A Hypoimmune Pluripotent Stem Cell Platform Enabling Vascularized Cellular Therapeutics for Catastrophic Extremity Injury

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Introduction

Catastrophic extremity injuries remain a major source of morbidity among warfighters, resulting in limb loss or complex reconstructive procedures with limited long-term success. Effective cell therapies require both restoration of vascular function to support tissue repair and durable integration of therapeutic cells. However, immune-mediated injury remains a central barrier, compromising graft survival and vascular stability. Current approaches rely on systemic immunosuppression, which carries substantial risks and is not scalable for widespread clinical deployment.

We developed a hypoimmune pluripotent stem cell (PSC) platform to enable vascularized cellular therapeutics that both repair injured tissue and evade immune rejection without the need for immunosuppression. Our approach is distinct from conventional strategies that broadly disrupt antigen presentation. Instead, we implement targeted engineering of adhesion pathways, specifically via ICAM-1 disruption, to reduce innate and adaptive immune cell engagement while preserving cellular identity and function.

Using PSC-derived endothelial cells (ECs, the primary interface for the immune system), we demonstrate that this platform technology supports key functional properties required for vascular repair, including barrier formation and compatibility with tissue integration. In parallel, hypoimmune ECs attenuate both adaptive and innate immune responses, exhibiting reduced T cell adhesion, activation, and proliferation, as well as diminished neutrophil activation and oxidative responses under physiologically relevant flow conditions. These effects are particularly relevant in traumatic injury, where neutrophils are early responders that drive inflammation, vascular dysfunction, and graft failure.

This platform enables ECs to serve both as direct allogeneic vascular therapeutics to improve perfusion and tissue salvage and as foundational components of multicellular grafts for complex tissue reconstruction in the future. More broadly, this work establishes a modular strategy for generating immune-compatible, functional cellular therapies that directly address a critical limitation in regenerative medicine for trauma.

By combining restoration of vascular function with targeted immune modulation, this approach has the potential to improve outcomes following catastrophic extremity injury and expand the feasibility of advanced reconstructive strategies relevant to military medicine.

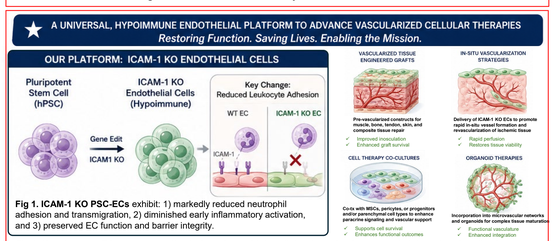

Fig 1. ICAM-1 KO PSC-ECs exhibit: 1) markedly reduced neutrophil adhesion and transmigration, 2) diminished early inflammatory activation, and 3) preserved EC function and barrier integrity.

Fig 3. Hypoimmune ECs are a versatile platform for multiple cellular therapies for traumatic injury. As a front line for immune cell encounter, hypoimmune ECs can be incorporated into multiple PSC-derived regenerative cellular therapy candidates to protect the graft from early innate, as well as longer-term adaptive immune responses.[3] Representative therapies are shown.

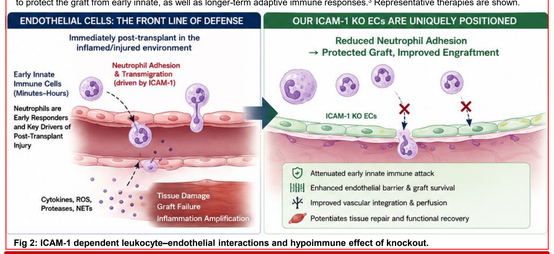

Fig 2: ICAM-1 dependent leukocyte–endothelial interactions and hypoimmune effect of knockout.

Methods

Flow cytometry, cell-cell adhesion assay, biofabrication, fluorescence microscopy (IF), mouse hind-limb injection, bioluminescence imaging (BLI).

Discussion

ICAM-1 KO hypoimmune PSC-derived ECs hold great promise for improving graft survival in PSC-based vascular therapies, as they:

- Maintain key endothelial functions (acetylated-LDL uptake, nitric oxide production, cord formation) (Fig. 4B-D).
- Attenuate cytotoxic T cell responses *in vitro* (Fig. 6).
- Reduce neutrophil adhesion and transmigration *in vitro* under physiologically relevant conditions (Fig. 7-8)
- Reduce neutrophil-derived ROS oxidative stress responses (Fig. 9).

Acknowledgements

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References: 1) Hu et al. 2023 *Nature Biotechnology;* **2)** Goushki et al. 2024 *Stem Cell Research & Therapy;* **3)** Saha et al. 2025 *Nature Communications* **4)** *Jose et al. 2017 Adv Healthc Mater*

Results

ICAM-1 KO PSCs differentiate into functional endothelial cells (PSC-ECs) that lack surface and soluble ICAM-1 expression

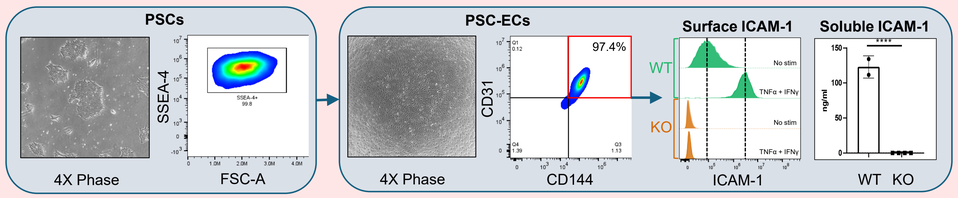

Figure 4: (A) Overview of the differentiation of pluripotent stem cells (PSCs) into endothelial cells (ECs). PSCs first undergo mesoderm induction driven by activation of WNT and TGF-β/BMP signaling, followed by specification into vascular progenitors. At the vascular progenitor stage, CD34⁺ cells are enriched by magnetic-activated cell sorting (MACS). Purified CD34⁺ cells are subsequently exposed to pro-angiogenic cues, such as VEGF and FGF, to promote endothelial maturation. Mature endothelial cells express high levels of canonical endothelial markers CD31 (PECAM1), CD144 (VE-cadherin). ICAM-1 knockout PSC-derived ECs lack both membrane-bound and soluble ICAM-1 expression, confirmed by flow cytometry and Luminex-based analysis. *Legend continued in next panel, right.*

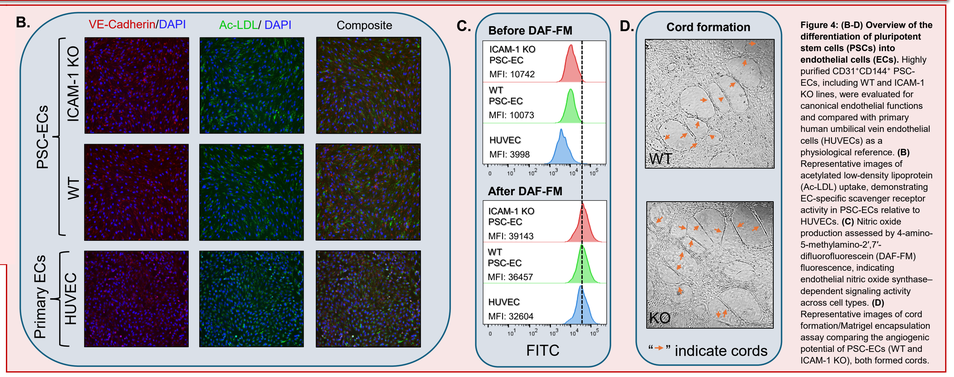

Figure 4: (B-D) Overview of the differentiation of pluripotent stem cells (PSCs) into endothelial cells (ECs). Highly purified CD31⁺CD144⁺ PSC-ECs, including WT and ICAM-1 KO lines, were evaluated for canonical endothelial functions and compared with primary human umbilical vein endothelial cells (HUVECs) as a physiological reference. **(B)** Representative images of acetylated low-density lipoprotein (Ac-LDL) uptake, demonstrating EC-specific scavenger receptor activity in PSC-ECs relative to HUVECs. **(C)** Nitric oxide production assessed by 4-amino-5-methylamino-2',7'-difluorofluorescein (DAF-FM) fluorescence, indicating endothelial nitric oxide synthase–dependent signaling activity across cell types. **(D)** Representative images of cord formation/Matrigel encapsulation assay comparing the angiogenic potential of PSC-ECs (WT and ICAM-1 KO), both formed cords.

ICAM-1 KO PSC-ECs form 3D lumens *in vitro*

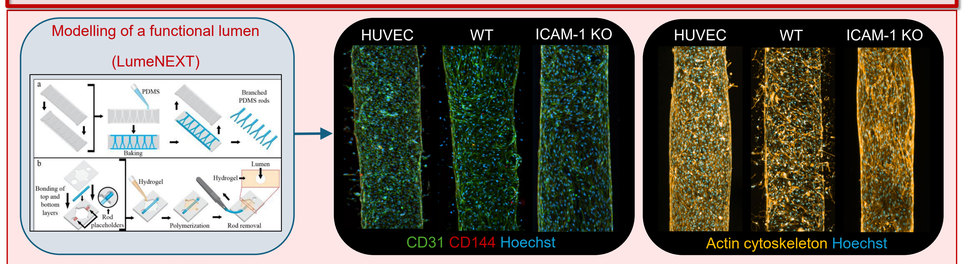

Fig 5: Assembly of LumeNEXT devices.[4] A lumen mold was filled with PDMS to create the top and bottom layers of the device. A PDMS rod was placed between the two layers, and the open chamber was filled with collagen. The rod was removed leaving an opening where ECs can be seeded to create a 3D model of a lumen. The 3D vessel was characterized by staining for canonical endothelial markers (CD31 and CD144) and endothelial actin cytoskeleton.

Absence of ICAM-1 signal from PSC-ECs results in lower clonal proliferation and activation of alloreactive T cells

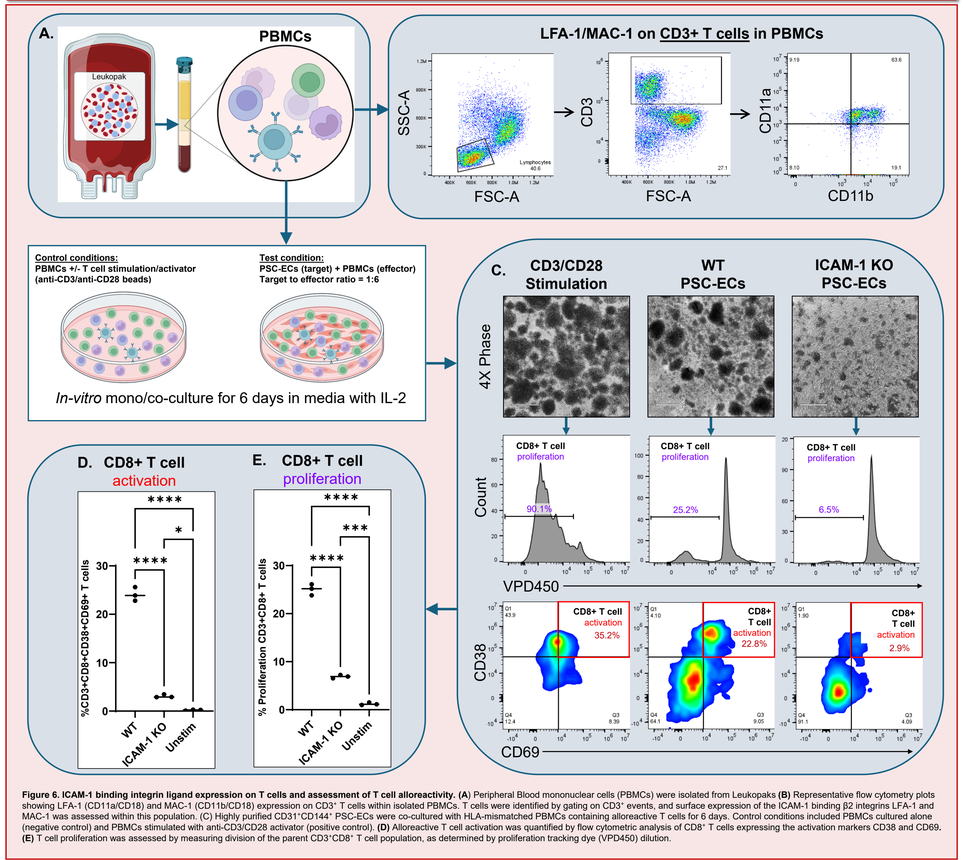

Figure 6. ICAM-1 binding integrin ligand expression on T cells and assessment of T cell alloreactivity. (A) Peripheral Blood mononuclear cells (PBMCs) were isolated from Leukopaks **(B)** Representative flow cytometry plots showing LFA-1 (CD11a/CD18) and MAC-1 (CD11b/CD18) expression on CD3⁺ T cells within isolated PBMCs. T cells were identified by gating on CD3⁺ events, and surface expression of the ICAM-1 binding β2 integrins LFA-1 and MAC-1 was assessed within this population. (C) Highly purified CD31⁺CD144⁺ PSC-ECs were co-cultured with HLA-mismatched PBMCs containing alloreactive T cells for 6 days. Control conditions included PBMCs cultured alone (negative control) and PBMCs stimulated with anti-CD3/CD28 activator (positive control). **(D)** Alloreactive T cell activation was quantified by flow cytometric analysis of CD8⁺ T cells expressing the activation markers CD38 and CD69. **(E)** T cell proliferation was assessed by measuring division of the parent CD3⁺CD8⁺ T cell population, as determined by proliferation tracking dye (VPD450) dilution.

Lack of surface ICAM-1 in PSC-ECs result in diminished firm adhesion of neutrophils to the EC surface under dynamic shear flow

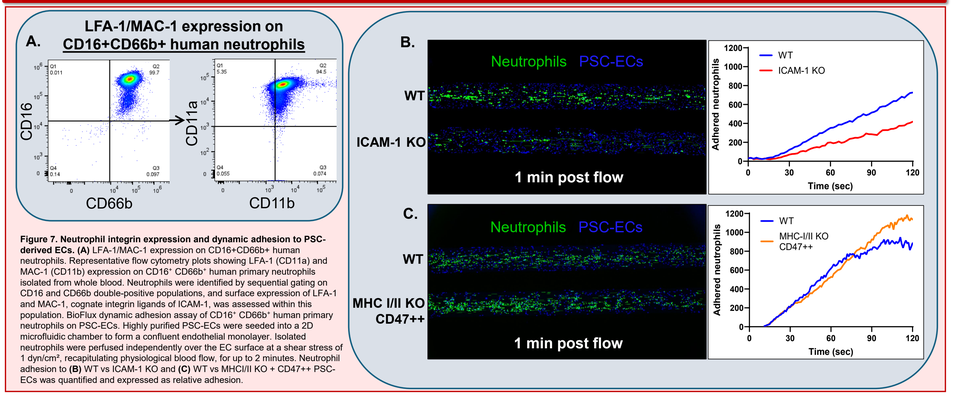

Figure 7. Neutrophil integrin expression and dynamic adhesion to PSC-derived ECs. (A) LFA-1/MAC-1 expression on CD16⁺CD66b⁺ human neutrophils. Representative flow cytometry plots showing LFA-1 (CD11a) and MAC-1 (CD11b) expression on CD16⁺ CD66b⁺ human primary neutrophils isolated from whole blood. Neutrophils were identified by sequential gating on CD16 and CD66b double-positive populations, and surface expression of LFA-1 and MAC-1, cognate integrin ligands of ICAM-1, was assessed within this population. BioFlux dynamic adhesion assay of CD16⁺ CD66b⁺ human primary neutrophils on PSC-ECs. Highly purified PSC-ECs were seeded into a 2D microfluidic chamber to form a confluent endothelial monolayer. Isolated neutrophils were perfused independently over the EC surface at a shear stress of 1 dyn/cm², recapitulating physiological blood flow, for up to 2 minutes. Neutrophil adhesion to **(B)** WT vs ICAM-1 KO and **(C)** WT vs MHCI/II KO + CD47++ PSC-ECs was quantified and expressed as relative adhesion.

ICAM-1 KO reduces neutrophil transmigration across PSC-ECs

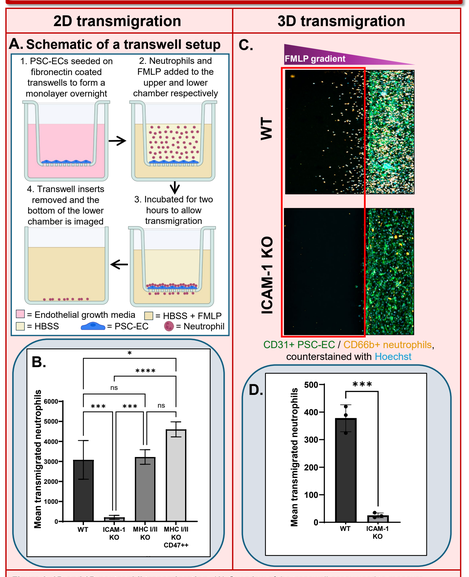

Figure 8. 2D and 3D neutrophil transmigration. (A) Overview of the transwell assay used to measure neutrophil migration across TNFα activated PSC-EC monolayers toward chemotactic (FMLP) gradient. **(B)** Quantification of transmigrated neutrophils. **(C)** Representative image of transmigrated CD66b⁺ neutrophils (orange) 12 h after extravasation across a 3D PSC-EC lumen (CD31⁺, green). Lumens assembled as shown in Fig 5. The transmigrated region (collagen matrix) is outlined in red. **(D)** Quantification of migrated neutrophils.

ICAM-1 KO reduces oxidative stress in neutrophils co-cultured with PSC-ECs

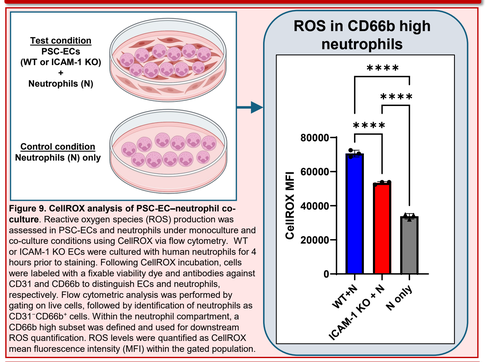

Figure 9. CellROX analysis of PSC-EC–neutrophil co-culture. Reactive oxygen species (ROS) production was assessed in PSC-ECs and neutrophils under monoculture and co-culture conditions using CellROX via flow cytometry. WT or ICAM-1 KO ECs were cultured with human neutrophils for 4 hours prior to staining. Following CellROX incubation, cells were labeled with a fixable viability dye and antibodies against CD31 and CD66b to distinguish ECs and neutrophils, respectively. Flow cytometric analysis was performed by gating on live cells, followed by identification of neutrophils as CD31⁻ CD66b⁺ cells. Within the neutrophil compartment, a CD66b high subset was defined and used for downstream ROS quantification. ROS levels were quantified as CellROX mean fluorescence intensity (MFI) within the gated population.

ICAM-1 KO PSC-ECs engraft similarly to WT counterpart

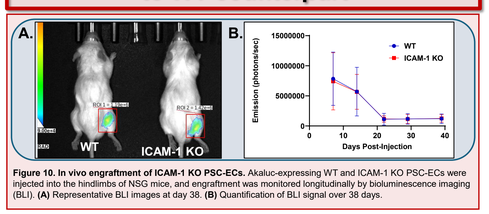

Figure 10. In vivo engraftment of ICAM-1 KO PSC-ECs. Akaluc-expressing WT and ICAM-1 KO PSC-ECs were injected into the hindlimbs of NSG mice, and engraftment was monitored longitudinally by bioluminescence imaging (BLI). **(A)** Representative BLI images at day 38. **(B)** Quantification of BLI signal over 38 days.