

Xenotransplantation: The Science, the Advantages, the Ethics

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Abstract

Xenotransplantation is a breakthrough medicinal technology that is an attempt to change the lives of millions of people. The problems in the current organ transplant system risk the lives of patients each and every day. Intense waiting periods and extremely costly procedures exemplify the stress and pressure that these patients face as an attempt to save their own lives. Xenotransplantation is the idea of growing human organs in a different species, using incredible stem-cell and CRISPR technology. This can introduce an answer to some of the issues in the current transplant system. Many technical and ethical issues are becoming relevant with the introduction of this new medical phenomenon. If these barriers can be overcome, xenotransplantation can offer a quicker, cheaper, and more effective option for patients in need of an organ transplant.

Introduction

Thousands of sick patients are in desperate need of an organ transplant, often having their entire lives depending on this one vital procedure. Before they can undergo treatment, a proper organ donor must be found, and the process is harder than ever imagined. Numerous factors affect the long waiting time, a period that can frequently become over a year. The main slow-down is the simple shortage of organs ready for transplant. In response to the overwhelming need for organ donations, new medical research is being explored as an attempt to alleviate the high demand. Xenotransplantation poses as a possible solution to this problem and has the capabilities to be very helpful in the medical world; releasing the great amount of stress and anxiety involved in the classic organ transplant system that is now in place. However, this new medical process introduces new biomedical and ethical issues. Scientists and doctors are currently exploring the possibilities of this new technology, weighing the costs against the benefits; an important attempt to improve the medical world of organ transplantation.

History of Organ Transplants

The concept of organ transfer in a procedural sense has been around for centuries, in-fact cases of organ transplantation dates back to ancient times. There is even written proof and documentation from archeological records that suggest that organ and tissue transplantation is thousands of years old. However, modern-day organ transplantation is a relatively new medical phenomenon that has saved the lives of millions of people from end-stage organ failure.

In the early 1900's, Dr. Alexis Carrel experimented with kidney transplantations in cats, becoming the first surgeon to explore vascular surgery (Howard et.al., 2012). Carrel's journey to organ transplantations started with success in vascular surgery and suturing of vessels. These experiments also advanced the knowledge of organ preservation, showing that human tissue could be stored in either saline solution, the patient's serum, or defibrinated blood, until it is needed. Carrel then went on to organ transplantations, experimenting with kidney transplantations between dogs, and from dogs to cats. He realized that the animals only lived with the transplant for a short period of time and he knew that there was some sort of rejection going on in the host's body. Alexis Carrel made extraordinary

advancements in the medical exploration of organ transplants and created the possibility for more research in this area (Rothwell, 2011). In 1923, Dr. Harold Neuhof wrote a book called *The Transplantation of Tissues*, which revealed the work of many different tissue transplants including skin, cornea, muscle, pancreas and nerve tissue (Howard et.al., 2012).

Soon after, the first human kidney transplant was recorded using a kidney from a donor that had died six hours prior. The blood type of the donor was type B and the recipient was type O, which prevented the kidney from functioning, leading to the patient's death. However, this may not have been the only cause for the disfunction of the kidney as other trials have also been proven unsuccessful. In 1953, a kidney transplant was done from mother to son which only lasted three weeks before the recipient rejected the donor organ. In the beginning years of the 1950's, Dr. David Hume performed almost ten kidney transplantations which are now considered the first of their kind in the modern era of transplantation. However, none of the recipients had a long-term survival after their kidney transplant.

These and other trials revealed that such organ transplants would not be possible without immunosuppressant drugs. With this development, organ transplant became a real option for patients with organ failure. Dr. Joseph Murray performed the first successful kidney transplant in 1962, leading into many other victorious cases around the world. The '60's were very progressive years for this specific research and medication, as the first lung and heart transplants were also done during this time (Howard et. al., 2012).

Problems With the Current Organ Transplant System

As immunosuppressant drugs allowed organ transplantation to start becoming a viable option for victims of organ failure, the need for transplantable organs became overwhelming. In the past, kidneys from living donors were being used, but this supply is limited and not nearly enough to provide for all in need. Even with deceased donors for transplants, the available donor organs, unfortunately, do not cover the demand (United Network for Organ Sharing, Data, 2018).

Although most technical problems involved in the process have been solved, organ transplantation is not seen as a long-term cure for patients as there are still biological issues and financial problems.

The biggest and most complicated problem is the patient's rejection of the new organ. When a patient undergoes an organ transplant surgery, the body's immune system becomes stimulated against the "foreign" organ and tries to kill it. Patients are usually given strong medication to suppress their entire immune system, but that can become counterproductive as the patient is now vulnerable to other diseases. Different drugs, such as Cyclosporin, have been approved to control much of the rejection, but transplantations can still have those dangerous effects (MTF Biologics, 2017). Although they have been shown to improve symptoms, these drugs can have many negative side effects including tremors and seizures.

Another problem is the long waiting period that patients wait in order to receive a proper match for donation. There are simply not enough donors to fill the demands, causing many victims to die in the 'waiting room'. Although the shortage of organs is the main cause for the waiting time, the length that the patient waits can vary, depending on a number of factors. The blood or tissue type, the size of the organ, and the medical urgency of the situation are some factors that will fluctuate the waiting time. In any case, the patient almost always needs to wait for a proper donor before proceeding with the transplant. This is not practical in many cases when the organ transplant is extremely urgent. On average, twenty people die every day while waiting for an organ donor (United Network for Organ Sharing, Data, 2018).

Also, organ transplants are extremely costly and many people simply cannot afford such medical procedures. Kidney transplants cost over four hundred thousand dollars, and a heart transplant goes up to over one million (Rapp & Vandermeij, 2017). This cost does not even include the health maintenance of such a procedure, nor the cost of immunosuppressant drugs that cost as much as ten thousand dollars per year, for life (Gordon, et. al. 2008).

Methodology of Xenotransplantation

Looking for a solution to this unmet medical need, researchers have come up with a new method known as Xenotransplantation, which is the process of "grafting or transplanting organs or tissues between members of different species." Xenotransplantation is using stem-cell and CRISPR technology to grow human organs in a host of a different species. This idea comes from chimerism, the ability to create a "living thing that is composed of cells from two or more organisms" (Dunlap, 2017). The basic procedure is to use the pig as a host by cutting out the HOX genes that code for growing certain organs and the genes that code for porcine microorganisms from their genome. HOX genes are the sequence of genes in the genome that directs the body organization in an embryo (KhanAcademy, Khanacademy.org, 2018). Using CRISPR-Cas9 technology, described below, genetic engineers can knock out the genes in the pig that code for the development of specific

organs, and replace them with specialized human stem cells that will grow human organs instead. The hypothesis is that infusing differentiated human stem-cells into this embryo will result in the pig growing a human organ instead of its own.

CRISPR-Cas9 Technology

CRISPR-Cas9 stands for Clustered Regularly Interspaced Short Palindromic Repeats, and is breakthrough technology in genome editing. CRISPR is a special region in DNA that is characterized by having repeated nucleotides and spacers. This unique DNA region was first recognized in bacterial genomes and has since been adapted in the laboratory as the CRISPR Cas9 complex (Vidyasagar, 2018). Cas9 is a protein that acts as the molecular scissors that can cut the DNA in a double-strand break. When combined with a guide RNA (sgRNA), it forms the Cas9 Complex. The Cas9 first binds to a sequence in the genome and the guide RNA unwinds the double helix. The guide RNA is precisely designed to match up with the specific strand of DNA that needs to be edited. Then, the Cas9, with the help of its nuclease domains, cuts the DNA, creating a double-strand break in the double-helix. The DNA tries to repair itself but the reparations are usually flawed which inevitably shuts off that particular gene. This is why CRISPR is "a great tool for knocking out specific genes" (NatureVideoChannel, 2017).

Stem-Cell Research

Another biomedical technique is somatic cell nuclear transfer, known as SCNT, or cloning. Conceptually, the nucleus from a somatic (body) cell replaces the nucleus in a fertilized oocyte of another individual. This hybrid blastocyst is capable of generating a stem cell line" (Columbia.edu, 2018). The created stem cells will be identical to the donor and have many uses in the medical field. One such example would be to use these cloned cells to create human organs in pigs a step in the xenotransplantation process. The idea is to fuse the stem cells from the patient into a porcine embryo, hoping that the pig will grow human organs that can then be used as the transplant for the intended patient. In a few short months, the patient could potentially be on his/her way to a complete recovery. This will only be a possibility, however, if the barriers of Xenotransplantation are overcome.

Brief History of Xenotransplantation

Aside from cross-species blood transfusions, which were around since the 17th century, skin grafts are considered the first attempted trial in xenotransplantation. Skin grafts from animals to human patients were performed as early as the 19th century. Frog skin was the most popular species to was the most frequent source of the donor material to the skin grafts. Different attempts at xenotransplantation were tested, mostly using primates as the donor animal. Out of thirteen trials, only one kidney transplant from a chimpanzee to a human patient

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was successful with the patient living for an entire nine months before dying as a result of rejection of the kidney. In 1964, the first heart xenograft from a chimpanzee to a human was performed. The patient died within two hours of the operation. Later, in 1992, a doctor named Starzl performed a baboon to human liver transplant, with the patient surviving for seventy days. Along with the numerous failures, some of the early xenotransplants resulted in temporary patient survival. But, in general, the organs were rejected in an even more serious case than with a same-species transplant. The rejections of this kind are so severe that immunosuppressive drugs are not strong enough to stop the rejective response to the xenografts (Cooper, 2015).

The Advantages of Porcine Xenotransplantation

Recently, the focus has been on the use of organs from the pig and hog family, porcine, as they are comparable to human organs in size, anatomy and physiology, compared to other species (Niu, Wei, & Lin, 2017). Pigs, compared to other species such as baboons, would be a valuable candidate for organ xenotransplantation. Pigs can be bred in large herds and with a relatively short maternity period, making them a more available option. In addition, they are not a costly animal to breed (Cooper, 2015).

The advantages of xenotransplantation from pigs would be enormous, starting with the obvious increase in availability of organs for transplantation. Right now, there is a shortage of human organs for transplants, but using organs from another species will solve this problem, it will also make immediate transplant surgery possible for patients with an urgent situation. In addition, organs that are retrieved from human cadavers have already experienced the trauma of brain death which may lead to other issues later on. With animal donor organs, the organs will be removed from a healthy pig that is under anesthesia, avoiding the problem altogether. Another important solution that xenotransplantation brings is the decrease of the risk of a pathogen being transferred from the donor to the patient. Diseases such as the West Nile Virus and rabies have been passed through organ transplant surgery resulting in a number of deaths. The pigs intended to be used for xenotransplantation are being raised in the best conditions and are being supervised closely which reduces the likelihood of any illness to be transferred from donor to patient (Cooper, 2015).

Zoonoses and Porcine Endogenous Retrovirus (PERV)

CRISPR-Cas9 can be used in xenotransplantation research by knocking out specific genes in the pig that cause rejection when transplanted into human patients. Zoonoses are diseases that are transferred between humans and animals, caused by bacteria, parasites, and viruses. These diseases pose the major barrier preventing xenotransplantation from becoming proto-call in the medical world. The potential transfer of porcine related

infections into the human genome is a major risk of xenotransplantation. The pig herds intended to be used for medical reasons have been raised under the most sanitary conditions and have been screened for most pathogens and viruses that can possibly harm the recipient. However, porcine endogenous retroviruses that lie in the porcine genome have the capability to hurt human health. PERV are viral elements in the porcine genome, and being that they are innately a part of the DNA, the retroviruses cannot be eliminated by means of selective breeding or drugs. PERVs have the ability to recombine with other genetic elements of the recipient's genome. Similar to pigs, humans have their own set of endogenous retroviruses, known as human endogenous retroviruses, or HERVs. If the PERV were to come in contact with the human endogenous retroviruses, the former genes can recombine and disturb the human genome. Naturally, the HERVs are attempted to be halted by some sort of stop codons or deletions, but some bypass the stop codons and actually "play an important role in human physiology as well as in pathogenesis" (Machnik, et al., 2014).

Studies of HERVs have concluded that class I of HERVs group together with γ - and ϵ -retroviruses, and during a xenotransplantation it is these genes that can recombine with PERVs that also belong to the γ -retroviral group (Machnik, et al., 2014). Studies have recently confirmed that two out of three PERV types, namely PERV-A and B, have been seen to replicate in human cells during in-vitro research, meaning in a clinical setting. To date, no PERV infection of human cells have been documented in-vivo, but the possibility of this occurring needs to be taken into real account as new pathogens can be created at the point of interaction, and these new pathogens can cause unpredictable damage (Prabha & Verghese, 2012).

Recent discoveries, however, have shown major advancements in this area. Scientists have used CRISPR technology to successfully cut out sixty-two PERV sequences from a porcine genome. The nuclei of these cells were then transferred into enucleated pig oocytes by somatic cell nuclear transfer, and then implanted into surrogate female pigs. The results were astonishing with thirty-seven piglets being born, all with inactivated PERVs in the genome. This research greatly expands the possibilities in the realm of xenotransplantation, although other issues are yet to be resolved (Denner, 2017).

The Issue of Rejection

With all the prementioned advantages, there are still barriers preventing pig organ xenotransplantation from becoming the standard medical procedure. Scientifically speaking, the biggest barrier to overcome is the problem of rejection; the patient's natural response to an alien in the body. Firstly, within seconds or minutes of the transplant, the possibility of hyperacute rejection can happen. This type of rejection occurred when pig organs were transplanted into nonhuman primates. The

preformed antibodies of the receiver attached themselves to the pig's vascular endothelium and as protocol reaction to a pathogen, the cascade system was initiated. This caused the endothelial cells to convert into procoagulant phenotypical cells, resulting in hyperacute rejection. Attempts have been made to prevent this by depleting the anti-pig antigens, or the complement system from the recipient's serum, however, there are still other forms of rejection that may occur. Acute humoral xenograft rejection may occur during the first few days after the transplant, and cellular rejection can become a problem even weeks after the operation. This rejection is acute-cell mediated, meaning that different cells in the patient's body recognize the foreign organ as unfamiliar and begins to attack it. The cells involved are usually the natural killer cells, macrophages, and cytotoxic T cells. It is believed that even immunosuppressant drugs cannot prevent this rejection from being triggered. If the recipient is able to survive with the graft for a longer period of time, chronic rejection may occur. The exact cause of this type of rejection is not completely understood. However, the main point regarding all types of rejections is that the antibodies or killer-cells in the recipient attack the donated graft, resulting in a slow, but ultimately fatal destruction of that organ (Esker & Cooper, 2010). The information regarding the rejection or acceptance of a pig organ in a human patient is extremely limited, as only a few procedures have been tested so far. The scientists and doctors cannot study this further until a patient survives the procedure for a longer time (McLean & Williamson, 2004).

Rejection of the organ can occur in any transplant, regardless of whether the graft is from the same species or not. The difference is that in a xenotransplant rejection, the immunosuppressant drugs are not strong enough nor sophisticated enough to significantly extend the patient's survival time.

Potential Solution for Xenograft Rejection

Genetic Engineering of the pigs is the newest and most successful method to date to prevent or mitigate the rejection of the organ graft. One major advancement is the creation of a genetically engineered pig that expresses a human complement-regulatory protein. This protein is found in the pig's vascular endothelial cells and protects the tissue of this donated organ from being attacked by the pre-formed antibodies during hyperacute rejection (Bloom, Moulton, McCoy, Chapman, & Patterson, 1999). Genetic engineers have also produced pigs which have had the gene for $\alpha 1,3$ -galactosyltransferase removed. The gene for $\alpha 1,3$ -galactosyltransferase codes for the production of the enzyme that adds Gal $\alpha 1,3$ Gal oligosaccharides to different basic glycoproteins and glycolipids in pigs. In general, Gal is a major target for the human antibodies, as it is seen as a major invader. The removal of this gene, and therefore the halt of the Gal production in the pigs, has significantly reduced the hyperacute rejections that usually occur during the pig to nonhuman primate

xenograft trials (Esker & Cooper, 2010).

The results of this research have been positive; the length of survival time increasing by the use of the genetically engineered pig organs. A pig heart of this type lasted for 3-6 months, kidneys for close to three months, and livers for days. The extension of the survival period is proof of the success of the genetic research (Esker & Cooper, 2010).

There are also genetic solutions being studied that will disable the natural killer cells and the macrophages from attacking the xenograft, although they have yet to be tested in pig to non-human-primate transplant. Genetic engineers are working on producing pigs that are HLA-E or HLA-G transgenic as these immunoregulatory molecules are expected to stop natural killer cell cytotoxicity (Esker & Cooper, 2010).

The Issue of Coagulation Dysregulation

Another barrier that has arisen during the xenotransplantation trials is the development of thrombotic coagulopathy and consumptive coagulopathy, different types of coagulation dysregulation. Coagulation dysregulation is irregular blood clotting in areas of the circulatory system. The xenograft recipient will usually develop one of these, or possibly both, which poses a big limitation to the survival time of the graft. In heart transplants from genetically modified pigs to baboons, thrombotic coagulopathy is the predominant symptom, while in the kidney graft, the baboon developed consumptive coagulopathy. It is thought that physiological differences between pigs and primates are the causes for these problems, although the exact reason for the coagulation dysregulation development remains unclear. It is known, however, that activated endothelial cells of the donated organ are the cause for inflammation and coagulation in the recipient. Ischemia, or inflammation, is inevitable in most types of organ transplants, but various types of intertwined factors can be the cause of graft endothelial cell activation (Cowan, Robson, & d'Apice, 2011). One proposition suggests that the endothelial cells activated by either the antibodies or the start of the complement, increase the activity of TF, tissue factor. The introduction of the TF into the portal vein activates the instant blood-mediated inflammatory reaction, known as IBMIR. Characteristics of IBMIR are platelet binding, complement activation, and thrombosis (Esker & Cooper, 2010).

Ethical Problems With Xenotransplantation

In addition to the biological barriers of Xenotransplantation, problems regarding ethical and moral topics come into question when researching the possibilities for the future. Transplantation in general has a public risk associated with the administration of immunosuppressant drugs. These drugs are known to lower the patient's ability to fight off infections, and therefore increase the risk of contamination. For the patient, the risks involved need to be weighed by their own physician; however there are public

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concerns involved as well. The new genetic engineering, plus the previously used immunosuppressant drugs, have the potential to “open the way for the emergence of new viral mosaics into the general population” (Anderson, 2006).

The opposing side of the ethical debate brings up valid points as well. With thousands of people in desperate need of organ donations, is it ethically fair to deprive them of the possible solution that can potentially save so many lives? The introduction of xenotransplantation can greatly reduce the waiting period for an organ donation, and additionally provide the patient with a long-term graft. The immunosuppressant drugs have saved so many people from death; is it morally okay to ban them on the slight chance that they can cause an infection outbreak? These questions are some of the many that have come up in discussion over the last couple of years, as the medical world is coming closer to introducing this new phenomenon to regular medical protocol.

Another ethical problem involved in xenotransplantation is the issue of personal privacy and confidentiality. This question arises due to the necessity of monitoring of the patient after an organ transplant, going as far as to institute a lifetime surveillance of the patient. This surveillance has become a part of the system as an attempt to prevent pathogenic diseases from spreading through organ transplant surgery. As previously discussed, the pathogenic risks involved in xenotransplantation are very great and have caused medical outbreaks in the past. In fact, in the year 2000, a hepatitis C breakout was reported due to an infected donor that was mistaken to be healthy. Since then, tremendous precautions have been taken while looking for organ donors, as the transfer of pathogens through transplants can lead to numerous fatalities. Opponents of the monitoring system believe that this provision is an invasion of the patients' privacy, as well as the privacy of their family and friends. Here lies the question whether or not the invasion of privacy is considered to be ethical for the safety of the patient and the “problem of maintaining ethical standards in situations such as this is vexing” (Anderson, 2006).

The only possible way to effectively protect society from infectious diseases via xenotransplantation is if the national and international powers decide on a logical system, keeping the ethical debates in mind. The World Health Organization has previously come together to discuss this exact topic, although no agreement was yet to be decided. To date, the only guidelines put in place are those of the Food and Drug Administration's Recombinant DNA Advisory Council. The RAC approved gene recombination studies to be done at Harvard and MIT, advancing the medical research of this field. The RAC's permission was granted with the consent of the citizens of Massachusetts, justifying the ethical questions involved in such research (Anderson, 2006).

The American Society of Transplant Physicians voice their concern of infectious diseases being spread internationally. They feel that United States regulations are not strong enough in

comparison to the severity of the risk involved. It is presently legal for patients in need of an organ donation to receive organs from live donors living in impoverished countries, where infections are known to be rampant. For example, there is a website named Liver4you.org which arranges liver donations from people in the Philippine Islands. Bringing these diseases across to the United States through the organ transplant poses as a major threat to the wellbeing of society (Anderson, 2006).

Often, doctors and surgeons are also researchers of new xenotransplantation medication, which poses additional ethical questions regarding the split between their medical responsibilities and their personal studies. The physicians can frequently be confronted with “the well-being of the patient in direct opposition to the advancement of academic medicine” (Anderson, 2006). An example of what might result from such a situation is a doctor convincing a patient to participate in a clinical trial that he/she would otherwise not join. An infamous case of this sort, known as the Baby Fae case, took place in 1984 when a young child was used in a medical trial of a xenotransplantation using a baboon heart. The parents were encouraged by optimistic doctors who hoped to provide the baby with “immediate and long-term survival” but she only survived for four weeks following the graft. Medical researchers have become cautious getting involved in xenotransplantation studies in fear of this sort of issue cropping up, in addition to the history of medical science misconduct (Anderson, 2006).

Additionally, with the introduction of stem cells into the pig embryo, the possibility of creating a pig with human elements presents an ethical issue. The ethical debate discusses the possibility of human gametes to be created and an embryo to be formed as an interspecies chimera. Although it is agreed upon that the embryo would not be able to develop, the mere creation of it poses as an ethical question. Also, the differentiation of human stem cells needs to be studied in depth as the question arises of what is stopping the stem cells from entering the pig's brain, or other areas, which will blur the margin of what is considered human and what is considered animal? Is it morally permissible to perform a procedure that can potentially give a pig, human, high-level brain functions? These ideas are unlikely in the scheme of things, but pose concerns in the biomedical world nonetheless (Masaski & Nakauchi, 2017).

Conclusion

Scientific researchers are constantly trying to better the medical world; looking for solutions and applying their incredible knowledge to helping humanity. The biomedical field is exploring the possibilities of genetic engineering combined with stem-cell research, hoping for xenotransplantation to one day become a standard medical procedure for patients with organ failure. When it comes to genetic engineering, the possibilities are endless; however, xenotransplantation has many barriers to

overcome before it can become the standard medical procedure. However, the latest research may explore the best course of action regarding this dynamic research. Researchers have proposed for xenografts to be used for patients in the waiting period that are waiting for an organ donor. This way, although xenotransplantation is not yet a final solution, it will at least minimize the number of deaths of patients, and the health risks for waiting for a proper transplant. This change in protocol has the tremendous potential to save millions of lives, giving the patient the ability to live the few months until they can receive a proper transplant. Researchers are only beginning to uncover the technology's tremendous potential and long-term survival of xenografts may be a few years away. Even so, it is possible that for now, temporary xenotransplantation can be the life-saving procedure that patients are hoping for (Servick, 2017).

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