Biomaterials 147 (2017) 116–132

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Vascular smooth muscle cells derived from inbred swine induced pluripotent stem cells for vascular tissue engineering

Jiesi Luo [a, b], Lingfeng Qin [c], Mehmet H. Kural [d, e], Jonas Schwan [f], Xia Li [a, b], Oscar Bartulos [a, b], Xiao-qiang Cong [a, b, g], Yongming Ren [a, b], Liqiong Gui [d, e], Guangxin Li [c, h], Matthew W. Ellis [a, i], Peining Li [j], Darrell N. Kotton [k], Alan Dardik [c, d], Jordan S. Pober [d, l, m], George Tellides [c, d], Marsha Rolle [n], Stuart Campbell [f], Robert J. Hawley [o], David H. Sachs [o], Laura E. Niklason [b, d, e, f], Yibing Qyang [a, b, d, m, *]

[a] Yale Cardiovascular Research Center, Section of Cardiovascular Medicine, Department of Internal Medicine Yale School of Medicine, New Haven, CT 06511, USA
[b] Yale Stem Cell Center, New Haven, CT 06520, USA
[c] Department of Surgery, Yale University, New Haven, CT 06520, USA
[d] Vascular Biology and Therapeutics Program, Yale University School of Medicine, New Haven, CT 06520, USA
[e] Department of Anesthesiology, Yale University, New Haven, CT 06519, USA
[f] Department of Biomedical Engineering, Yale University, New Haven, CT 06519, USA
[g] Department of Cardiology, Bethune First Hospital of Jilin University, ChangChun, 130021, China
[h] Department of Vascular Surgery, The First Hospital of China Medical University, Shenyang, 110122, China
[i] Department of Cellular and Molecular Physiology, Yale University, New Haven, CT 06519, USA
[j] Department of Genetics, Yale University, New Haven, CT 06519, USA
[k] Center for Regenerative Medicine, Boston University and Boston Medical Center, Boston, MA 02118, USA
[l] Department of Immunobiology, Yale University, New Haven, CT 06520, USA
[m] Department of Pathology, Yale School of Medicine, New Haven, CT 06520, USA
[n] Department of Biomedical Engineering, Worcester Polytechnic Institute, Worcester, MA 01605, USA
[o] Center for Transplantation Sciences, Massachusetts General Hospital, Boston, MA 02129, USA

ARTICLE INFO

Article history:
Received 14 April 2017
Received in revised form
5 September 2017
Accepted 17 September 2017
Available online 19 September 2017

Keywords:
Tissue engineering
Vascular tissue
Induced pluripotent stem cell
Smooth muscle cell
Inbred swine

ABSTRACT

Development of autologous tissue-engineered vascular constructs using vascular smooth muscle cells (VSMCs) derived from human induced pluripotent stem cells (iPSCs) holds great potential in treating patients with vascular disease. However, preclinical, large animal iPSC-based cellular and tissue models are required to evaluate safety and efficacy prior to clinical application. Herein, swine iPSC (siPSC) lines were established by introducing doxycycline-inducible reprogramming factors into fetal fibroblasts from a line of inbred Massachusetts General Hospital miniature swine that accept tissue and organ transplants without immunosuppression within the line. Highly enriched, functional VSMCs were derived from siPSCs based on addition of ascorbic acid and inactivation of reprogramming factor via doxycycline withdrawal. Moreover, siPSC-VSMCs seeded onto biodegradable polyglycolic acid (PGA) scaffolds readily formed vascular tissues, which were implanted subcutaneously into immunodeficient mice and showed further maturation revealed by expression of the mature VSMC marker, smooth muscle myosin heavy chain. Finally, using a robust cellular self-assembly approach, we developed 3D scaffold-free tissue rings from siPSC-VSMCs that showed comparable mechanical properties and contractile function to those developed from swine primary VSMCs. These engineered vascular constructs, prepared from doxycycline-inducible inbred siPSCs, offer new opportunities for preclinical investigation of autologous human iPSC-based vascular tissues for patient treatment.

1. Introduction

Every year millions of patients with cardiovascular disease

* Corresponding author. Yale Cardiovascular Research Center, Section of Cardiovascular Medicine, Department of Internal Medicine Yale School of Medicine, New Haven, CT 06511, USA.
E-mail address: yibing.qyang@yale.edu (Y. Qyang).

http://dx.doi.org/10.1016/j.biomaterials.2017.09.019
0142-9612/

require vascular constructs for bypass surgery or replacement of defective blood vessels in the United States [1–3]_ENREF_18. Tissue-engineered vascular constructs grown using vascular smooth muscle cells (VSMCs) isolated from primary tissue hold great potential as tools for surgical replacement of the affected vessels in these patients [4–6]. However, the development of engineered vascular constructs for clinical application using primary VSMCs has been hampered by limited accessibility to patient VSMCs, finite expandability of primary VSMCs, and functional disparities between VSMCs derived from different donors [5–7].

Human induced pluripotent stem cells (iPSCs) can be derived from patients' own somatic cells by ectopic expression of stem cell factors, self-renew, and differentiate into virtually every cell type in the human body including functional VSMCs, thereby providing an unlimited cell source for generating autologous vascular constructs for disease treatment [8–13]. Human iPSC-based engineered vascular constructs may be of particular importance to patients with dysfunctional vascular cells due to diseases or aging. In such patients, allogeneic, decellularized vascular grafts might not be an optimal treatment option since patients' own defective vascular cells might not effectively remodel the implanted grafts into functional vessels. Our recent studies revealed that human iPSC-based engineered vascular constructs remained intact and were patterned with active vascular remodeling after implantation into rat abdominal aorta, setting the stage for developing iPSC vascular tissue constructs for potential clinical use in the future [13]. However, before therapeutic application of autologous, patient-specific iPSC vascular tissue constructs, it is necessary to establish an autologous, large animal iPSC model to evaluate the safety and efficacy of these iPSC vascular constructs.

Preclinical evaluation of human iPSC-based vascular tissue constructs using a non-human primate model may provide useful information. However, such allogeneic, human to non-human primate implantation requires sustained immunosuppression, and does not mimic clinical application of autologous, patient-specific iPSC-based vascular constructs. Additionally, the non-human primate model may also be limited by significant economic burden and potential challenges of ethical issues. In contrast, swine are an excellent preclinical model for developing therapeutic applications due to their similarity to human physiology and organ size, affordability, and considerably lesser level of ethical concerns [14–16].

Generation of swine iPSCs (siPSCs) has been reported [15,17,18]. However, the continual expression of ectopic reprogramming factors in cells derived from these siPSCs may inhibit cellular differentiation and maturation and increase the risk of tumorigenesis. Additionally, siPSCs generated from swine somatic cells with outbred origin would require sustained immunosuppression when siPSC-derived tissue constructs are implanted *in vivo*, potentially confounding the interpretation of research results. Thus, an inbred siPSC line based on an inducible expression of reprogramming factors is needed to evaluate the safety and efficacy of iPSC-derived therapies.

In this study, we have established the first doxycycline-inducible siPSC lines from fetal fibroblast cells of the Massachusetts General Hospital (MGH) miniature swine that are extensively inbred and therefore accept tissue and organ transplants without immunosuppression [19]. Our results revealed that the use of a doxycycline-inducible approach greatly reduced the adverse effects caused by reprogramming transgenes, since removal of doxycycline during differentiation resulted in elimination of transgene expression and enhancement of differentiation into the three germ lineages. Functional VSMCs differentiated from inbred siPSCs can be utilized to develop vascular tissues for cell-based therapies in a preclinical swine model, which helps to derive fundamental knowledge for clinical application of autologous iPSC-derived VSMC tissue constructs in patients with vascular disease (Supplementary Fig. 1). However, an efficient approach to derive functional siPSC-VSMCs is lacking in the field. Based on the enhanced lineage differentiation via doxycycline withdrawal in combination of the addition of ascorbic acid, we developed a robust approach to derive functional VSMCs from siPSCs, and then generated for the first time swine vascular tissue constructs by either seeding siPSC-VSMCs onto biodegradable polyglycolic acid (PGA) mesh or allowing siPSC-VSMCs to self-assemble into tissue rings in agarose molds. Our studies have laid the groundwork for inbred siPSC-based tissue engineering and implantation studies, which closely resemble patient-specific, autologous iPSC-based therapeutic applications. It is anticipated that the knowledge derived from inbred doxycycline-inducible siPSC model would be readily utilized to guide autologous, patient iPSC-based tissue engineering and therapeutic intervention during human clinical trials (Supplementary Fig. 1), thus providing the field of regenerative medicine with a powerful tool for preclinical investigations.

2. Materials and methods

2.1. Animal use

The study was approved by the Yale University Institutional Animal Care and Use Committee. All animal care complied with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Culture of primary inbred swine embryonic fibroblasts

Swine embryonic fibroblasts (SEFs) (isolated from inbred miniature swine at generation eleven of inbreeding) were provided by Drs. David Sachs and Robert Hawley, Transplantation Biology Research Center, Massachusetts General Hospital, Harvard Medical School, Cambridge, MA [19]. SEFs were cultured in fibroblast medium (Dulbecco's Modified Eagle Medium (DMEM; high glucose, ThermoFisher) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco), 2 mM L-glutamine (ThermoFisher), 1% (v/v) non-essential amino acid (NEAA, ThermoFisher), 1 mM sodium pyruvate (ThermoFisher) and 1% (v/v) penicillin/streptomycin (pen/strep, ThermoFisher)). Medium was changed daily, and the SEFs were routinely passaged at 80% confluence every 4 or 5 days.

2.3. Derivation and maintenance of inbred doxycycline-inducible siPSCs

The plasmid vectors including the Tet-hSTEMCCA-loxP polycistronic cassette encoding human OCT4, SOX2, KLF4 and c-MYC (hSTEMCCA) and the construct encoding the reverse transcription tetracycline activator (rtTA) were provided by Dr. Darrell Kotton, Center for Regenerative Medicine (CReM) of Boston University and Boston Medical Center, Boston, MA. The hSTEMCCA and rtTA vectors were packaged into lentiviral particles and collected as previously described [10]. To generate siPSCs, 8000 SEFs/cm^2 (below passage 3) were seeded onto 6-well dish two days prior to viral infection (day −2). On day 0, fibroblast medium was replaced with medium containing lentiviral particles (1 mL fresh fibroblast medium mixed with 1.4 mL viral supernatant containing hSTEMCCA and 0.6 mL supernatant containing rtTA, plus 5 μg/mL polybrene (SigmaAldrich)). Viral infection was subsequently repeated at 12 h, 24 h and 36 h after the first infection. 48 h after the first infection, medium was switched to pluripotency-promoting medium (Knockout DMEM (ThermoFisher), 10% Knockout Serum Replacement (KOSR, ThermoFisher), 10% (v/v) FBS, 20 ng/mL human leukemia growth factor (LIF, Peprotech), 20 ng/mL human basic