Development of a Novel Serum-free Directed Differentiation Protocol for Functional hiPSC-derived Endothelial Cells

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Abstract

Endothelial cells are specialized cells that line the interior surface of blood vessels, forming a dynamic and selective barrier between the bloodstream and surrounding tissues, thereby playing a central role in regulating tissue metabolism and physiology. Induced pluripotent stem cell (iPSC)–derived endothelial cells have broad applications in disease modeling, drug discovery, toxicity screening, regenerative medicine, and tissue engineering, enabling the study and restoration of vascular function in both research and therapeutic contexts. Here, we implemented High-Dimensional Design-of-Experiments (HD-DoE®) to develop a reproducible, serum-free differentiation protocol that can generate highly pure, functional endothelial cells. These cells maintained high viability (>80%) after cryopreservation. Flow cytometry analysis confirmed the identity of the cells, with endothelial markers such as CD31, CD144, CD34, KDR and CD105 all above 90%. Expression of non-endothelial markers such as PDGFRβ (pericytes), CD324 (epithelial) and Tra1-60 (undifferentiated) were all below 5%. Function of endothelial cells was demonstrated through Matrigel® tube formation, transwell invasion, transwell migration, and ac-LDL uptake assays. The cells were also responsive to inflammatory stimuli, as demonstrated by upregulation of adhesion molecules E-selectin and ICAM-1 (38- and 70-fold increases, respectively) upon 25ng/mL TNFα stimulation for 24h. To support proliferation of these cells, we evaluated expansion of TrailBio® Endothelial Cells in both commercially available VascuLife® media and internally developed TrailBio® Endothelial Medium. In both media conditions, cells expanded an average 4.3-fold per passage while maintaining high endothelial marker expression (93% CD31+), low PDGFRβ expression (4%), and functionality including Ac-LDL uptake and Matrigel tube formation over at least three passages. Overall, TrailBio® Endothelial Cells provide a reliable, scalable, and functional platform for vascular research, drug discovery, and regenerative applications.

1. Endothelial cell differentiation

2. Highly pure endothelial cells ready for immediate use

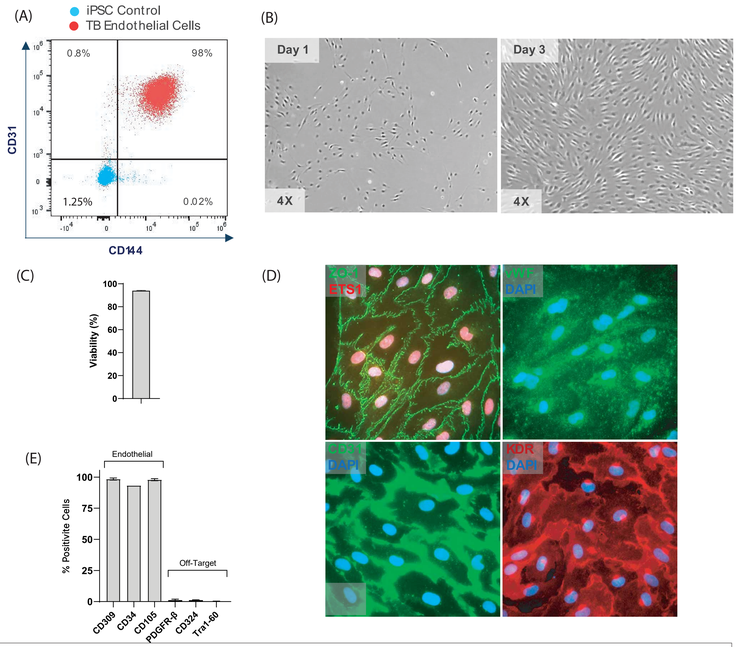

Fig 1. (A) Flow cytometry shows >98% of TrailBio® Endothelial Cells co-express CD31 and CD144, confirming a highly pure endothelial population. (B) Cells rapidly recover endothelial morphology within 24 hours post plating and reach confluence by day 3. (C) Cells demonstrate >90% viability post-thaw (n=3). (D) Immunofluorescent staining confirms expression of endothelial and maturation-associated markers, including ZO-1, vWF, CD31, and KDR. (E) Additional endothelial markers (CD309, CD34, CD105) are highly expressed, while off-target markers (PDGFRβ, CD324, TRA-1-60) remain minimal.

3. Robust endothelial function including migration, invasion and tube formation

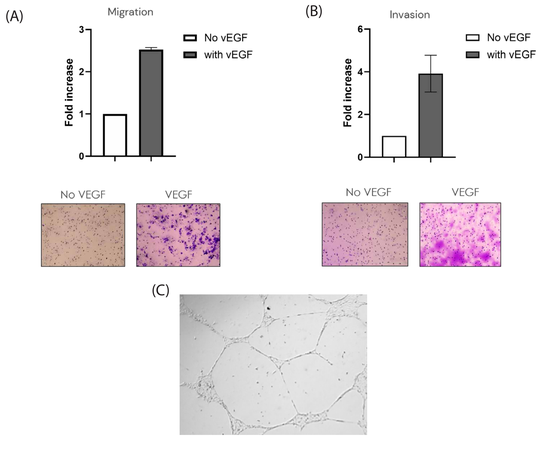

Fig 2. (A) In transwell migration assays, cells demonstrate a ~2.5-fold increase in migration in response to VEGF compared to control conditions, visualized by crystal violet staining of migrated cells (n=2). (B) In Matrigel-coated invasion assays, cells show ~4-fold increased invasion in response to VEGF, with invading cells detected by crystal violet staining, indicating intact proteolytic and invasive capacity. (C) TrailBio®Endothelial Cells form interconnected tubule networks on Matrigel, recapitulating key aspects of vascular network formation. Tube formation was observed as early as 4 hours post-seeding, with more extensive network development by 24 hours.

4. Physiologically relevant inflammatory activation and endothelial uptake function

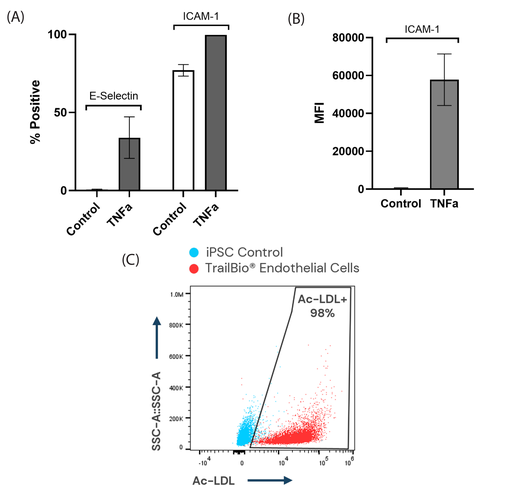

Fig 3. (A) Following TNF-α stimulation (25 ng/mL, 24 hours), TrailBio® Endothelial Cells show increased expression of E-selectin and ICAM-1 compared to control, indicating activation of endothelial adhesion pathways. (B) ICAM-1 expression intensity (MFI), as measured by flow cytometry, also increases following TNF-α stimulation, indicating increased ICAM-1 expression per cell. (C) Flow cytometry shows ≥98% of cells uptake Ac-LDL, confirming intact scavenger receptor activity.

5. Endothelial identity and function are preserved after 3 passages

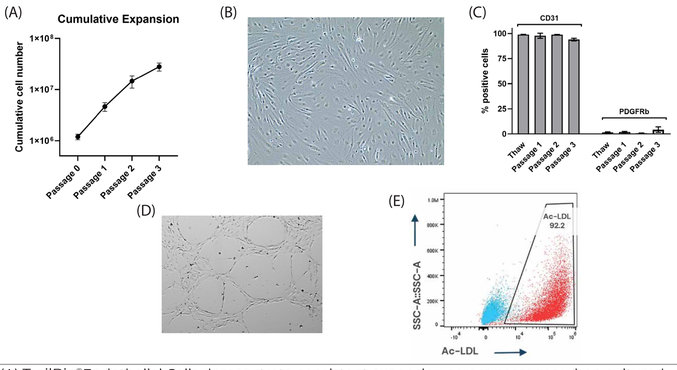

Fig 4.(A) TrailBio®Endothelial Cells demonstrate consistent expansion across passages when cultured in VascuLife® VEGF Endothelial Medium . Cells are passaged every 3-4 days to ensure cultures do not become overconfluent. (B) Cells retain characteristic endothelial cobblestone morphology following expansion. (C) Flow cytometry from thaw through Passage 3 shows that CD31 expression remains high (≥90%) while PDGFRβ expression remains low (<5%) (n=3), indicating stable endothelial identity during expansion. (D) Expanded cells retain the ability to form interconnected tubule networks. (E) Expanded cells maintain Ac-LDL uptake, confirming preserved endothelial function.

6. Summary

- **Physiologically Relevant:** iPSC-derived endothelial cells provide a reproducible model for vascular research.
- **Highly Pure:** >98% CD31/CD144 co-expression immediately post-thaw.
- **Ready-to-Use:** Cryopreserved cells recover full functionality rapidly upon thawing.
- **Stable Identity:** Maintain cobblestone morphology, tubule formation, and marker purity (CD31+/PDGFRβ-) across 3 passages.
- **Functional Activity:** Verified Ac-LDL uptake, robust tube formation, and VEGF-driven migration/invasion.
- **Inflammatory Response:** Rapid upregulation of ICAM-1 and E-selectin post-stimulation.
- **Reproducible:** Developed using HD-DoE® to minimize lot-to-lot variability.