STEMdiff™ Blood Vessel Organoid Kit Supports Efficient Generation of Vascular Organoids from Human Pluripotent Stem Cells

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INTRODUCTION

Blood vessels are a fundamental part of all organ systems and have critical roles in multiple diseases, including diabetes, atherosclerosis, and cancer. The blood vasculature is composed of endothelial cells that form luminal tubes, and pericytes covering the endothelial wall. In vitro models of vascular biology involve co-culturing endothelial cells with pericytes but do not fully recapitulate their three-dimensional (3D) organization and functionality. A novel culture system where human pluripotent stem cell (hPSC)-derived blood vessel organoids are used to model the structural and functional features of blood vasculature was recently reported.[1,2] Based on these publications, we have developed the STEMdiff™ Human Blood Vessel Organoid Kit to enable robust and reproducible generation of blood vessel organoids in culture.

METHODS

Aggregates derived from hPSC lines are first generated in STEMdiff™ Blood Vessel Organoid Aggregation Medium, followed by generation of mesoderm and vascular progenitor cells using STEMdiff™ Blood Vessel Organoid Mesoderm and Vascular Induction Media, respectively. Vascular aggregates sprout into vascular networks and mature into stable blood vessels when grown within the extracellular matrix in STEMdiff™ Blood Vessel Maturation Medium. Vascular networks were exposed to high-glucose diabetic-mimicking conditions at day 8 - 9 and deposition of extracellular matrix was evaluated at day 15 - 18.

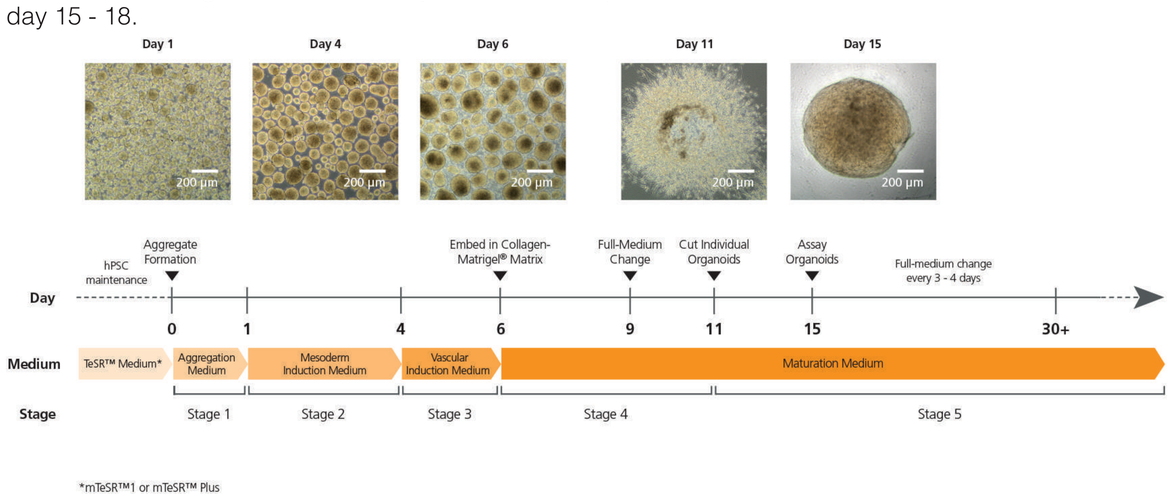

FIGURE 1. STEMdiff™ Blood Vessel Organoid Kit Protocol

mTeSR™1-maintained hPSCs are seeded as single cells at 0.2 - 0.4 x 10^6 cells/well and mTeSR™ Plus-maintained hPSCs are seeded as single cells at 0.1 - 0.2 x 10^6 cells/well in 6-well ultra-low attachment plates in STEMdiff™ Blood Vessel Organoid Aggregation Medium, then incubated at 37°C for 1 or 2 days. Differentiation is then initiated by replacing the medium with STEMdiff™ Blood Vessel Organoid Mesoderm Medium and continuing incubation for 3 days. On day 4, vascular induction is initiated by replacing the medium with STEMdiff™ Blood Vessel Organoid Vascular Induction Medium, then incubating at 37°C for 2 days. Vascular aggregates are then embedded in a Matrigel®/collagen sandwich; aggregates sprout into vascular networks and mature into stable blood vessels when grown in STEMdiff™ Blood Vessel Organoid Maturation Medium for 5 days. Percentage of endothelial cells (CD31⁺ and CD144⁺) and pericytes (CD140b⁺) was evaluated via flow cytometry. Formation of lumen and 3D organization of endothelial cells and pericytes were analyzed using a confocal microscope.

RESULTS

FIGURE 2. Vascular Aggregates Sprout Into Vascular Networks and Form Mature Blood Vessel Organoids in STEMdiff™ Blood Vessel Maturation Medium

(A) Vascular aggregates sprout into vascular networks after 5 days in STEMdiff™ Blood Vessel Maturation Medium and a Matrigel®/collagen sandwich. Vascular sproutings mature into blood vessel organoids when cultured in STEMdiff™ Blood Vessel Maturation Medium. Magnification: 10X; Insert: 25X; anti-human CD31 (**red**).
(B) 3D reconstruction of z stack planes shows complex vasculature is formed after 22 days in STEMdiff™ Blood Vessel Maturation Medium and free-floating conditions. Magnification: 10X; anti-human CD31 (**green**).

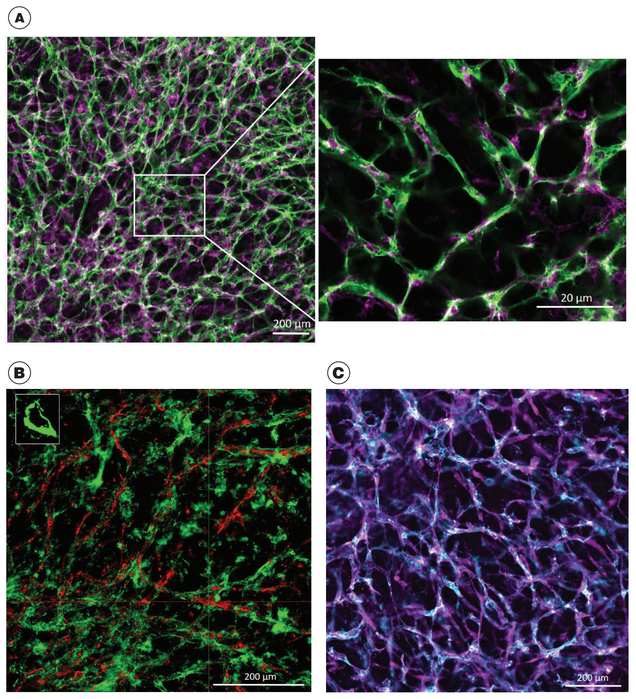

FIGURE 3. Vascular Networks Mature into Stable Blood Vessels When Cultured Within the Extracellular Matrix in STEMdiff™ Blood Vessel Maturation Medium

(A) hPSC-derived blood vessel organoids are composed of hCD31⁺ cells (**green**) and hPDGFRβ⁺ cells (**magenta**); small quadrant shows tight endothelial and pericyte interactions. **(B)** hPSC-derived blood vessel organoids are composed of hCD31⁺ cells (**red**) and deposited collagen IV (**green**) (3D reconstruction of optical Z stacks); small quadrant shows blood vessel lumen.
(C) hPSC-derived blood vessel organoids are composed of hCD31⁺ cells (**blue**) and alpha-smooth muscle actin cells (**magenta**).

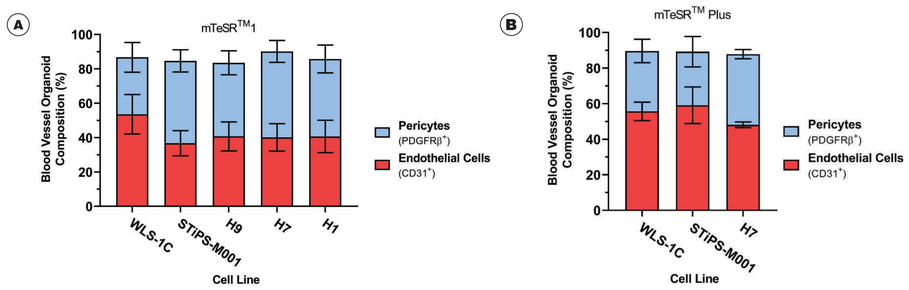

FIGURE 4. Blood Vessel Organoids Contain Both Endothelial Cells and Pericytes

(A) Induced pluripotent stem (iPS) and embryonic stem (ES) cell-derived blood vessel organoids contain endothelial cells (42.92 ± 3.97% CD31⁺, n=3 - 4) and pericytes (43.10 ± 3.31% CD140b⁺, n=3 - 4) in mTeSR™1-maintained cell lines WLS-1C (iPS), STiPS-M001 (iPS), H9 (ES), H7 (ES), and H1 (ES).
(B) iPS and ES cell-derived blood vessel organoids contain endothelial cells (54.29 ± 3.72% CD31⁺, n=3) and pericytes (34.58 ± 3.49% CD140b⁺, n=3) in mTeSR™ Plus-maintained cell lines WLS-1C (iPS), STiPS-M001 (iPS), and H1 (ES).

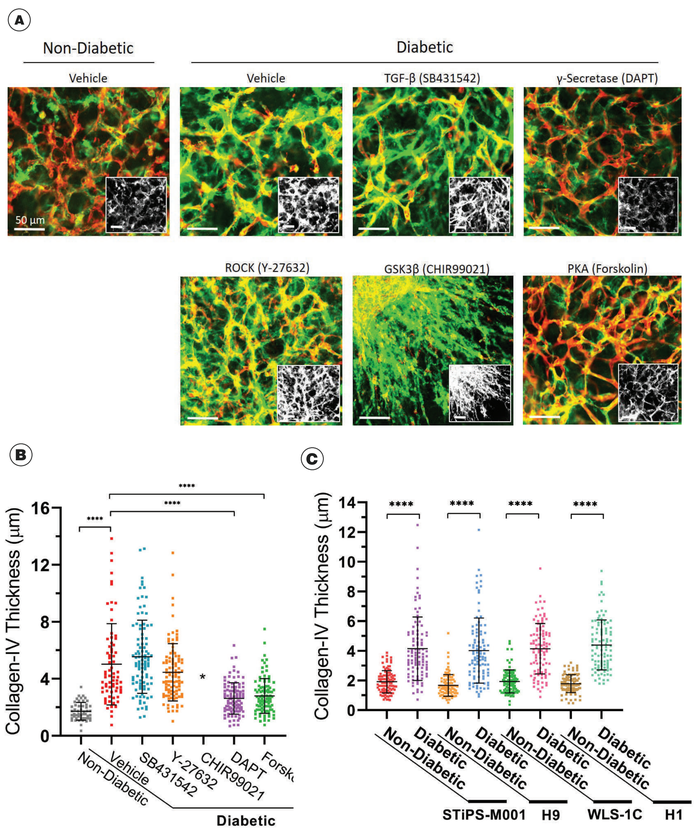

FIGURE 5. Deposition of Extracellular Matrix

(A) Diabetic conditions were applied to vascular networks, then collagen IV deposition was evaluated. Confocal microscopy revealed the formation of a complex network of tubes composed of CD31⁺ cells (**red**). Increased expression of collagen IV (**green**) was found in diabetic vascular networks compared to non-diabetic. Multiple small molecules were used in diabetic organoids to block signaling pathways involved in diabetic vasculopathies. Only addition of γ-secretase inhibitor DAPT and protein kinase activator (PKA) forskolin significantly reduced expansion of the collagen IV basement membrane. **(B)** The thickness of the collagen IV coat of individual vessels was measured in optical cross-sections (n=50 for non-diabetic, n=73 for diabetic (vehicle) & n=100 for diabetic (SB431542, Y-27632, CHIR99021, forskolin, and DAPT) blood vessel lumina from one independent experiment. Data are mean ± SD *P < 0.0001; two-tailed Student's t-test). **(C)** The thickness of the collagen IV coat of individual vessels was measured in optical cross-sections (n=100 for non-diabetic & n=100 for diabetic blood vessel lumina from one independent experiment in two iPS cell lines & two ES cell lines. Data are mean ± SD *P < 0.0001; two-tailed Student's t-test).

Summary

These data demonstrate that STEMdiff™ Blood Vessel Organoid Kit supports:

- Reproducible generation of blood vessel organoids from multiple ES and iPS cell lines
- Formation of mature blood vessel organoids containing endothelial cells forming a lumen surrounded by perivascular cells
- Generation of blood vessel organoids that can be used to model diabetic conditions in vitro

References
1: Wimmer RA et al. (2019) Nat Protoc 14(11): 3082–100.
2: Wimmer RA et al. (2019) Nature 565 (7740): 505–10.