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Pigs as Models of Preclinical Studies and In Vivo Bioreactors for Generation of Human Organs

Yujiro Kawai, Shugo Tohyama, Hideyuki Shimizu,
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Abstract

Pigs are valuable and essential large animal models for human medical applications, including for stem cell therapy. Moreover, substantial effort has been made to directly engraft genetically engineered pig organs in the human body and to use pigs as in vivo bioreactors for the growth and development of human cells, tissue, or organs. However, engraftment of human cells in pigs has not yet been achieved. Although severe combined immunodeficient pigs have been developed, which can accept human biological materials, these pigs do not have practical value at present owing to difficulty in their care. To overcome these current limitations, we have proposed the generation of operational immunodeficient pig models by simply removing the thymus and spleen, enabling the long-term accommodation of human tissue. In this review, we summarize research progress on xenotransplantation animal models that accept human cells, tissues, or organs.

Keywords: regenerative therapy, transplant, bioreactor, immune tolerance

1. Introduction

Organ transplantation is often the only possible treatment for a patient with organ failure. The organs are donated from either living or deceased donors, and thus the number of transplantable organs is limited and insufficient to meet the clinical demand. Consequently, some illegal or unethical transplantations along with transplant commercialism and tourism have emerged, representing a worldwide problem.

The discovery of the potential of pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced PSCs (iPSCs), to regenerate tissues or organs offers new hope to overcome this situation. Human ESCs [1] and iPSCs [2] are now widely used to generate tissues or organs, and techniques for the in vitro production of specific cell types have been developed [3]. However, these strategies still have several limitations for clinical application, including the size, maturity, function, and risk of tumor formation after transplantation [4].

To solve these problems, large animal models for the transplantation of human PSC-derived cells, tissues, or organs are required. In this review, we summarize the animal models currently used in the development for xenotransplantation and highlight the efficacy and prospects of pig models to accept human tissues or organs.

2. Xenotransplantation in small animals (mouse and rat)

Immunosuppression is a key requirement for an animal to accept human tissues or organs, as a functional immune system will result in the host animals rejecting the human grafts. The nude mouse was the first immunosuppressed animal model developed in 1962 [5]. Nude mice lack T cells and can therefore accept human tumor cells. Subsequently, severe combined immunodeficient (SCID) mice were developed in 1983, which lack both B cells and T cells [6]. McCune et al. [7] successfully transplanted a human fetal thymus, liver cells, and lymph node into SCID mice, resulting in the differentiation of human T cells and B cells. However, the rate of engraftment of human cells in these mice was low due to maintenance of their natural killer (NK)-T cell activity. Gerling et al. [8] developed NOD/SCID mice by crossbreeding SCID mice with NOD mice, a diabetes model due to autoimmunity in the pancreas, which also show low NK-T cell activity and macrophage function [9]. Combining the low activity of NK-T cells and macrophages in NOD mice with the lack of B cells and T cells in SCID mice, the use of NOD/SCID mice improved the engraftment rate of hematopoietic stem cells [10]. Ito et al. [11] produced NOG mice as a crossbreed of NOD/SCID mice and gamma(c)(null) mice, which completely lack NK-T cells, and achieved a dramatically improved engraftment rate of human hematopoietic cells.

We previously reported the successful transplantation of rat cells into SCID mice [12]. Isolated hepatocytes obtained from the rat liver were injected into urokinase-type plasminogen activator (uPA)/SCID mice, in which urokinase-type plasminogen accumulates specifically in the native liver causing the damaged liver. The mice served as bioreactors to allow the transplanted rat hepatocytes to proliferate in the mouse host, resulting in more than 95% of cells in the mouse liver being of rat origin. Oldani et al. [13] successfully developed a mouse-rat chimeric liver, which was transplanted in rats. They injected hepatocytes isolated from Lewis rats into C57Bl/6$^{Fah-/-Rag2-/-Il2rg-/-}$ mice to create chimeric livers, which were transplanted into rats with or without immunosuppression. Without immunosuppression, the recipient rats died from acute rejection, whereas rats with immunosuppression survived for more than 112 days and maturation of rat bile ducts was observed 4 months after transplantation. We also demonstrated that the nude rat model could serve as an in vivo bioreactor. Liver grafts from Syrian hamsters were transplanted into nude rats that administered several immunosuppressive agents, including tacrolimus and mycophenolate mofetil (MMF). After auxiliary xenogenic partial liver transplantation, regeneration of the liver graft was observed, and its weight increased from pre-transplant to 7 days after transplantation [14].

These immunodeficient mouse models, including SCID, NOD/SCID, and NOG mice, are useful for research on regenerative medicine using human PSCs, allowing for evaluation of teratoma formation to confirm the differentiation of the cells into the three germ layers [1]. In addition, these models are widely utilized for evaluation of tumorigenicity in human PSC-derived cells after transplantation [4], since human PSC-derived cells or tissues have a risk of tumor formation from contamination of undifferentiated PSCs [15, 16]. Small animals such as mice and rats are widely applied as models in cell transplantation research owing to their ease of handling. However, small animals have limitations in terms of the number of cells that can be transplanted and evaluation of therapeutic efficacy, that is, a human clinical application might require the transplantation of several hundreds of million cells, which is impossible to accomplish in small animals. Moreover, large animal models are required for accurate evaluation of the efficacy of cell transplantation.

Furthermore, large animal models are expected to play roles as bioreactors for functionally mature human tissues or organs.

3. Xenotransplantation in middle and large animals (monkey and pig)

Chong et al. [17] transplanted human ESC-derived cardiomyocytes into the hearts of pig-tailed macaques as a nonhuman primate model. The main advantage of this model is that the hearts are much larger (37–52 g) than those of mice (0.15 g), rats (1 g), and guinea pigs (3 g), which allowed for the transplantation of 1×10^9 cells into the infarcted myocardium and subsequent engraftment. The macaques were administered methylprednisolone, cyclosporine, and abatacept (a CTLA4 immunoglobulin) to prevent immune rejection. The efficacy of human ESC-derived cardiomyocytes in the infarcted hearts of pig-tailed macaques was demonstrated, and maturation of the transplanted ESC-derived cardiomyocytes was observed [18]. However, compared to an adult human, pig-tailed macaques are still relatively small (5.2–12.6 kg), and the heart is much smaller than that of a human (300 g).

Pigs are a suitable animal for preclinical studies and in vivo reactors in terms of their size and anatomy that correspond well to those of humans. To establish an immunosuppressed state that allows for transplantation of human PSC-derived cells or tissues into host pigs without rejection, SCID pigs were also developed [19]. Suzuki et al. [19] generated cloned pigs by serial nuclear transfer using fibroblasts with disruption of the X-linked interleukin 2 receptor subunit gamma (*IL2RG*) gene, as this mutation is known to cause X-linked SCID in humans. The SCID pigs accepted human cells, indicating their potential in preclinical studies and as in vivo reactors with human PSCs. However, raising these pigs is a technical challenge; among the 31 cloned piglets produced, only four survived for over 1 year. In addition, SCID pigs must be raised under meticulous hygiene conditions, which impose a further cost for their establishment and maintenance. Therefore, it is not practical to use SCID pigs as models in preclinical studies and in vivo reactors.

Total thymectomy is an alternative strategy to create immunosuppressed pigs that can accept human cells. Binns et al. [20] first proposed the concept of achieving immunosuppression by performing thymectomy in neonatal pigs in 1972. Microminiature pigs (MMPs) are smaller than domestic or ordinary miniature pigs and are thus suitable model animals for preclinical studies [21]. To develop immunodeficient MMPs, we performed thymectomy in neonatal pigs, which were transplanted with human hepatocytes that could engraft in the pig liver without any immunosuppressive agents [22]. To further improve the immunodeficient pig model, we performed splenectomy along with the thymectomy in 6–7-month-old miniature pigs and administered several immunosuppressive agents, including tacrolimus, MMF, and prednisolone, via a stomach tube [23]. This so-called operational immunodeficient miniature pig (OIDP) model allowed for the successful implantation of artificial human vascular tubes created by a three-dimensional bioprinting. Moreover, the human tube was inserted between the carotid artery and jugular vein to act as a shunt, and blood flow was observed for 3 months without immune rejection.

As mentioned above, establishment of a chimera is a potential strategy for growing human tissues or organs in large animals. Matsunari et al. [24] demonstrated that blastocyst complementation can be applied to large animals by creating chimeric pigs. Specifically, they generated embryos from clones of porcine somatic cells, which showed an apancreatic phenotype, and their complementation

with allogenic blastomeres resulted in the development of a functional pancreas. Wu et al. [25] reported a successful pig-human chimera that was created by introducing human PSCs into fertilized pig eggs. Therefore, when combined with blastocyst complementation, human organs can be created in a human-pig chimera; however, these methods are associated with serious ethical and legal problems. Alternatively, the introduction of human-derived cells to pig fetuses can lead to immune tolerance, allowing for the acceptance of human PSC-derived tissues or organs.

4. Immune tolerance induction for xenotransplantation

Immune tolerance is defined as a lack of an immune response against particular antigens. In general, the immune system has tolerance to self-antigens and only responds to non-self-antigens, which is a challenge for transplantation, as the grafted cells or tissues are rejected and not able to survive in the host body. The phenomenon of immune tolerance was first described in 1945 in which anastomosis in the placenta was observed in twin calves, and they accepted each other's skin grafts [26]. Hasek et al. [27] subsequently confirmed this phenomenon in chicken and duck by producing parabiosis in fertilized eggs. In 1953, Medawar et al. [28] established actively acquired tolerance by implanting a live antigen in the fetuses of mice or embryonic chicks. Using this method, Binns et al. [29] also tried to create immune tolerance in pigs by implanting bone marrow cells or lymphocytes from another pig into fetal pigs, resulting in prolonged survival of skin graft in the treated pigs.

In addition to these examples, induction of immune tolerance to human cells or tissues has been attempted in other animals. Kenneth et al. [30] transplanted human mesenchymal stem cells into fetal sheep early in gestation. Despite the xenogeneic condition, the human mesenchymal stem cells engrafted and survived in multiple tissues for up to 13 months after transplantation. These strategies of injecting human cells into a fetus were proven to result in immune tolerance to human cells after birth.

As MMPs have emerged as suitable candidates for immune tolerance induction to accept human cells, tissues, and organs owing to their useful applications in preclinical studies and in vivo reactors, it may be possible to create MMPs with immune tolerance to human cells by injecting a human antigen into pig fetuses without requiring the need to create human-pig chimeras [31].

5. Conclusions

Our newly developed OIDPs can accept human cells, tissues, and organs derived from human PSCs. These models will allow for long-term observation after the transplantation of human PSC-derived cells or tissues to better evaluate the safety and efficacy of the procedure. Moreover, if human cells, tissues, and organs are transplanted into piglets, they will grow in vivo along with the growth of the host pig. These grafts will then mature and be of suitable size with appropriate function for human application. Therefore, pigs can be suitable models for preclinical studies and serve as in vivo bioreactors for developing human tissues or organs (**Figure 1**). Transplantable MMPs without immunosuppressive agents are expected to be developed in the near future as promising and valuable animal models for researchers, which can dramatically promote regenerative medicine and organ transplant therapies with human PSCs.
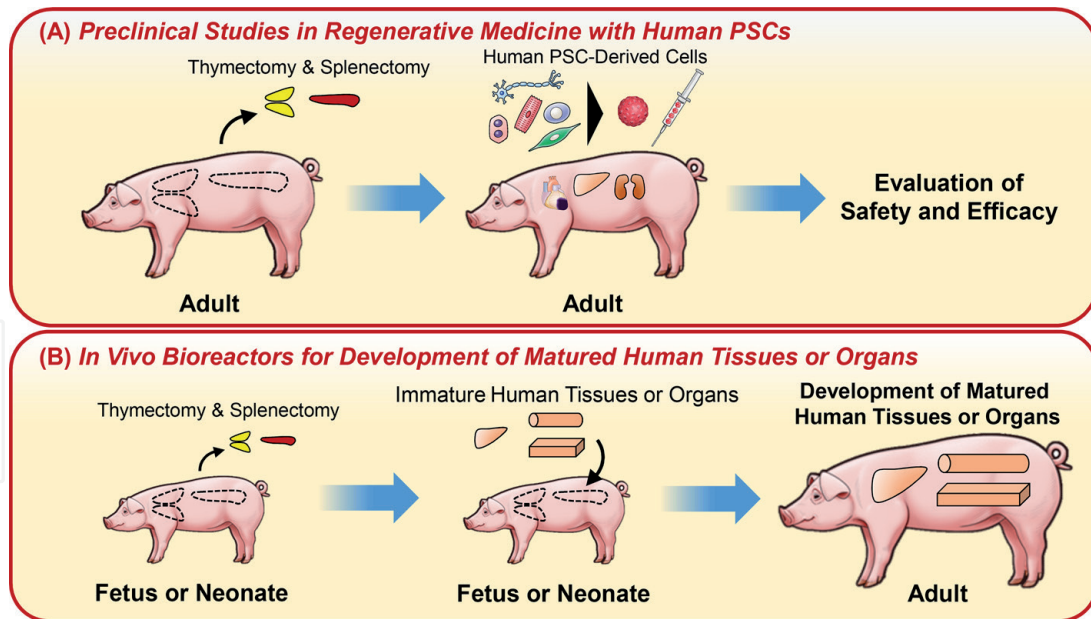
Figure 1.
Schema of pigs as models of preclinical studies and in vivo bioreactors. (A) Adult operational immunodeficient miniature pigs (OIDPs) are useful for preclinical studies in regenerative medicine with human PSCs, enabling evaluation of the safety and efficacy of cell transplantation. In particular, after transplantation of human PSC-derived spheroids or organoids into the OIDPs, the risk of tumorigenicity can be evaluated. (B) Fetal or neonatal OIDPs are also useful as in vivo bioreactors, facilitating the efficient in vivo growth of immature human tissues. After immature human PSC-derived tissues or organs are transplanted into OIDPs, they will mature along with the growth of the host.

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