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CRISPR/Cas Technology and Pigs: The Path to successful Xenotransplantation

1. Preface

We chose the topic of xenotransplantation because it is a new field of medicine and most likely realizable in the near future, therefore we might even be directly affected by it, which is why knowing all about it is an advantage.

What really makes it fascinating is the use of modern gene editing technology, CRISPR/Cas, to enable a safe transplant. The key advantage of this new scientific method is that it has the potential to solve the problem of there not being enough human donors to supply for the increasing need of transplant organs. This thought caused a spark of interest, which eventually led to us choosing it as the topic of the following paper

After some research we also came upon some questions we would like to be able to answer, or at least speculate about:

- Is there a risk of creating pandemics?
- Is breeding pigs ethically acceptable?
- What progress was made in the technique of Xenotransplantation?
- What future research steps lie ahead?

2. Xenotransplantation: an introduction

Xenotransplantation is the process of replacing damaged human cells or organs with living ones of a different animal or human cells bred *ex vivo* (=outside the living organism). However it is often accompanied by issues such as viruses found in the genetic code of the donor-animal, so-called Retroviruses.
In recent years, scientists have been working on a technique to safely transplant pig organs into humans, a feat that proved to be more difficult than expected because the porcine genome also contains Retroviruses that could be potentially harmful to humans. These are called Porcine Endogenous (=included in the genetic code) Retroviruses; PERVs for short. The reason they chose pigs is because the organs have a similar size and function to human organs.

To remove these PERVs from a pig's genetic code, researchers have to use a special gene-editing technology: CRISPR/Cas
So far there have been no alternative methods that enable safe pig to human xenotransplantation.

3.1 What is CRISPR/Cas?

CRISPR stands for *Clustered Regularly Interspaced Short Palindromic Repeats,* which essentially describes a replicate of a virus 'built' into the genetic code of bacteria within an organism so they can recognise it and come up with their own immune response to protect themselves against said virus. This immune response comes in the form of a CRISPR/Cas-Complex made out of RNA molecules and Cas proteins, the RNA molecules serve to identify and find the virus while the Cas proteins splice it, thus rendering the attacking virus useless. This was first successfully achieved in December 2010 by Sylvain Moineau from the University of Laval in Quebec City, Canada. However the first time it was used for targeted genome editing was three years later in 2013 by Feng Zhang at the Broad Institute of MIT and Harvard. Previous attempts were launched in the 1990s but the lack of the knowledge we have today and technology prevented successful completion.

3.2 How can this be used in research?

Thanks to the discovery of CRISPR/Cas, researchers were able to use computers to synthesize the RNA sequence complementary to the DNA section that they want to remove from the genome of a pig, for example. This so-called Guide RNA sequence is then bound to the Cas protein and used to target and cut the Retrovirus from the pig's DNA. This creates damage in the DNA molecule which needs to be fixed. There are two different mechanisms the body can use to do that. It can either switch off the targeted gene or replace it with a new sequence.

This illustration shows a simplified overview of how CRISPR/Cas works. The guide RNA unwinds the targeted part of the DNA and because it is linked to the guide RNA, the Cas9 enzyme (a synthesized version of Cas) is able to cut the right section out of the genome.

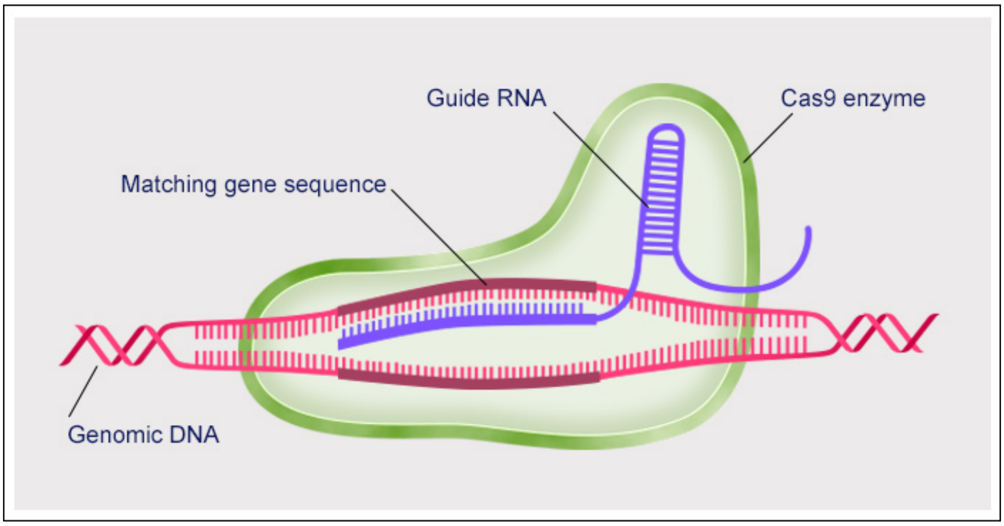
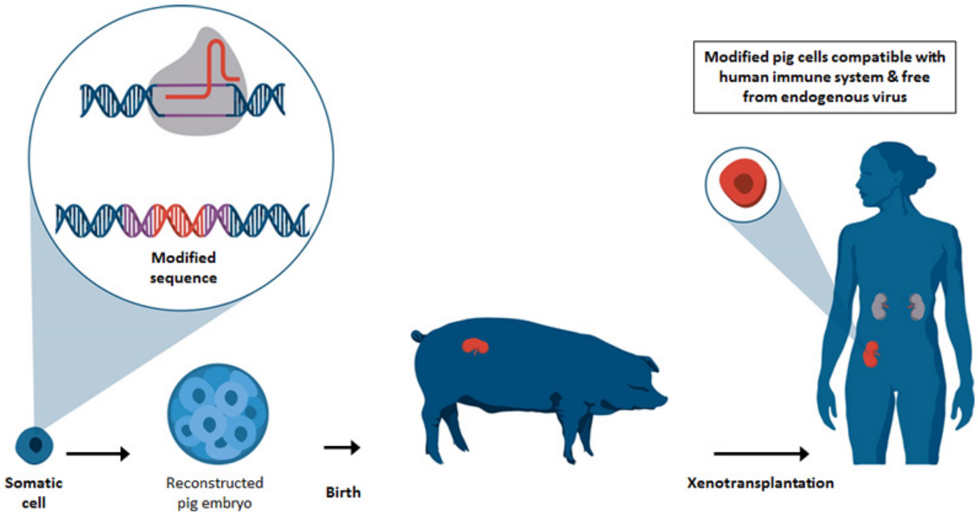


Image Source: Crispr-cas9-at-work-data.jpg

3.3 Practical application of CRISPR/Cas9

As previously mentioned, pig DNA contains potentially harmful retroviruses that can be removed using the CRISPR/Cas9 technology. Once these are removed, the pig cells and organs can be used to replace damaged human ones. This hasn't gone into clinical trials yet because often there are Off-Target-Effects, where the wrong part of the DNA is removed, which makes producing enough viable pigs incredibly difficult.



The image to the left shows the basic, theoretical steps leading from the CRISPR/Cas9 edited cell to a successful transplantation.

Image Source: Modified-pig-cells_new_sm.jpg

4. Interview with Prof. Dr. Niemann

Since 2008 Prof. Dr. Niemann is the head of the Institute of Farm genetics (ING) of the Friedrich-Löffler Institut in Mariensee Germany. The institute of Farm genetics deals with genetically targeted animal breeding. The main research focus relies on the biotechnological developments related to animal breeding and the investigations and characterization of genetic resources. Prof. Dr. Niemann (see image on the right) is a veterinarian and currently working as a researcher at the institute. He led further projects which dealt with embryo transfer, more precisely with the cryoconservation (=freezing) of embryos from angora goat. He is currently working on the development and realization of xenotransplantation. The following interview with him on the topic of xenotransplantation using organs from CRISPR/Cas edited pigs was conducted on February 2nd, 2018.

Inga & Olivia: Is xenotransplantation also possible for children? (would the organs grow with the body?)

Prof. Dr. Niemann: Unclear at the moment, there is some preliminary evidence that the transplanted organs would grow with the recipient, but no final conclusions yet.

Can you get all the organs from one edited pig?

Theoretically possible, but very unlikely. It is conceivable that for example a heart and a kidney from the same ultimate donor pig will be used for transplantation into two different recipients.

Could the rest of the pig be consumed?

Theoretically yes, but the current Gene technology law forbids this, that means that the carcasses have to thrown away.

How expensive is a genetically edited pig? (breeding, feeding, “upkeep”)

Very difficult to calculate at present, should be expensive initially, but once the ultimate pigs have been produced, it should be rather cheap. There are established systems to keep pigs under largely sterile conditions. There are theoretical considerations that a porcine xenotransplant would be significantly cheaper than keeping patients with kidney failures on dialysis, or patients with heart failures in the intensive care unit.

Human organs are donated (i.e. ‘free’) would pig organs cost a lot more money? Only accessible for the wealthy? Would insurance cover it?

These are all questions that would have to answered when clinical application is reality. The general consensus is that it ultimately would be cheaper because there are no constraints and pigs would be available in great abundance. Once it is an effective treatment, the health insurance will cover it.

When do clinical trials start?

There are already advanced clinical trials with porcine islet cells (phase II and III) and heart valves are just on the rise. Solid organs will take a bit longer, my estimation is within 7-10 years.

How would PERVs affect humans?

Research has shown that PERV risk is negligible, You can also knock the PERV loci out as recently shown by group at Harvard.

Is there a difference between the body accepting a human and a pig organ? (other than the source)

Research is directed to insert enough genetic modifications into the porcine genome that the remaining immune response can be controlled by a conventional immune suppressive treatment

Do you think that gene-editing of pigs is animal cruelty? How so/not?

I don't think that it is animal cruelty, gene editing is a technology to inducing precise genetic modifications, this is necessary for producing the right pigs.

It is known that the organs were implanted in chimpanzees in the early phases of research. Additionally, the pigs are held in very sterile, unnatural conditions. Is that all really worth it, if convincing humans to donate could be a lot less expensive and time consuming?

Porcine organs are transplanted usually in baboons as model for humans to get insight into the in vivo situation after transplantation. All efforts to increase the willingness to donate human organs have been unsuccessful thus far.

5.1 Progress & Future Research Steps

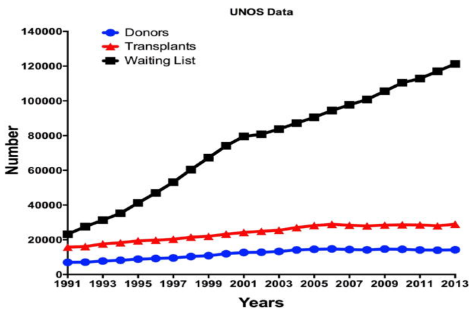
Taking our research and the information from Prof. Niemann into account it seems like the application of CRISPR/Cas to allow pig to human xenotransplantation is very close to realization in the field of clinical medicine. As of now scientists still need to prove to their respective governments that the method is safe and beneficial to the healthcare system. This entails a much smaller failure rate (Off-Target Effects). They therefore need to improve the synthesized Guide-RNA to target the correct DNA sequence and perhaps develop another more accurate Cas protein.

5.2 Ethical discussion

When looking at the ethical aspects of xenotransplantation, it becomes apparent that the benefits as well as the concerns are similar to those of transplantation in general. Scientific discussions deal with highly sensitive topics such as death and the role of medicine in interfering with the ‘cycle of life’. In the following paragraphs the pros and cons of xenotransplantation will be discussed regarding ethical aspects.

5.2.1 Ethical benefits:

1) **Availability.** Organs would be available whenever required. Unfortunately patients on the waiting list for an organ transplant still die or suffer unnecessarily prolonged pain even in this time of modern medicine. Having access to pig organs would significantly reduce those figures.



The graph on the left shows data on the availability of transplant organs from 1991 to 2013 collected by the UNOS. It is evident that the amount of people waiting for an organ transplant (black line) has increased in recent years whereas the amount of people prepared to donate organs (blue line) has not.

Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4684733/

2) **Reduction of illegal activity regarding organ donation.** The illegal sale of human organs is a huge issue, where people from third-world countries sell their organs (mostly their own kidneys) for a living. These transplantations are arranged by criminal organizations, which benefit financially from the desperation of others. Additionally, there are no health and safety regulations. Patients would nonetheless be taking a huge risk that the obtained organs may be diseased, causing the transplant to fail. With this in mind, using organs from genetically modified animals, instead of those illegally donated by humans, can end or at least limit this type of trade to a minimum.

3) **Potential to open up new areas of research.** Successfully completed pig to human transplants would create an almost entirely new field of research and open the gates for further gene editing technology/research with this goal in mind, perhaps allowing for other species to become useful.

5.2.2 Ethical concerns

1) **Animals are not objects, they are living beings.** Pigs shouldn't be considered as donors because the definition of donation involves voluntarily giving something. Critics argue that, as they are not fit to communicate (or even develop) their own decisions they should not be exploited in such a way. Additionally their lives would be limited to a confined, sterile location to prevent any epigenetic changes. An animal-friendly alternative could be to find a way to grow entire human organs *ex-vivo* (=outside the living organism) from the patient's tissue for autotransplantation.

2) **Religious aspects.** Many religions such as mainstream branches of Islam forbid the slaughtering of pigs for personal use. Due to the fact that there are 1,6 billion muslims worldwide, pig/human xenotransplantations would be limited to non-muslim countries.
3) **Risk of disease transmission.** There are concerns indicating, that with the transplantation of animal-cells there would also be a high risk of transferring diseases or retroviruses which are harmless for pigs but potentially life-threatening to humans.
4) **There is a risk of rejection.** There is a high likelihood that the immune system of a patient would directly attack the newly implanted organs, causing the operation to fail. This would bring the patient in a situation risking his life, with the possibility of death.

5.3 Our opinion: We support the concept of xenotransplantation

We think that xenotransplantation is a concept which opens up great possibilities such as independence from human donors, stopping criminal organisations, thus preventing the exploitation of innocent people, and supporting new scientific methods, which could help even more people in need of medical attention. We also believe that in contrast to some farms, the laboratories in which the genetically edited pigs are kept, do not expose them to potentially painful diseases and injuries. Although there is a risk of organ rejection, one may argue that this is also possible for human to human transplants. Furthermore the pigs won't transmit any PERVs, because of the aforementioned CRISPR/Cas method. Clinical trials on humans will only start after all potentially harmful viruses have been edited out of the porcine genome with 100% accuracy. We are aware of the fact, that the animals utilized for xenotransplantation would not survive most procedures, which one should not support in general. However, taking into consideration that one single genetically modified pig could save the lives of up to five people, we conclude that the medical benefits outweigh the ethical concerns.

6. Summary

To summarize, xenotransplantation from pigs to humans is a process, which could only be considered safe once all porcine endogenous retroviruses (PERVs) have been removed. This is done using a complicated scientific procedure called CRISPR/Cas. It uses synthesized RNA segments to identify and locate the corresponding DNA segment one wishes to remove. The RNA is bound to a protein abbreviated as ‘Cas’ which has the ability to splice out the targeted segment. Scientists have tried this on many pigs, triggering an ethical debate over whether it’s morally sound. We concluded that the benefits, such as significantly reducing the donor organ deficiency, outweigh the drawbacks, such as unnatural living conditions of pigs bred in the labs and the considerable failure rate.

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