better. Which applies to all people suffering diseases. And if we lived forever would we still want to have kids? For me as a father, that's a very personal topic. Kids make you think that at least one part of you can live forever when you hand over your

genetics to a different person. Also, something that really drives humanity, is the fact that we die. If we lived forever we probably had no motivation to get things done. Why should you stand up in the morning when your time is unlimited?

Do you feel responsible for you research? It's clear that everything can be used for good or can be misused. If I invent a ladder. And someone jumps down, this surely wasn't the sense of it. But we should definitely discuss these things in society. Even if it's difficult because there's only a minority that understands certain specific detailed things. That's why we need to discuss these things in a broader aspect. Scientists should more go out and talk about their research. Lots of misunderstandings come from the misconception that scientists can do whatever they want and are not obliged to any ethical rules, which isn't true. I don't know anyone who isn't obliged to ethical rules, but of course there are some who don't care and just want to get famous. For sure I would feel responsible for accidents. We try to prevent this from happening. We normally think things roughly through and then it gets checked by other teams

Do you recommend your Job? It's a long way to get here, but it is super exciting. You discover something new everyday. You could compare it to playing room escape or bringing puzzle pieces together. First, you try to make sense out of something, then when you make it, this opens a completely new view and brings new questions. I'm often still thinking at home or on vacation about these questions. I really like it but you also need to be ready to get frustrated.

6 Discussion

6.1 How far is Ips-cells research ?

Currently scientists are able to produce ips-cell lines from humans and animals like monkeys and mouses. They are also able to make them from different kinds of adult cells. Progress was made in using this technology to regenerate tissue. Rhesus monkeys who had a spinal cord injury could be partially regenerated from their damage by using derived neural progenitor cells, which were made with ips-cells. [7] Other uses for the ips-cells are using these to grow tissue or even organelles to simulate diseases in them. (Disease in a dish) These simulations are crucial to the development of treatments against multifactorial diseases like alzheimer. Work is also done in non-integrative methods like with miRNA.

6.2 What are scientists still working on ?

Ips-cell research still has a long way to go, until it is effective enough to be able to be applied to real life medicine. It is still a challenge to investigate the effects of factors on diseases in a dish as the scientists do have to fully identify the cellular factors of the patients and the used controls. We also do not know all of the differentiation factors, to differentiate into any cell. Also since these methods can only produce one cell type at once, we do not have any possibility to make a whole organ out of ips-cells, only organelles. Although there is evidence that ips-cells are able to "give birth" to a whole organism [8], as several researchers are trying to create a full-term embryo (the experiments are conducted with mice). Also, as mentioned before in this paper, different kinds of cells from which ips are formed lead to possible risks in terms of the appearance of tumors.
To sum up: Ips-cell technology is almost equivalent with embryonic stem cell research, but until then, there is still a good amount of research that needs to be done.

6.3 What speaks against the usage of the IPSC-technology, ethically ?

One of the counter arguments against ips, is the risk that these technology has, because of the tumor risk. [9]
There are people who fear that this technology will be abused to make clones (which is illegal) or to artificially create whole organisms (which is ethically pretty delicate) [10]
Because of some misleading information in the internet many, especially religious extremists believe that the simulation of embryonic stem cells is the same as creating an embryo or life in a petri dish. According to them ips is just as bad as the embryonic stem cell technology.
Others are afraid of this technology's potential, because with the regenerative applications the lifespan of a human can possibly be increased tremendously. This leads to demographic and political problems. Already now we have problems with paying the rent for the next generation as a consequence of the large amount of elderly people.

6.4 What speaks for the usage of the IPSC-technology, ethically ?

This technology seems to avoid any of the problems that ES-technology has. One of the major points is that there is no organism killed in the entire process. Also no new life is created. You just force adult cells to divide and alter in a specific way, which is then called reprogramming (or differentiation later).

In our current perspective it is the only solution to many problems. Therefore, it is ethically very correct to give this technology a try and seize the chance find a treatment with very little to no side effects.
This leads to our next point: This technology has no negative effect on the patient if everything works fine. To claim the technology would be harmless is a lie, because it isn't perfect yet and there has to be more research done. As the patient is his or her own donor there is no need for looking out if the cells can match. This fact let's us avoid the problems we have with organ donation/transplantation.

7 Summary

We explain how IPS cells are very broadly applied, a very important resource and researching topic in stem cell technology.IPS cells are used for stem cells therapy disease model production and to replace embryonic stem cells. This way ethical problems are avoided. The engineering technique for the IPS cells is explained and certain factors like efficiency and speed of reprogramming. The quality in IPS cells can also vary and some have a higher chance of forming a tumor than others. The most common cell type used in IPS cell research are the human fibroblasts. To get it into the cell we mainly use viruses. These viruses contain genes that create transcription factors. An embryonic environment is simulated. IPS cell research is already so far that it is possible to regenerate tissue. Scientists are still working further by trying to apply this technology to real life medicine. Interview with Dr. Christoph Patsch: Dr. Christoph Patsch works at Roche as a team leader. In 2006 he did his PhD in molecular biomedicine and after that went to Basel (to Roche) to do his postdoc and has been working as a team leader for two years now. He and his team have about 300 ips-lines (question 4 in the interview explains the term) to work with. Most of them come from external collaboration partners like the Harvard stem cell institute. They use IPS cells to try to mimic diseases in the petri dish to study the disease or find disease modelling compounds to develop drugs. He also explains that IPS cells can develop into any cell type whereas the adult cells are limited. Additionally, you can derive patient specific cells and you can derive samples of cells no one would want to donate. He also thinks that regeneration of the body will be very possible in the future since it already works to regenerate the brain and the eyes partly by replacing cells by new ips derived cells. He ends the interview by recommending his job saying that even though It's a long way to get there its super exciting and you discover something new everyday. "You could compare it to playing room escape or bringing puzzle pieces together. First you try to make sense out of something, then you make sense out of it and this opens a completely new view and brings new questions. (...) but you also need to be ready to get frustrated."

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Here we want to warmheartedly thank Patsch and his team with which we corresponded, especially Markus.

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