Theriogenology xxx (2016) 1–5

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Review

# Growing human organs in pigs—A dream or reality?

Hiroshi Nagashima*, Hitomi Matsunari

*Meiji University International Institute for Bio-Resource Research, Kawasaki, Japan*

*Keywords:*
Organ regeneration
Chimera
Blastocyst complementation
Genome editing

A B S T R A C T

Organ transplantation has been the last line of therapy for saving patients experiencing end-stage organ failure. However, the success of organ transplantation is critically dependent on the availability of donor organs. There are high expectations for research on organ regeneration as a solution to the donor shortage issue faced by transplantation medicine. Thus, generation of human organs from pluripotent stem cells is now one of the ultimate goals of regenerative medicine. In recent years, several approaches to using pluripotent stem cells to generate organs of complex structure and function have been developed. Reproductive biology plays an indispensable role in the development of innovative organ regeneration researches. In this review, we discuss the potential of the animal biotechnology aiming at making human organs using pigs as a platform.



## 1. Introduction

Regenerative medicine, including cell transplantation therapy and tissue engineering, offers an effective approach for developing treatments of intractable diseases. Still, organ transplantation has been the last line of therapy in saving patients experiencing end-stage organ failure when other therapies are not effective. However, the growing shortage of organ donors throughout the world is still a major challenge. One proposed solution for this serious problem is xenotransplantation, in which organs of genetically modified pigs are used for clinical application [1–3]. As pigs share many anatomic and physiological characteristics with humans, xenotransplantation has been an important subject of research in the last decades [1–3]. In addition, an innovative approach of generating human tissues and organs from pluripotent stem cells (PSCs) has been extensively studied [4–8]. However, human organs with complex physiological functions and three-dimensional structures are extremely difficult to build in an artificial culturing environment. Therefore, the use of animal fetuses is a promising alternative strategy that may circumvent the problems of *in vitro* organ generation by supporting organ development from xenogeneic PSCs in a natural physiological environment. Yokoo et al. [9,10] have shown that the mechanisms supporting kidney growth in animal fetuses could be used to build kidney tissue that originates from human PSCs. They transplanted human bone marrow–derived mesenchymal stem cells into kidney anlagens of rat fetuses (E11.5), cultured the fetuses *in vitro* for 2 days, and then extracted the primordial kidney (metanephros). These metanephroi were grown further either *in vitro* for 6 days or as transplants into omental adipose tissue of immunosuppressed adult rats. The analysis of the developed renal tissue confirmed that exogenous human mesenchymal stem cellss participated in nephrogenesis, suggesting a possibility of regenerating humanized organs by using primordial animal organs as a scaffold.

Several studies have been conducted with the aim of producing transplantable organs based on the mechanisms of organogenesis in heterologous animal species [9–15]. Recently, a new approach using blastocyst complementation has been developed and attracted considerable attention (Fig. 1) [16–18]. It is based on generating animal fetuses deficient in a specific organ and using the empty space in the fetal body as a niche for the growth and differentiation of allogeneic or xenogeneic PSCs, ultimately to form a solid organ.

* Corresponding author. Tel./fax: +81 44 934 7824.
*E-mail address:* hnagas@meiji.ac.jp (H. Nagashima).


http://dx.doi.org/10.1016/j.theriogenology.2016.04.056

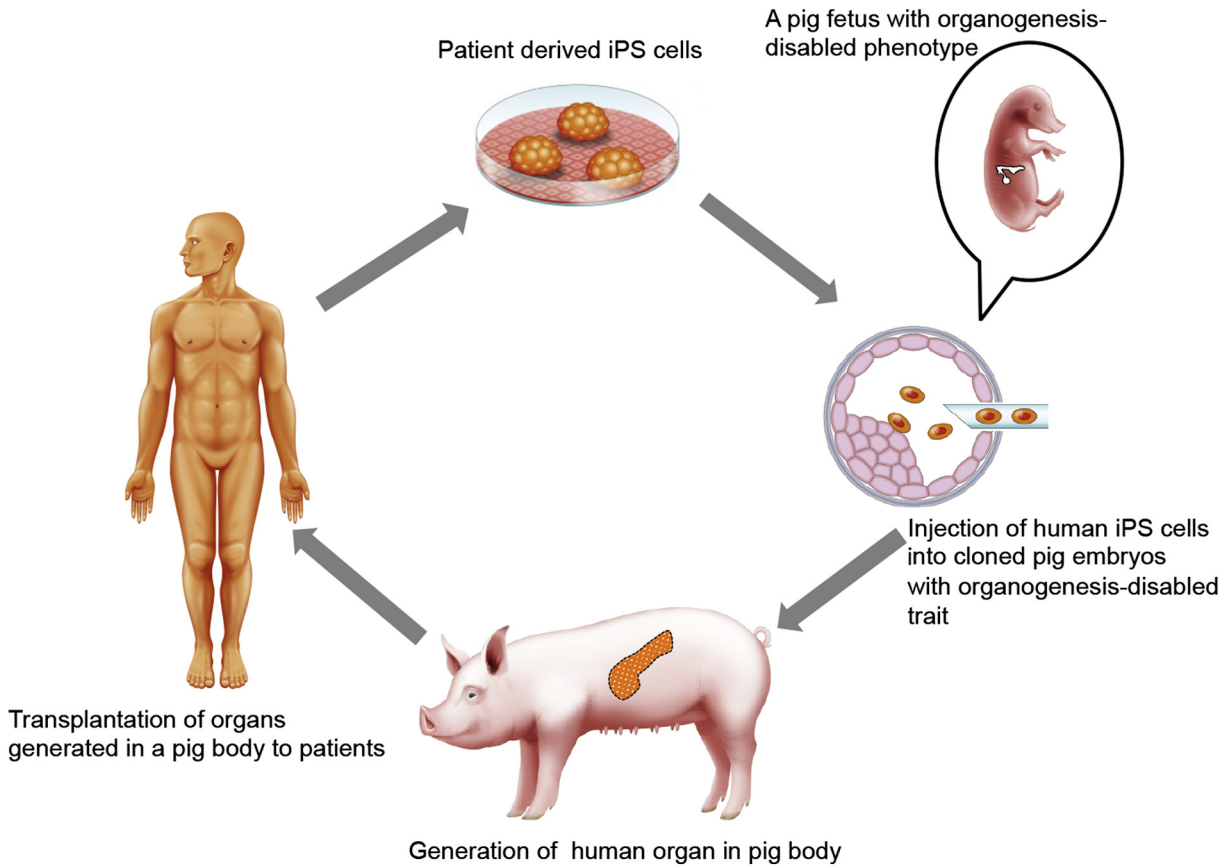


**Fig. 1.** Schematic presentation of blastocyst complementation: generation of human organs using organogenesis-disabled pigs as a platform. iPS cells, induced pluripotent stem cells.

## 2. Generation of the rat pancreas in a mouse

The pancreatic and duodenal homeobox 1 (*Pdx1*) gene is known as a master regulator of pancreas formation. *Pdx1* knockout ($Pdx1^{-/-}$) mice exhibit a phenotype of pancreatic agenesis. Kobayashi et al. [16] have reported that these apancreatic mice can support the formation of the pancreas from rat PSCs.

The apancreatic $Pdx1^{-/-}$ mice die within a few days after birth. However, injecting induced PSCs (iPSCs) from healthy rats into the $Pdx1^{-/-}$ mouse blastocysts has been shown to result in the generation of chimeric mice carrying the pancreas developed from rat iPSCs. These research findings demonstrate the principal possibility of exploiting an empty developmental niche (organ niche) in the organogenesis-disabled fetus for organ formation from xenogeneic PSCs, which can be applied to the generation of human organs. Using the *in vivo* environment provided by the animals relatively physiologically compatible with humans would be the key to organ regeneration. Thus, this approach would offer a definitive solution to the problem of shortage of transplantable organs. Therefore, we conducted a basic study aimed at developing a system for organ regeneration in pigs, which uses blastocyst complementation [17].

## 3. Producing pigs with an apancreatic phenotype

We generated transgenic (Tg) pigs that express Hairy enhancer split-1 (*Hes1*) under the control of the *Pdx1* promoter [17]. The *Pdx1-Hes1* Tg midgestation fetuses were confirmed to lack the pancreas, indicating that the animals exhibited an apancreatic phenotype. Next, we established primary fibroblast cultures from the Tg fetuses and used them for somatic cell–based cloning. The cloned fetuses and offsprings showed the apancreatic phenotype.

For the production of Tg pigs, we used intracytoplasmic sperm injection–mediated gene transfer [19,20]. In this method, frozen sperm heads that were thawed and pre-incubated with the gene construct were microinjected into oocytes for fertilization; as a result, foreign genes were incorporated into the genome of the host egg. With pigs, a system for mass *in vitro* production of mature eggs with high developmental ability has been established. The intracytoplasmic sperm injection–mediated gene transfer is, therefore, a very effective practically applicable method for producing Tg fetuses.

Pigs with the apancreatic phenotype can also be generated by methods other than Tg approach. Recently, we have reported, for the first time, that genome editing with zinc-finger nuclease is effective for gene knockout in