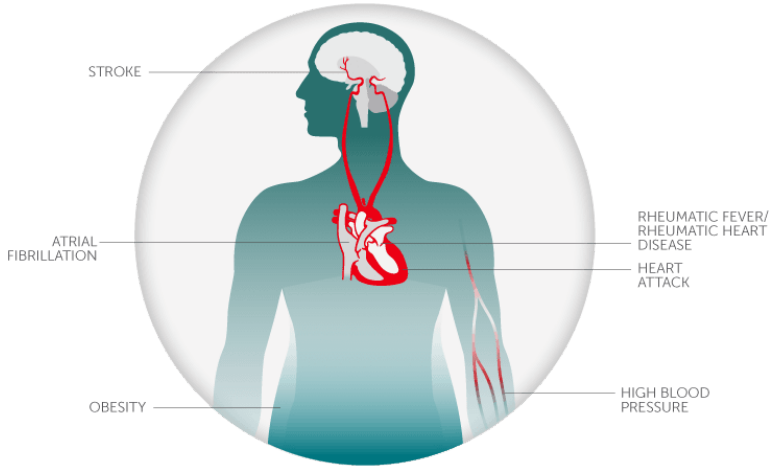
Term Paper Biology

Blood Vessel development and cardiovascular diseases



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Preface

Genetic engineering is a very broad and hot field, which made it especially hard for us to come up with a topic and not feel bad about all the other topics, which we couldn't do further research on. But our interest shall not end with the date we hand this paper in.
In the beginning we were completely overwhelmed with the content and new groundbreaking research that has been done and is being done at the time. We were very much shocked positively as well as frightened just by the doors that the invention or better said, the discovery of CRISPR, an ancient immune system of bacteria has opened. For us both, who never before had looked into this section of biology, it seemed just like watching the start of a sci-fi movie, it just seemed surreal. We were immediately sucked into the world of microbiology and genetic engineering and Biohacking. We had heard of the fictional dream or fear of designer babies and cloning before, but seeing how it works and realising that this scientific as well as medical progress might be closer than we have thought was just flabbergasting.

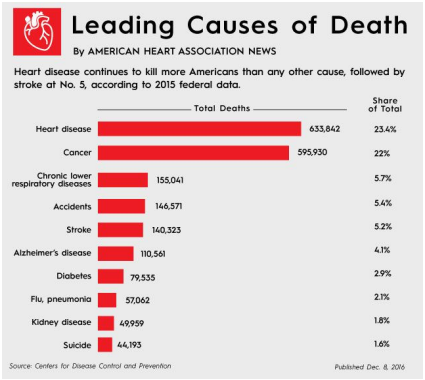
Our motivation was pretty much over the roof and the big problem was to select a topic, at which we would take a closer look. It was important to choose a topic which had at least a certain relevance to it. That means that we sadly had to leave the field of the glowing beer as well as of the glowing plants behind and look at more serious experiments.

At the end we decided to take a closer look at gene engineering techniques as well as an application we thought of as relevant, which was fundamental research concerning blood vessels. This branch of research has the end goal of gaining more knowledge in terms of how to interfere with blood vessels using genetic engineering techniques and thus gaining insights to fight the number one reason for mortality, cardiovascular diseases.
We had certain questions to begin with throughout the writing of this paper.
We of course asked ourselves:

1. How is it even possible to interfere with genes and how do scientists go on and actually modify certain genes?
2. Are there other ways of modifying genes without using CRISPR that are perhaps even better?
3. What tools do you need to perform such experiments and how long does it take to actually see results?
4. How does the process of "from the idea to the end application" look?
5. What problems come along with such extreme progress in genetic engineering and what questions do we need to discuss?

Introduction

What are cardiovascular diseases?
To further understand why we chose this specific path of research, we first need to know a few things that put it in context. In this paper we chose cardiovascular diseases, which is a possible application of the research of blood vessels.
Cardiovascular diseases (CVD) are the leading cause of death worldwide. These are diseases that involve the heart or blood vessels[1].
There are many different types of cardiovascular diseases like Coronary artery disease, stroke, and peripheral artery disease. They mostly involve atherosclerosis, which is the building up of plaque in the arteries.



What causes CVDs?
Cardiovascular diseases often result from plaque build up and are often a sign of an unhealthy lifestyle. That means CVDs can be caused or are more probable to happen to those who are keeping bad habits like smoking, poor diet, lack of exercise and excessive alcohol consumption [2]. There are also a lot of predispositions that promote atherosclerosis like obesity, high blood cholesterol, high blood pressure and/or diabetes.

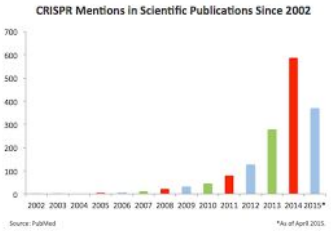
But because there are so many things we do in our everyday life that have an influence on the possibility of us suffering from a cardiovascular disease, studies have shown that on average an estimated number of 90%[3] of all CVD are preventable, with an overall healthy lifestyle. But of course for some people, who are predispositioned, this won't be the case. So the question remains, what methods exist or what is being done to improve or heal people who have a high risk or already are suffering from CVDs. That brings us to the blood vessels.

[1] http://www.who.int/mediacentre/factsheets/fs317/en/
[2] https://www.medicinenet.com/heart_disease_coronary_artery_disease/article.htm
[3] McGill HC, McMahan CA, Gidding SS (March 2008). "Preventing heart disease in the 21st century: implications of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study". *Circulation*. **117** (9): 1216–27. doi:10.1161/CIRCULATIONAHA.107.717033. PMID 18316498.

Why is research concerning blood vessels interesting?
First let's look at one of those CVDs a little closer like i.e. in case of a stroke[4]: the blood flow to the brain is impacted by this plaque build up, resulting in a cell death. However there are two types of strokes: the ischemic, due to a lack of blood flow and the hemorrhagic due to a bleeding. But both of them are closely linked with the blood vessels of the affected person. If we look at the ischemic stroke, where due to a clutting up of a blood vessel, not enough blood is supplied to the brain, then we could induce blood vessels and blood vessel growth. This would be a much more elegant way of targeting the issue. If we turn the idea around it gets also very interesting.
What if we could stop Blood vessel development in for example cancerous tissue? Then you would take away the supply from the cancer and prevent it from growing.

Description of engineering technique

There are a lot of genetic engineering techniques[5], with which you can manipulate genes.
The current uprising of CRISPR[6] also made it much easier to interact with genes so we were sure to face it in this branch of research. But to our surprise not all of them worked with CRISPR. The People who worked with it were even in the minority. We will come back to that later.



What is CRISPR?
To fully explain what you can do with CRISPR the size of this paper wouldn't suffice. So we only look at the knockout princip and will shortly look at the possibilities of its use. CRISPR or Clustered Regularly Interspaced Short Palindromic Repeat is basically a system to cut out specific parts of DNA. It was found in bacteria where it acted as an immune system against viruses. It consists out of a protein, called CAS9 (cuts DNA) and a so called guide RNA (gRNA(selects the piece of DNA that is being cut)), which can be designed and modified itself. There is also the possibility of adding things to the system but we will come to that later. But what made CRISPR such an easy tool is, that it helped to knock out[7] genes very efficiently and easy, which enables you to find out faster what a gene does.

But how does it work?

[4] https://en.wikipedia.org/wiki/Stroke
[5] http://www.semencespaysannes.org/images/imagesFCK/file/juridique/europe/mars_2016_position_societe_civile_europeenne_ntmg/joint_position_new_techniques_of_genetic_engineering_march_2016.pdf
[6] https://www.youtube.com/watch?v=6rC4kiZJZyY
[7] https://en.wikipedia.org/wiki/Gene_knockout

1. First you need to know what gene you want to target. It can be any sequence with about 20 nucleotide genes but it has to be unique compared to the rest of the genome and has to be adjoining to a Protospacer Adjacent Motif (PAM), which is sort of a certain codon that the protein (in this cas Cas9) needs for binding and cutting the gene.
2. Once the system targets such a piece of DNA, it forms a ribonucleoprotein complex, which essentially shifts Cas9 from an inactive, non-DNA binding protein into an active DNA-binding protein. Then Cas9 splits a given locus(place), if the gRNA sequence shares enough homology with the targeted DNA.
3. After the gRNA binds to the targeted DNA strand in the 3′ to 5′ direction, Cas9 now cuts the targeted DNA, which results in a double stranded cut.
4. Now the cell is in alert mode and wants to repair the DNA sequence. There are two ways how this can be done: The more efficient but tending to cause errors **non-homologous end joining (NHEJ) pathway** or the less efficient but error safe possibility the **homology directed repair (HDR) pathway.** The first is the one that happens frequently and often causes nucleotide insertions or deletions in this double stranded break.
5. If everything goes well up on this point, the ideal end result is a loss-of-function mutation in the gene. (knock out)

What did Mr. Schellinx use instead and why?
In the case of Mr. Schellinx, he decided in contrast to some of his colleagues not to use CRISPR. He thinks that it can be useful to simply perform a knockout on a gene with it, but that it gets complicated very fast, as soon as you want to make more specific changes or add a gene or even knockout a gene in later development of the organism. Therefore he uses a different technique called induced knockout[8]:

1. He designs a gene and puts on both ends a certain codon or sequence, that is called LoxP.
2. He then puts the gene in a plasmid, which essentially is a circular DNA strand that is often found in bacteria. This plasmid now acts as a sort of transport vehicle of the gene (vector).
3. Then he uses E.coli bacteria that act as a host.
4. These bacteria need to take up this plasmid which is usually initiated with a heat or an electro shock.
5. After those bacteria take up the plasmid, he can modify the genes further.
6. Then he gets rid of the bacteria and purifies the plasmids.
7. Afterwards he goes to the zebrafish and let's them mate.
8. During the first 20 minutes after fertilisation, he needs to inject his plasmid together with a certain protein, paste it in the genome of the zebrafish.
9. Later he can even after the whole fish is developed make certain changes to the genes. That means for example induce blood vessel growth after the heart and other organs are developed.

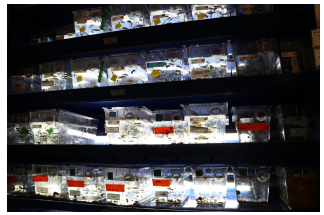
[8] https://www.genoway.com/services/customized-mouse/knockout-models/conditional-ko-time.htm

Documentations and pictures of research

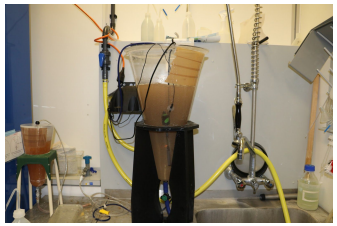
We went to the Biozentrum Basel to interview Niels Shellinx, who studied Biomedical Sciences in Belgium and came to Switzerland to do his PhD. He and his team do research on Blood Vessel Development, called Angiogenesis. They use Zebrafishes as the model organism. Niels also gave us the chance to take a closer look at their work environment and showed us some genetically modified specimen under the microscope. These are the most important parts of the interview:

What is the main goal of your project?

Niels: "First of all, we know that the highest mortality rate by any disease is linked to Cardiovascular diseases. If we know exactly how blood vessels work, meaning which genes are important and how the proteins that are formed enable the cells to make a stable blood vessel, we can find ways to maybe cure it. You can induce or stop blood vessel development. Inducing could be very interesting in the case of an heart attack or stroke instead of doing a bypass operation. Stopping the development could be useful in cancerous tissues for example. It wouldn't cure it, but the cancer wouldn't be able to grow. But this is the far future. You first have to know how the biological system works. And we research that here on zebrafish."



Why did you choose Zebrafish?



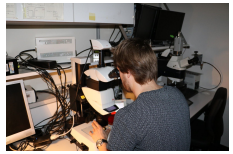
Niels: "Because zebrafish have a few advantages over mice. They grow outside the mother, so you can see every little detail and secondly you can easily manipulate them on a genetic level. The embryos are completely transparent in the first week, which helps us to observe them pretty clearly. But a disadvantage is that they are evolutionary farther away from the human being than mice.

So after you crossed them and they mated you have 20 minutes time before the first fertilized cell divides. You only want to modify the first cell so you gotta be quick."

Could you maybe try to explain to us in a simple way the procedure of this project?

Niels: ”On our main project we first make a tool. And if we want to address the function of a specific gene, we want to get it damaged, we call this a gene Knock-out. But the problem is, if you damage a gene and then cross the specimen and look at the embryos, they will always have this damage from the very beginning on. So what I’m doing now is, I make a gene myself and on both sites of this gene I put two small DNA sequences.
These two sequences are recognised by a protein which cuts them out. Then the gene is lost but now the trick is that you can induce this protein whenever you want. You can say at any time of the development “I want to know what this gene does”. So you activate the protein who then cuts it out. In that way I can study the function of genes.”

And in what context is this technique applied? Would it be possible to perform this on humans?



Niels: “The question we ask ourselves is very easy: “How do epithelial cells form stable blood vessels?” But it’s much too early for medicine. You first need applied research. Otherwise you cannot build on it. So this is the very start of it. It goes from the “Biomedizin Department” here in Basel to the Pharma Industry that picks up the ideas and uses it for drug development etc. But we’re still at the lowest level.“

For the modification of the gene, do you ever use CRISPR?

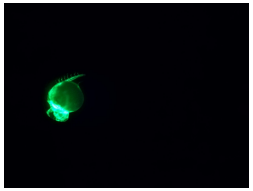
Niels: “I used it once. It helps to Knockout a gene, but to put something in a gene is very difficult with it. I do it differently, I do my modifications in a bacterium. In that bacterium I have a big Plasmid with DNA, in which my gene is put inside. Then I use the bacteria as a host to manipulate.”

Were there any complications during the process and how did you deal with them?

Niels: ”Oh of course! Out of all ideas 90% aren’t going to work, forget it. In my case I have problems with the “gene-reading”, because the protein doesn’t stop at the right moment, so I get things in my gene that I don’t want. I also had problems with my bacteria that didn’t do the correct recombination. But there’s always a reason, because biology is logic.”

What is the result of your research/studies?

Niels: “Our main project isn’t really finished at the moment but currently I have my Plasmid and injected it. Unfortunately as I mentioned the stop part doesn’t work efficiently. If everything works we’ll have the first inducible gene in zebrafish which you can knockout whenever you want.”



Discussion

The zebrafish lab, in which Niels works is a fundamental research lab. This means that there is not any big progress at which you can look at. The main goal of this kind of research is, as he explained in the interview, to learn more about blood vessel development. We already discussed why this is such an important topic and the application in CVDs more in depth. But what did they find out?
Actually Niels has not yet finished his work with the zebra fish, and he didn’t go very deep in what he actually found out. But basically with his method he was able to explore the function of VE-Cadherin, which is a protein that gets expressed by a certain gene, that enables cells to adhere in a homologous manner. Because he could just knock out the gene in different stages of the life of his fish, he could insert the manipulated gene, which he put into the cell at the beginning of the fish’s life, into the genome. This is also a progress he made with his applied technique. If it works it would be the first induced knockout in fish. We asked him about the future of the project, which as it turned out didn’t make a lot of sense, because of the fact that it is fundamental research, which has the goal of exploring things. He personally will not do any further research in this field after his Phd. When we talk about genetic engineering, we always have to look at ethical aspects too. Changing specific organisms by manipulation can sometimes go well and sometimes not. As a result animals and other organisms can die. The question we ask ourselves is: is it okay to use animals as experimental objects and is the suffering justified by the positive outcomes that never can be guaranteed?
The answer is very subjective. We thought it is a bit different in our case. As often said before, this research lab we wrote about works with zebrafish and we thought that maybe fish don’t experience the same kind of pain as we do. As this is partly true, we then found out due to newer research that has been done, fish do experience pain[9]. We asked Niels what he thought of this ethical problem concerning experiments on animals. He said that he thinks, that if the research helps to find out things about us and our environment that seriously improves or safes the life of a human being, it is justified. But he is strongly against any harm of animals that could be circumvented. We could agree to this, but we also think that there is even a next distinction to make. Not every life form or animal experiences pain in the same way. There are a lot of animals that don’t even have a consciousness, which means that their experience of pain is very likely different as well as might be not as bad as in animals with a conscious mind. Therefore performing an experiment on a fish is probably more ethical than performing it on a chimpanzee. To draw a conclusion, it remains a question, but it seems like the positive outcome on our site is over weighing the negative aspects, if there are certain restrictions.
This brings us to our next question, that is: Is genetic engineering as it's being done in america ethically supportable?
In the United States it is allowed to perform genetic experiments on humans as well as on animals. With the uprising of CRISPR, a lot of people started to do their own experiments at home, without a professional lab, some even without a degree. Now there is, as always, a

[9] https://www.sciencedaily.com/releases/2013/08/130808123719.htm

flipside to that coin. On one side, you could say that, if science is more liberal, the outcomes could be huge. This is kind of like the area of the computer or the internet, everybody is trying it out and playing with it. This could bring our knowledge extremely forward. But on the other side there are massive disadvantages. For example there are a lot of inexperienced people playing with this extremely powerful method, which is like giving everybody a pandora's box and expecting them to be clever enough not to open it. Niels meant, that he is a more liberal guy and thinks if people want to experiment with themselves, they should. But what if i. e. someone creates a virus, we haven't seen before or makes an already resistant bacteria more harmful or even more resistant? That is a question which we will probably see being solved in front of our eyes in the next couple of years. Because the fact, that everybody can hack biology in the United States as he likes, won't change soon.

Summary

It was hard for us to choose a specific topic for this paper. But after some research we stumbled across CRISPR and were immediately impressed by the many possibilities it offers. We found out that cardiovascular diseases are the number one cause of death worldwide and that a lot of researchers are working on solutions to treat it properly. Many achieve this by interfering blood vessel development using genetic engineering techniques. We asked ourselves how genes are modified with CRISPR and what tools are used and what complications appear during the process. We found out that there are many different versions of cardiovascular diseases and that most of them are caused by a building up of plaque in the arteries called atherosclerosis. This is often the result of an unhealthy lifestyle. In a stroke f. e. atherosclerosis prevents the blood flow to access the brain, which leads to a cell death. We knew that If one could induce or stop blood vessel development one would be able to easily experiment with it in order to find methods to heal such diseases.
One of the possible methods is CRISPR which was found in Bacteria, where it acted as an immune system and consists of a protein called CAS9 and a guide RNA. They locate the specific DNA branch and then cut it out. The Result would be a so called "loss-of-function-mutation".But there's yet another way as Mr. Shellinx showed us. He puts a codon on both ends of his gene and uses a plasmid as a transport vehicle, which enters the host (e.coli bacteria in this case) through an electric shock. There he can modify his gene unproblematically. After two Zebrafish mated, he injects his modified gene after the first 20 minutes of the first fertilisation in the genome of the zebrafish. Now he can even after the whole fish is developed make certain changes to the genes. He told us additionally that there are some complications with the protein which doesn't stop at the right time and that the project is still not finished yet. We further discussed the meaning and relevance of animals as experimental objects and whether their pain or death can be justified. We came to the conclusion that it can be allowed if the research seriously improves or saves the lives of human beings and helps us to understand nature better.
We also looked at the more liberal laws of the USA and think that science could benefit from this open system of research if it is done cautiously. But there is also a big potential of it to go in a wrong direction. But we'll have to wait to see further development.

References

Pictures:

1. http://www.championadvocates.org/en/whf/cvd-focus-areas
2. https://news.heart.org/wp-content/uploads/2016/12/1208-news-mortality_Graphic.jpg
3. https://motherboard-images.vice.com/content-images/contentimage/21355/1430338249394545.png
4. Fish Lab in the Biozentrum
5. Picture of the fish food
6. Mr. Shellinx while observing a modified specimen
7. Genetically modified zebrafish under the microscope