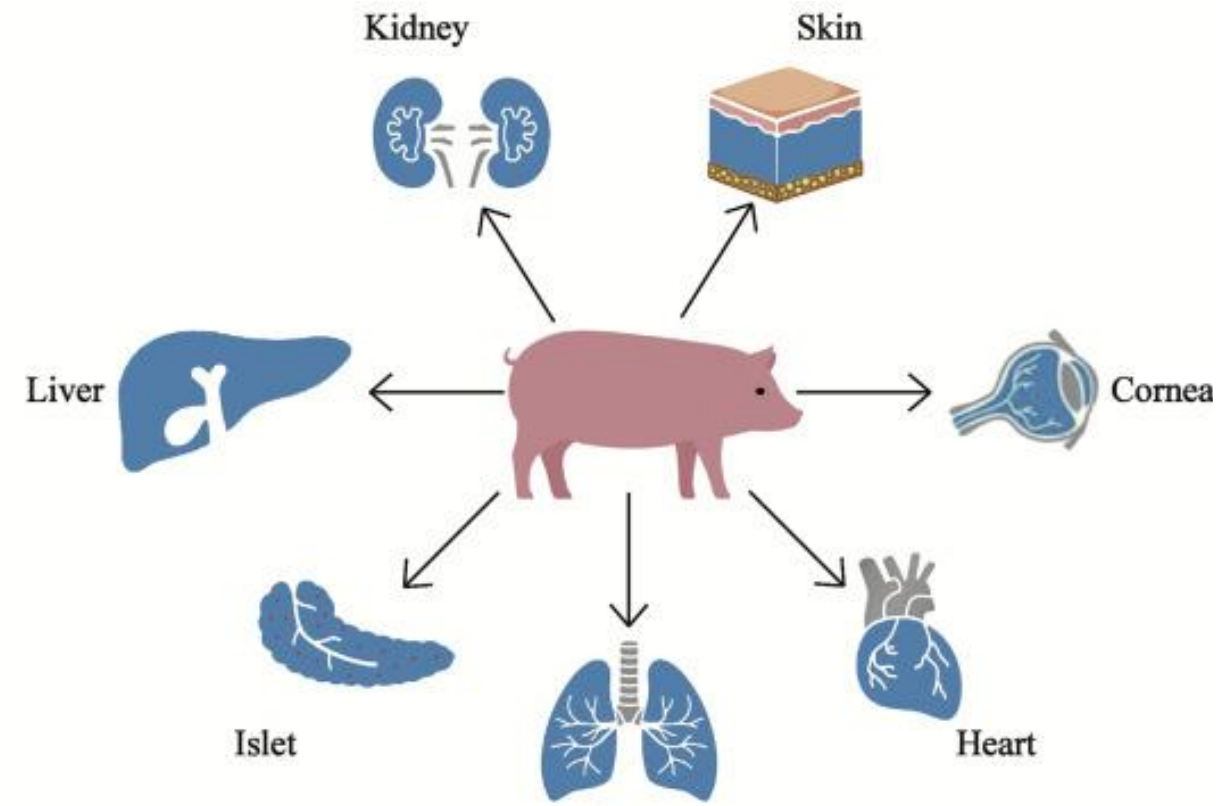
Biology Term Paper

Genetic Engineering in Xenotransplantation

Gymnasium Kirschgarten



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11.2.2022

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1.Preface:

While searching for an interesting topic for our term paper, we came across many different and very interesting articles. We were very interested in the medical applications of genetic engineering to combat genetic hereditary diseases. However, there is very little public information on this research. Then we noticed a headline in the newspaper which said that a pig's heart can be transplanted into a human and be fully functional. Of course, we were immediately intrigued and wanted to learn more. If this really is possible, many lives can be saved in the future. After reading more on the subject we discovered that by using the CRISPR/Cas9 method, doctors successfully implanted a genetically modified pig heart into a human in the U.S. in January of 2022. A pig's heart is now beating inside a human. How is such a transplant even possible, and how is the pig heart modified to meet human needs and physiology? Will it become normal in the future to transplant not only human organs but also those of pigs? And will researchers also try and succeed to modify other species organs to fit into our bodies? There are also certain critical questions concerning xenotransplantation[a] which came to our mind after reading the article. Such as whether this method is justifiable on an ethical and moral basis and also what the possible consequences of this method may be?

2.Introduction:

We all know that organ donations are possible and can save lives. The main problem in most cases is that there are not enough donations. This has to do with the fact that not everyone who dies is an organ donor and that the number of patients who could benefit from a transplant is much larger. Some countries, e.g. Spain, try to overcome this limitation by a donor obligation[i].

The facts above explain why the donation of animal organs is of such a huge interest. Much research has been done and now, some weeks ago, more precisely on the 11th of January 2022[ii], in the US the first genetically modified pig heart could have been inserted in a human body. This was a big success and a breakthrough in medicine. Due to the fact that xenotransplantation nowadays is mostly proceeded on pigs, we mainly talk about pigs in this essay.

To minimize rejection and to optimize the organ for its use in humans, it is genetically modified. The method which scientist use is called CRISPR/Cas9. The first scientific documentation about the usage of CRISPR/Cas9 was published in 2012[iii]. Although, this specific method is fairly new, xenotransplantation in humans take place since the early 20th century.[iv]

An alternative treatment is the conventional transplantation from human organs. However, the number of people waiting for an organ transplantation is increasing constantly and the available transplants cannot fulfil the growing demand.

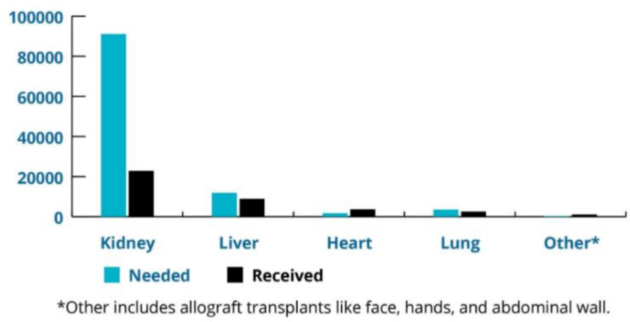


Figure 1: Patients on the waiting list vs. Transplants performed (2020)

[a] Xenotransplantation is the transplantation of living cells, tissues and organs between different species

3.Description of engineering technique:

3.1 The CRISPR/Cas9 technique in general[v]

The CRISPR/Cas9 technique is a tool to cut and modify DNA. It is often known as a "DNA-scissor". CRISPR is short for **c**lustered **r**egularly **i**nterspaced **s**hort **p**alindromic **r**epeats.

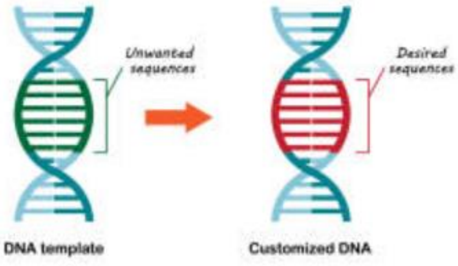


Figure 2

With this method we are able to edit the genome by removing or adding genes to the DNA strand. Scientist call this cutting out of a gene a "knock out" and the adding a "knock in". As a consequence, the proteins normally produced by these specific genes, are no longer being produced in the case of a knock out or new proteins are being produced in the case of a "knock in".

The two components needed for the CRISPR/Cas9 technique are the Cas9-protein and a guide-RNA. The guide-RNA is complementary to the sequence of the DNA, respectively a gene on the DNA strand that will be edited. The guide-RNA is attached to the Cas9-protein. When it recognises the DNA target sequence, the Cas9-protein breaks the double stranded DNA. The guide RNA attaches to the DNA target sequence. With the help of the PAM (protospacer adjacent motif) sequence on the DNA target strand, the Cas9-protein knows where to cut out the DNA target sequence. Now, the target sequence can be modified or completely eliminated. As well as it can be replaced by a completely new gene.

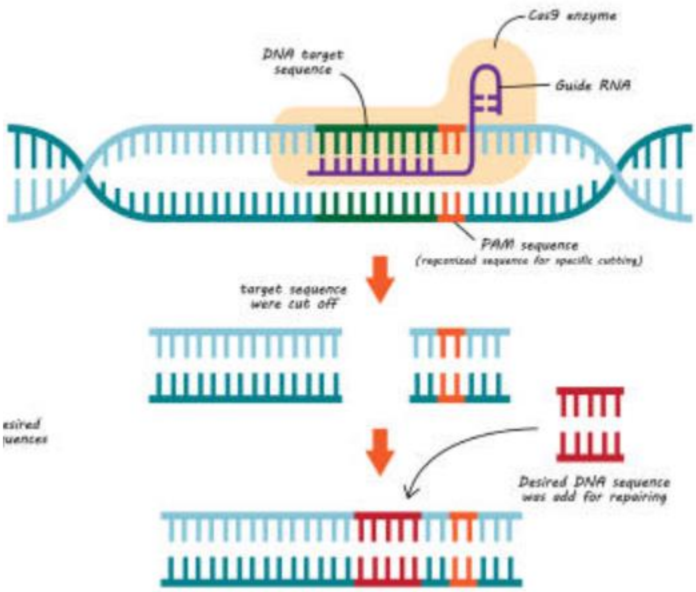


Figure 3: Visualization of the CRISPR/Cas9 technology

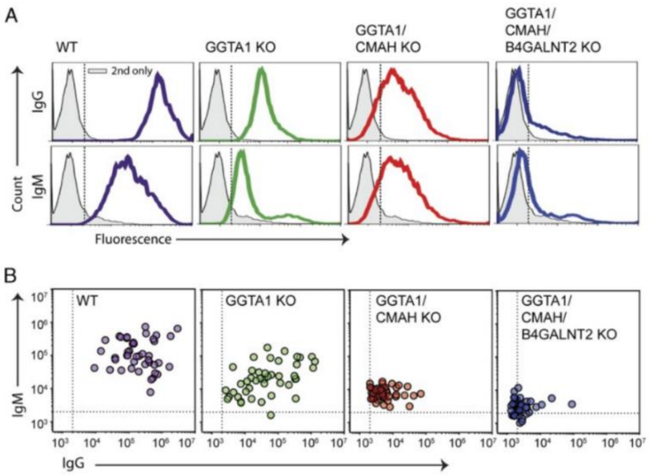
3.2 The CRISPR/Cas9 technique applied on Xenotransplantation[vi]

The genetic modification needed for a xenotransplantation in a pig is made before the fertilisation. Instead of sperm cells, normal cell tissues (fibroblasts) are being modified with the CRISPR/Cas9. Specific genes can now be "knocked out" or "knocked in". The newly modified nuclei of the fibroblasts are put into the oocytes of the sow with the help of a mechanic injection. This process is called SNCT (Somatic Nucleic Cell Transfer). Then the modified egg cell is implanted into the pig's uterus. The pig is artificially inseminated and the whole genetic modification happened before, outside the pig. The piglets are not born naturally but with the help of a caesarean section to increase sterility. The new born piglets are now genetically modified. After the birth, the sow is euthanized and the piglets are going to be raised sterile and without their mother. This is important to prevent further risk of virus contamination after the pig has been tested for viruses and mutations. When they are big enough, the organs are taken out of these pigs and are transplanted. Although the

CRISPR/Cas9 system can be used in vivo, for the modification of organs this process needs to happen in vitro. The *in vitro* method is much more precise than modifying a sperm cell *in vivo*, which is extremely unlikely to be fertilised.

One of the problems with xenotransplantation are the PERVs[vii], which are retroviruses and can also infect human cells. There are a few different types of this virus, including PERV-A and PERV-C. Most of them are not dangerous for humans, however the type PERV-C would endanger humans. One option to lower the risk of a PERV infection is using pigs that are tested negative. Alternatively, there is the possibility to knock out all the PERVs in the sow's genome. The problem being that knocking out all the different types of PERVs is not efficient due to the big effort it requires.

A big issue is, that our human body does not accept a pig's organ unconditionally. Consequences could be hyperacute, acute or chronic rejections or even blood clots as thrombosis. This rejection of the organ is caused by surface sugars sitting on a pig's organ, like the heart or the kidneys. The problem being that humans have antibodies against these surface sugars, which lead to a hyperacute rejection. So as soon as the organ is transplanted into the human body, our immune system fights against it. With the CRISPR/Cas9 system we are able to knock out the genes of these specific surface sugars, so the humans' antibodies do not work against the transplanted organ. Figure 4 shows how studies were made, to investigate whether the organ is being accepted and visualises how knock outs would influence it.



The graph in the left shows the organ without a knockout of any surface sugar. The grey area and the purple line are incompatible. This means that the human body does not accept the organ. In the second, third and fourth graph, single, double and triple knockouts were conducted. In the fourth graph, the grey area and the blue line are compatible, which means that no antibodies in the human body were activated to fight against the transplanted organ.

Figure 4: Study on the influence of knock outs

4. Interview with Dr. med. Guido Junge

Dr. med Junge was born in Germany and studied medicine and law in Bonn before completing his clinical training at the Charité in Berlin. There he worked as a specialist in transplant surgery and intensive care medicine. Each year, Dr. Junge transplanted 120-150 livers and kidneys each and performed several multivesicular transplantations (transplantation of several organs simultaneously). He then did his postdoctoral research in the US and joined Novartis where he works in clinical research. Currently, Guido Junge lives and works in Basel.

Figure 5: Dr. med. Guido Junge

Why are specifically pigs used as an organ donor?

In the first xenotransplantation in the 1960ies, one used a chimpanzee. They tried to take animals that were as close as possible to humans in terms of pedigree. At that time immunology was not well understood and after two hours to a few weeks the organs died. In addition, ethical questions came up: Can we breed monkeys only to take away their organs? Another question concerned the breeding potential: How many children can a monkey have? A pig can have between 8 and 12 piglets per litter. A monkey pregnancy, however, generates only one monkey. In addition, pigs reach reproductive maturity after 4-8 months, whereas it takes monkeys 3 to 4 years. Furthermore, pigs are accepted in society as breeding animals, since they are also used for meat production. The size, physiology, blood composition, etc. of pigs are not very far from humans.

What are risks when working with xenotransplantation? And how can the risk of transmitting viruses or other disease carriers be minimized?

There are three big areas where there are risks. One is the physiological problems that can occur. The first question, of course, is whether the anatomy fits at all. Are the organs about the same size that they even physically fit into the body. If we were to take an elephant's liver, we would not be able to fit a human being. Then there is the question of whether the physiology fits. The liver, produces proteins, various clotting factors and so on and does detoxification. Would a pig liver also replace what a human liver does in humans? Then the second question is about the incompatibility of immunology. We have an auto tolerance, but not a foreign tolerance, and if our body sees anything it doesn't know, it rejects it. Even in human organ transplants this is possible. Only in identical twins there can be no immunological reaction. The third big risk is that the pig lives together with certain viruses that we do not have (retroviruses). That scared people a lot at the beginning, because we all know our own history with retroviruses, which is HIV. People didn't know if these retroviruses in pigs were a danger. Either you can take only pigs that are negative for dangerous retroviruses, or you cut out all these genomes using CRISPR. However, this is very time-consuming. With the help of a negative mother pig, negative piglets can be raised in a sterile manner. The risk of Xeno zoonosis (transmission of an animal disease to humans) is relatively low in pigs, because they are kept in stables and that they are known for so long and treated with antibiotics. Monkeys are often captured from the wild and brought to the lab. So, you don't know what germs, bacteria or viruses they bring with them from the wild. The risk of such transmission is therefore much higher in wild animals than in pigs raised in controlled breeding.

Do you think that xenotransplantation will become a common treatment method soon, or does the technology still need more development?

There was a lot of excitement last year: In the USA, a gene-modified pig organ, a kidney, was implanted in a human for the first time, however into a brain-dead person. In Germany, that would be impossible. That was an isolated experiment. In order to become a standard treatment, you would need hundreds of patients for a large-scale study. It is certainly being worked on, but the health authorities are applying much higher criteria for such a transplant. It will not happen quickly, but it will probably develop. The question is: Will it become a standard procedure? Probably no. Xenotransplantation is very complex and expensive. The probability of survival is also much shorter than with a human organ.

What is your ethical position on the subject? Do you find such a transplantation justifiable?

In the case of humans, we all agree that everyone counts the same and that under no circumstances should you kill a human being for your own gain. If we were to breed apes for their organs, we *probably couldn't ethically defend that. But since we as humanity like to eat a lot of meat, the pig has a different value. So, if you are willing to eat them, you can raise them to save lives.*

Could you imagine claiming such a transplant yourself if necessary?

It is difficult because healthy people would naturally say that it is out of the question. The slightly ill would answer that they would think about it, and a seriously ill person would answer that if they didn't get a human, they would take one. The one in the ICU (intensive care unit) would say yes right away. When you're healthy, it's easy to say, of course I won't do it, but when you're facing death, your acceptance changes.

*This interview was translated from German to English

5. Discussion

5.1 What progress was made with the application of the chosen technique?

Due to the genetic modification of an organ the human body accepts it much better. Especially the invention of the method CRISPR/Cas9, was a big step for the research of xenotransplantation. The CRISPR/Cas9 is a breakthrough technology in the history of science, which allows to engineer genes so specifically. Therefore, it helped combat the rejection towards external organs in the human body.

Additionally, we have a big lack of people donating their organs. Therefore, taking organs from animals and doing a xenotransplantation can help us in the future to save lives. This opens new opportunities for transplantations.

5.2 What are future research steps?

The modification of a pig is elaborate because it is done it by artificial insemination and cannot be carried out it *in vivo*. It would be much more efficient to have a modified pig species that reproduces. But in this case, it's less sterile and harder to control. The risk of contamination and mutations increase[viii]. So, we have to find a way that permits an efficient but also hygienic and exact transplantation. An additional goal would be that organs from animals are accepted better in the human body and can help on a long-term period.

5.3 Discussion of ethical aspects:

A big majority of people eats meat without thinking much about the ethical question and do not ask themselves if it is justifiable. But when it comes to killing an animal and taking his heart for example, to save somebody's life, people get more critical and criticise the conditions for the animal. Is this not contradictory that we eat meat for pleasure but criticize the killing of the same animal for saving somebody's life? Is saving a human being not of greater benefit than the simple consumption of meet?

Furthermore, the breeding of pigs is much more efficient as the breeding of other animals or humans. It only takes some month until they can get pregnant and they can get several piglets at a time. Also, the acceptance of pigs as breeding animals in our society supports the justifiability of xenotransplantations with pigs as well.

Nevertheless, we have to ask ourselves if it is acceptable to kill an animal for saving a life at all. With each transplantation several animals are dying. On one hand the mother that does not give birth normally but through a c-section and on the other hand the piglet which we take out an organ from.

Animal activists would say it is not reasonable on a moral basis[ix].

The technique that is used namely CRISPR/cas9, as already mentioned before can't be done in vivo that's why it is even more complicated an elaborate. Additionally, the method is quite new and we don't know much about the long-term consequences.

Additionally, there are diseases among animals that humans normally do not get. Through xenotransplantations these diseases could be transferred to humans.

Although rejection is very likely and not all problems are solved yet, xenotransplantation can help patients that need rapidly an organ, bridging the time until they can get a human organ. And why not reduce the consumption of meat and instead see the chance of raising animals to take their organs and help someone in need?

6. Summary:

CRISPR/Cas9 opens up totally new and unexplored branches of medicine. As the number of people needing an organ donation increases and the number of organ donors decreases slightly, a different way to tackle the problem is very needed. Xenotransplantation is a creative and smart new possibility. However, it still requires a lot of work although some major milestones have already been reached, such as the transplantation of a modified pig heart into a human body. With the help of the CRISPR/Cas9 technique pig fibroblasts can be modified as required. With multiple precautionary measures the pigs are raised as sterile as possible to prevent the infection with PERV-C. Once adult, the organs can be transplanted. Pigs' anatomy, physiology and size fit very well to the human and they also become big enough quite fast and have multiple piglets at a time. Therefore, they are very well suited as organ donors and using primates is not the most obvious choice anymore. Additionally, pigs are commonly accepted as livestock and a big part of the population does enjoy eating a pork sausage from time to time. It appears to be more ethically justifiable killing pigs to save lives instead just having a nice meal. Using primates also arouses many ethical criticisms due to their close relation to humans. As there also is not one genetically altered species of pigs but each piglet has to individually be modified before fertilization, there is little risk of possible unwanted mutations, but costs increase very much due to the extra effort.

Although xenotransplantation is possible it will probably remain a rather futuristic idea due to the multiple reasons mentioned above and the preference of using human organs instead of those of any animal. If given the choice every patient will certainly choose the human organ over the pig organ. Researchers are now working on extending the time for how long a modified organ can fully function in the human body. They will for now probably focus on pigs as they hold the most potential. We'll have to wait to find out if some day lives really will be saved with xenotransplantation. It certainly is worth looking into what the future may hold.

7. References:

Graphs and figures:

Cover picture: https://ars.els-cdn.com/content/image/1-s2.0-S0169409X20300211-gr3.jpg (10.02.2022)

Figure 1:https://www.organdonor.gov/learn/organ-donation-statistics#numbers (04.02.2022)

Figure 2 + 3 : https://media.istockphoto.com/vectors/science-illustration-show-crispr-cas-9-work-for-cut-and-edit-dna-as-vector-id1279287335?k=20&m=1279287335&s=612x612&w=0&h=Vwvh0IfvfKWLfz-mts7UDkIZjvnTl0QuPnBz5y8Ojgc= (08.02.2022)

Figure 4 : Martens GR, Reyes LM, Li P, Butler JR, Ladowski JM, Estrada JL, Sidner RA, Eckhoff DE, Tector M, Tector AJ. Humoral Reactivity of Renal Transplant-Waitlisted Patients to Cells From GGTA1/CMAH/B4GalNT2, and SLA Class I Knockout Pigs. Transplantation. 2017 Apr;101(4):e86-e92. doi: 10.1097/TP.0000000000001646. Erratum in: Transplantation. 2018 Feb;102(2):e88. PMID: 28114170; PMCID: PMC7228580. (03.02.22)

Figure 5 : https://vitagate.ch/de/gesund_und_schoen/ratgeber/medikamente/pharmaforschung (10.02.2022)

Oral sources and links to sources on the internet:

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[i] Oral source: Dr. Med. Guido Junge (28.01.2022)
[ii] https://www.zdf.de/nachrichten/panorama/usa-herz-transplantation-mensch-schwein-100.html (7.2.2022)

[iii] https://de.wikipedia.org/wiki/CRISPR/Cas-Methode (7.2.2022)

[iv] https://en.wikipedia.org/wiki/Xenotransplantation (10.02.2022)

[v] https://www.youtube.com/watch?v=UKbrwPL3wXE (08.02.2022)
https://www.synthego.com/guide/how-to-use-crispr/pam-sequence (10.02.22)
Oral source: Dr. Med. Guido Junge (28.01.2022)

[vi] Oral source: Dr. Med. Guido Junge (28.01.2022)
[vii]https://www.frontiersin.org/articles/10.3389/fmicb.2018.00730/full (02.02.2022)

[viii] Oral source: Dr. Med. Guido Junge (28.01.2022)
[ix] https://biotechhealth.com/xenotransplantation/ (29.1.22)

Appendix:

The video recording contains all the oral sources and the interview. It is being sent separately, because of its big file size.