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Age-Appropriateness of Porcine Models Used for Cell Transplantation

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Abstract

Pigs have traditionally been used for preclinical experiments, and body size-matching is important for cell therapy in animal models used for preclinical trials. It has been shown that the efficacy of the transplanted cells is dependent on the response of the host heart and the age of experimental pigs.

Keywords

Stem cell, cell transplantation, pig, myocardial infarction, age

Translational research is important for clinical applications, and this has been scientifically established. Large animals used in clinical trials, such as primates, dogs, and pigs, differ significantly in size as compared with humans. It has been shown that body size-matching in animal models is essential for medical devices. Pigs have especially been considered essential for cell therapy tests in cardiovascular diseases. However, most investigators tend to use young pigs, because it is hard to handle adult miniature pigs weighing more than 40 kg. To investigate the profile of the pigs used in recent studies, we performed a literature review of publications from the past 3 years using three key words: cell transplantation, heart, and pig (Table 1)[1–16]. As expected, there were only a few reports describing the use of adult pigs older than 1 year of age[14], and most investigators used young domestic pigs in preclinical studies.

Recently, in a study using a swine myocardial infarction (MI) model, Gálvez-Montón et al. assessed myocardial function and scar evolution following the implantation of engineered bioactive impedance grafts made of a scaffold of decellularized human pericardium, porcine adipose tissue-derived progenitor cells, and a customized-design electrical impedance spectroscopy monitoring system[8]. In the above study, which used cross-bred Landrace × Large white pigs weighing 30.2 ± 3.6 kg, 1 month following the intervention, a significant improvement in left ventricular ejection fraction was detected via magnetic resonance imaging (MRI). Chang et al. also investigated whether injection of human cord blood mononuclear cells, when combined with hyaluronan hydrogel, could improve the efficacy of cell therapy in a miniature pig MI model[15]. The pigs were treated with cyclosporine and methylprednisolone to prevent rejection of human cell transplants. It was found that 2 months following the surgery, treatment with human mononuclear cells in hyaluronan hydrogel elicited the highest left ventricle ejection fraction. In this study, Lanyu minipigs (~5 months old and weighing 22.26 ± 0.78 kg) were used. In contrast, Natsumeda et al. used adult Göttingen minipigs (older than 1 year of age and weighing 25–30 kg)[14]. They evaluated the efficacy of combination cell therapy using autologous mesenchymal stem cells (MSCs) and cardiac stem cells (CSCs) and revealed that combination cell therapy synergistically reduced scar size and improved cardiac function.

It is well known that domestic pigs can grow quickly in size; however, the optimal weight for experiments ranges between 20 and 30 kg. In contrast, some investigators use 3–4-month-old domestic pigs in preclinical studies. However, an age of 3–4 months for a pig is equivalent to 6–7 years for a human. Certainly, it is unreasonable to use domestic pigs when their body size reaches that of a man older than 60 years of age. Additionally, it is difficult to justify the costs and the demerits involved in using adult mature miniature pigs. In contrast, there are possibilities that

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Table 1. Profile of Pigs Used in Recent Preclinical Studies of Cell Transplantation.

Author	Year	Cell Type	Pigs Used in Experiments	Duration of Observation	Cardiac Function	Reference
Blázquez R, et al.	2016	CDCs	Large white (3–4 months, 30–35 kg)	1 month	Not improved	PLOS One (2016)
Cai M, et al.	2016	MSCs from bone marrow	Chinese mini (10 months, 25 ± 5 kg)	1 month	Improved	Sci. Rep. (2016)
Chang MY, et al.	2016	Cord blood mononuclear cells	Lanyu mini (~5 months, 22.26 ± 0.78 kg)	2 months	Improved	Stem Cells Transl. Med. (2016)
Gómez-Mauricio G, et al.	2016	Modified MSCs from adipose tissue	Large white (3–4 months, 39 ± 9.72 kg)	1 month	Not improved	Stem Cell Res. Ther. (2016)
Kanazawa H, et al.	2016	CDCs	Yucatan mini (- months, 42–55 kg)	2 months	Improved	J. Am. Heart Assoc. (2016)
Kulandavelu S, et al.	2016	Modified CSCs	Yorkshire (- months, 20–30 kg)	2 months	Improved	J. Am. Coll. Cardiol. (2016)
Tseliou E, et al.	2016	CDCs	Yucatan mini (- months, 40–45 kg)	1 month	Improved	PLOS One (2016)
Bobi J, et al.	2017	MSCs from adipose tissue	Large white (3–4 months, 35.1 ± 2.7 kg)	2 months	Not improved	J. Am. Heart. Assoc. (2017)
Dariolli R, et al.	2017	MSCs from adipose tissue	MS60 EMBRAPA (- months, 15–20 kg)	3 months	Not improved	PLOS One (2017)
Gálvez-Montón C, et al.	2017	Progenitor cells from adipose tissue	Landrace × Large white (- months, 30.2 ± 3.6 kg)	1 month	Improved	Stem Cells Transl. Med. (2017)
Gálvez-Montón C, et al.	2017	Porcine iPSCs	Landrace × Large white (- months, 28.5 ± 3.3 kg)	3 months	Not improved	Tissue Eng. Part C Methods (2017)
Kim MC, et al.	2017	MSCs from adipose tissue	Yorkshire × Landrace (- months, 25 kg)	1 month	Improved	J. Korean Med. Sci. (2017)
Natsumeda M, et al.	2017	MSCs and/or CSCs	Göttingen mini (12–15 months, 25–30 kg)	3 months	Improved	J. Am. Coll. Cardiol. (2017)
Alvino VV, et al.	2018	Adventitial pericytes	Large white (- months, 34.8 ± 0.7 kg)	1.5 months	Not improved	J. Am. Heart Assoc. (2018)
Gao L, et al.	2018	Human iPSC-cardiac patch	Yorkshire (- months, 20–30 kg)	2 months	Improved	Circulation (2018)
Ishigami M, et al.	2018	Human iPSC-cardiac patch	Micro-mini (- months, 15–25 kg)	1 month	Improved	PLOS One (2018)

CDCs: cardiosphere-derived cells; CSCs: cardiac stem cells; iPSCs: induced pluripotent stem cells; MSCs: mesenchymal stem cells.

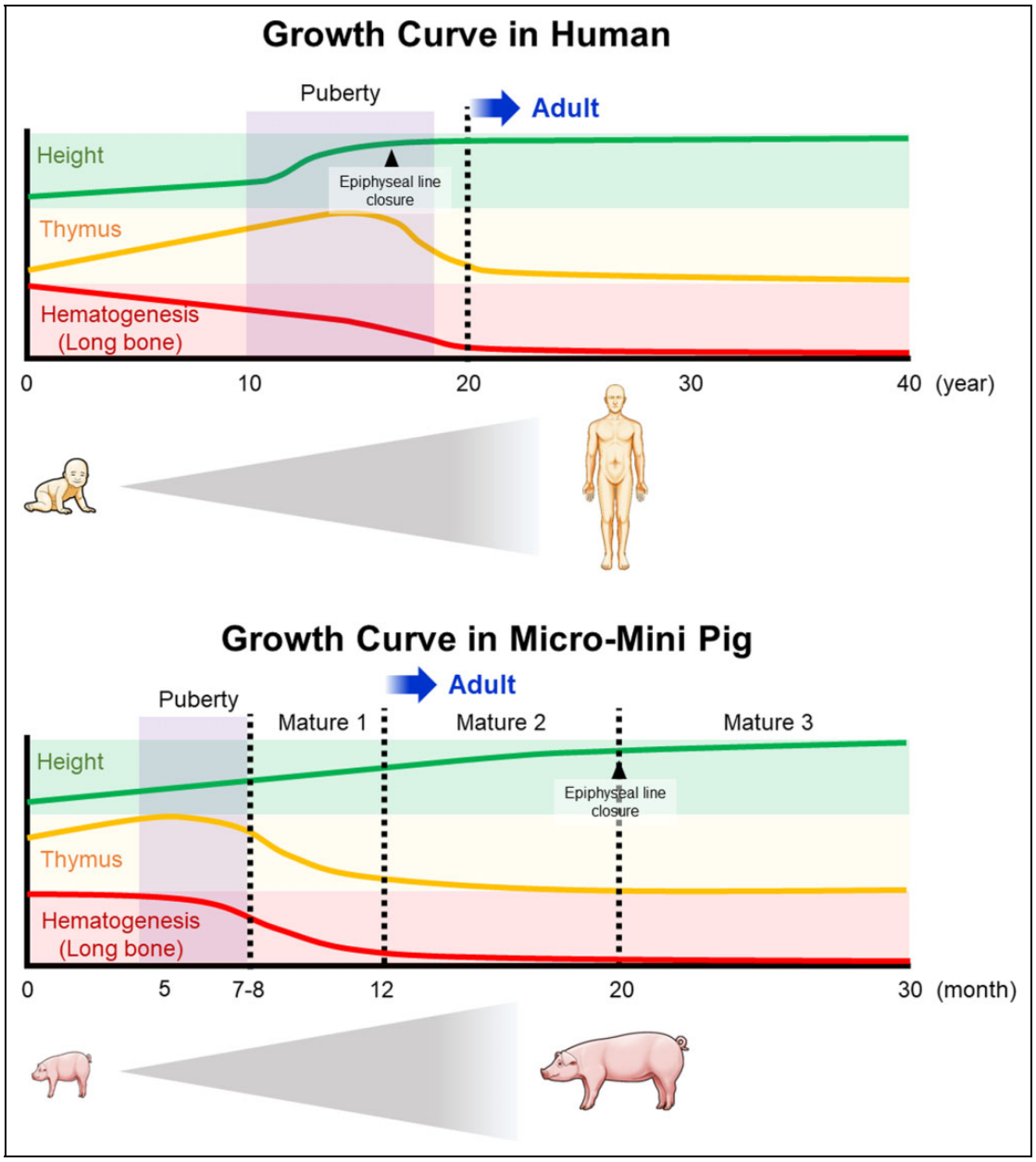

Fig. 1. Comparison of the growth curves in human and micro-mini pig. The growth curves with respect to height, development of thymus, and hematogenesis in humans and micro-mini pigs have been depicted. Micro-mini pigs take approximately 12 months after birth to reach an adult mature body weight of 20 kg. In addition, epiphyseal lines are closed at 20 months of age in micro-mini pigs, while in humans, they close at 15–17 years of age

the beneficial effects of transplanted cells may be dependent on the response of the host heart, such as through paracrine effects[16,17], indicating that the immature host heart will respond positively to the paracrine factors secreted from transplanted cells. Therefore, it is important to use adult pig models for accurate evaluations, because inaccurate evaluations will lead to failure in future clinical trials.

In our previous study, we investigated the smallest miniature pig, the micro-mini pig, as a preclinical model for cell therapy[18]. Based on the developmental profiles, we depicted the growth curve in humans and micro-mini pigs (Fig 1). Micro-mini pigs require approximately 12 months after birth to reach an adult mature body weight of 20 kg. In addition, epiphyseal lines close at 20 months of age in micro-mini pigs, while in humans, they usually close at 15–17 years of age. To establish an MI model in adult micro-mini pigs, cryoinjury-induced and ameroid constrictor-induced MI have been performed for preclinical studies[19]. Ishigami et al. also induced MI models in micro-mini pigs (weighing 15–25 kg) and demonstrated that the human pluripotent stem

cell (hiPSC)-derived cardiac sheet transplantation significantly improved cardiac function as compared with that of the sham group in 1 month[13]. They also revealed that left ventricular (LV) remodeling was attenuated in the treatment group. However, even with the use of immunosuppressive drugs, it is very difficult to engraft the human cells in pig models. To solve this, we established an athymic micro-mini pig model, a kind of immunodeficiency model[20]. We achieved neonatal thymectomy in infantile micro-mini pigs born via cesarean section and demonstrated that engraftment of transplanted human cells tended to exhibit a longer retention in thymectomized micro-mini pigs. For accurate evaluation of safety and efficacy in human cell transplantation, a thymectomized micro-mini adult pig is expected to be a promising model in the fields of cardiovascular and stem cell research.

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Ethical Approval

Ethical approval to report this case was obtained from the Keio University Institutional Animal Care and Use Committee.

Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with the Institutional Guidelines on Animal Experimentation at Keio University.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: E.K. is a medical advisor of Fuji Micra Inc., Shizuoka, Japan.

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