HHS Public Access

Author manuscript

Cell Stem Cell. Author manuscript; available in PMC 2021 February 06.

Published in final edited form as:

Cell Stem Cell. 2020 February 06; 26(2): 136–137. doi:10.1016/j.stem.2020.01.011.

Human iPSCs stretch to improve tissue engineered vascular grafts

Nadia Abutaleb, **George A. Truskey**

Department of Biomedical Engineering, Duke University, Durham, NC 27708

Summary

Human induced pluripotent stem cells (hiPSCs) provide a potentially unlimited cell source for producing autologous tissue-engineered vascular grafts (TEVGs), which currently suffer from low mechanical strength. Luo et al. (2020) describe optimized culture media and a mechanical stretching regiment to produce hiPSC-derived TVEGs with mechanical behavior similar to natural vessels.

With cardiovascular disease ranked as a leading cause of death worldwide, functional vascular grafts are an urgent clinical demand (Wu et al., 2018). Autologous and synthetic grafts are the clinical standard but suffer from several challenges. While often the best choice at present, autologous vascular grafts have limited availability, can undergo restenosis, and cause donor site morbidity (Pashneh-Tala et al., 2016). Synthetic vascular grafts made of polytetrafluoroethylene and polyurethane suffer from long-term calcification and occlusion from thrombosis and are limited to large vessels where thrombosis is minimized (Pashneh-Tala et al., 2016). These challenges have driven interest in tissue engineered vascular grafts (TEVGs) as an attractive alternative to create functional blood vessel replacements *in vitro* that maintain function after implantation.

TEVGs have been fabricated using primary human vascular smooth muscle cells (VSMCs), but these cells are limited in quantity and proliferation potential which affects extracellular matrix (ECM) synthesis and the mechanical strength of TEVGs (Poh et al., 2005). Human induced pluripotent stem cells (hiPSCs) present a potentially unlimited source of cells for TEVGs, but previously reported hiPSC-TEVGs suffer from limited differentiation of VSMCs (Atchison et al., 2017) which affects extracellular matrix synthesis, mechanical strength, and radial dilation after implantation (Elliott et al., 2019; Gui et al., 2016; Sundaram et al., 2014). In a study published in this issue of *Cell Stem Cell*, Luo et al. (2020) addressed these concerns by combining optimized culture media with cyclic mechanical stretching to produce hiPSC-TEVGs with high mechanical strength. The work represents a significant step forward for the field in the ability to generate functional TEVGs using hiPSC-derived cells.

Luo et al. (2020) began by optimizing a method to produce functional hiPSC-derived VSMCs for TEVG production. hiPSC-VSMCs expressed VSMC markers including α-

gtruskey@duke.edu, Tel: 919-660-5147.

smooth muscle actin, calponin, smooth muscle myosin heavy chain 11, and smoothelin, and relevant ECM markers including collagen 1, collagen 3, and elastin. Cell yield from this differentiation method was ñine-fold higher than the group's previously reported approach, and VSMC marker expression was comparable to primary VSMCs.

Based on previous work in which TEVGs cultured in media containing transforming growth factor-β1 (TGF-β1) and platelet-derived growth factor-BB (PDGF-BB) had limited mechanical strength (Gui et al., 2016), the authors modified the TEVG culture medium to enhance differentiation. hiPSC-VSMCs seeded onto polyglycolic acid (PGA) meshes and cultured in media containing TGF-β1 deposited more collagen, while hiPSC-VSMCs on PGA meshes cultured in media containing PDGF-BB had a higher proportion of apoptotic cells, indicating a detrimental effect on hiPSC-VSMC survival. Thus, TEVG medium containing TGF-β1 without PDGF-BB was chosen for optimal TEVG maturation.

To further mature the hiPSC-VSMCs, Luo et al. (2020) found that, as with primary VSMCs, uniaxial mechanical stretching of hiPSC-VSMCs enhanced VSMC and ECM marker expression, collagen deposition, focal adhesion marker expression, and adherens junction marker expression. Stretching also increased formation of filamentous actin bundles and promoted actin alignment perpendicular to the direction of stretching as expected for VSMCs. Stretched VSMCs produced higher levels of ATP, increased glucose consumption, and expressed higher levels of genes related to glucose metabolism. These benefits were further enhanced when the stretching regimen was coupled with the optimized TGF-β1-containing culture medium.

Next, Luo et al. (2020) used the optimized hiPSC-VSMC differentiation protocol, culture medium, and mechanical maturation regimen to fabricate TEVGs. PGA scaffolds were seeded with hiPSC-VSMCs and cultured statically for one week, then pulsatile radial stress was applied for seven weeks. At the end of this regimen, hiPSC-TEVG opaqueness, rupture pressure, and suture retention strength were comparable to native human saphenous veins used for autologous grafts and higher than human umbilical arteries (HUAs). hiPSC-TEVG wall thickness and collagen content was comparable to both HUAs and primary human TEVGs. hiPSC-TEVGs were also highly cellularized, expressed VSMC marker levels similar to native HUAs, and did not show any evidence of calcium deposition. They did not contain mature ELN fibers, which is consistent with previous reports of TEVGs made with both primary and hiPSC-derived VSMCs (Gui et al., 2016; Niklason et al., 1999). In comparison with previously reported hiPSC-TEVGs, these engineered vessels exhibit marked improvement in suture retention strength, nearly two-fold increase in rupture pressure, and ~3.5-fold increase in collagen content (Gui et al., 2016; Sundaram et al., 2014).

To evaluate *in vivo* potential, hiPSC-TEVGs were implanted as interpositional grafts into the abdominal aorta of nude rats. After 30 days, explanted grafts were all patent with minimal thrombosis and no evidence of rupture, aberrant deformation, radial dilation, longitudinal elongation, or wall thickening. No significant differences were observed in mechanical properties of explanted TEVGs compared with TEVGs prior to implantation, including maximum modulus, ultimate tensile strength, failure strain, and contractile function.

Explanted TEVGs showed no signs of calcification, maintained cellularity, expressed VSMC markers, and contained abundant collagen. However, mature ELN fibers were not detected and require further study. Contrary to pre-implantation TEVGs, most cells in explanted TEVGs were not positive for proliferation marker Ki67, indicating a shift to quiescence after implantation. Very few CD68+ cells were observed near explanted TEVGs, indicating minimal to no macrophage infiltration. To resist thrombosis and improve biocompatibility, TEVGs should promote luminal endothelialization (Wu et al., 2018). Notably, while implanted grafts were not pre-endothelialized, some portions of the luminal surface of explanted TEVGs showed coverage by host endothelial cells. Successful endothelialization is needed for longer-term function of the TEVGs.

The study by Luo et al. (2020) represents the most mechanically robust hiPSC-TEVGs reported to date, with properties approaching those of native human vessels. A high-yield differentiation protocol combined with optimized culture medium and a cyclic stretching regimen enhanced mechanical properties of the engineered grafts far beyond the field standard. This mechanical strength contributed to the success of the implanted TEVGs, showing high patency, no dilation or elongation, and maintained morphology and function after 30 days. To take the work a step further, Luo et al. (2020) included data showing successful differentiation of VSMCs from an HLA-engineered universally immunocompatible hiPSC line, with differentiated cells exhibiting VSMC and ECM marker expression, contractile function, and collagen deposition. Fully developing a ready supply of immune compatible cells would further advance this technology.

Future studies should compare the gene expression profile of the hiPSC-VSMCs in the TEVGs with primary VSMCs to identify which pathways may need to be stimulated to more closely mimic the *in vivo* state. Because of the importance of the construct, these hiPSC-VSMCs should be examined in TEVGs made with synthetic or ECM scaffold. Nonetheless, this work represents an important advance for functional TEVGs using hiPSC-derived cells and illuminates the possibility of large-scale production of hiPSC-TEVGs as an effective clinical therapy.

References

Atchison L, Zhang H, Cao K, and Truskey GA (2017). A Tissue Engineered Blood Vessel Model of Hutchinson-Gilford Progeria Syndrome Using Human iPSC-derived Smooth Muscle Cells. Sci. Rep. 7, 8168. [PubMed: 28811655]

Elliott MB, Ginn B, Fukunishi T, Bedja D, Suresh A, Chen T, Inoue T, Dietz HC, Santhanam L, Mao HQ, et al. (2019). Regenerative and durable small-diameter graft as an arterial conduit. Proc. Natl. Acad. Sci. U. S. A. 116, 12710–12719.

Gui L, Dash BC, Luo J, Qin L, Zhao L, Yamamoto K, Hashimoto T, Wu H, Dardik A, Tellides G, et al. (2016). Implantable Tissue-Engineered Blood Vessels from Human Induced Pluripotent Stem Cells. Biomaterials 102, 120–129. [PubMed: 27336184]

Luo J, Qin L, Zhao L, Gui L, Ellis MW, Huang Y, Kural MH, Clark JA, Ono S, Wang J, et al. (2020). Tissue Engineered Vascular Grafts with Advanced Mechanical Strength from Human Induced Pluripotent Stem Cells. Cell Stem Cell.

Niklason LE, Gao J, Abbott WM, Hirschi KK, Houser S, Marini R, and Langer R (1999). Functional arteries grown in vitro. Science. 284, 489–493. [PubMed: 10205057]

Pashneh-Tala S, MacNeil S, and Claeyssens F (2016). The Tissue-Engineered Vascular Graft-Past, Present, and Future. Tissue Eng. Part B 22, 68–100.

Poh M, Banik SSR, Mckee Bse JA, Klinger RY, Niklason LE, Poh M, Boyer M, Solan A, Dahl SLM, Pedrotty D, et al. (2005). Blood vessels engineered from human cells. Lancet 365, 2122–2124. [PubMed: 15964449]

Sundaram S, One J, Siewert J, Teodosescu S, Zhao L, Dimitrievska S, Qian H, Huang AH, and Niklason L (2014). Tissue-Engineered Vascular Grafts Created From Human Induced Pluripotent Stem Cells. Stem Cells Transl. Med. 3, 1535–1543. [PubMed: 25378654]

Wu J, Hu C, Tang Z, Yu Q, Liu X, and Chen H (2018). Tissue-engineered Vascular Grafts: Balance of the Four Major Requirements. Colloids Interface Sci. Commun. 23, 34–44.