Rapid, autologous, and confluent endothelialization yields enhanced functionality and hemocompatibility of co-cultured vascular wall constructs

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Introduction

- Stable, mature endothelial monolayers prevent thrombosis and mediate the vasoactivity of arteries [1].
- Endothelialized grafts have been shown to remain patent for longer than non-endothelialized synthetic grafts made of stiff materials such as polyethylene terephthalate [2,3].

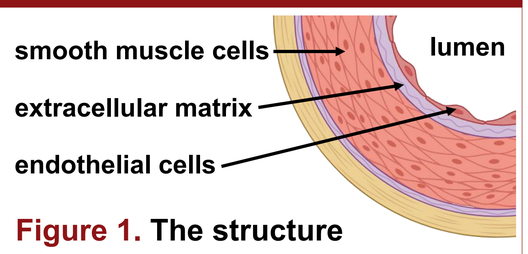
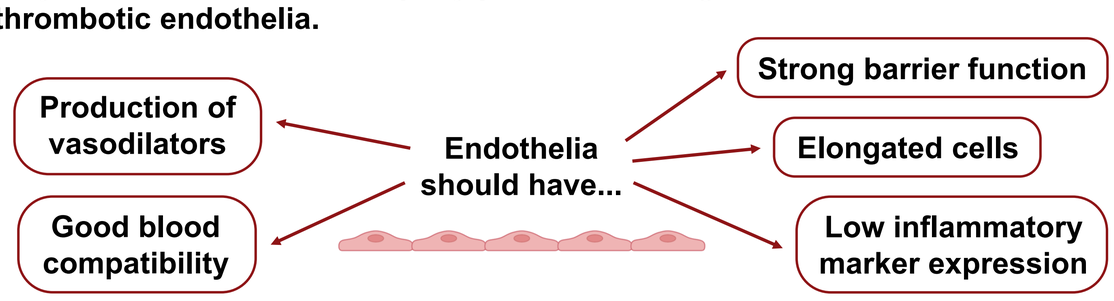


Figure 1. The structure of the arterial wall in the body.

- Sourcing expandable and autologous endothelial cells (ECs) has posed a consistent challenge.
- Adipose tissue is an abundant and dispensable source of human adipose tissue-derived microvascular ECs (HAMVECs) and adipose tissue-derived stromal cells (ASC) [4].
- HAMVECs alone take 14 days to fully endothelialize a polyurethane scaffold
- A confluent endothelium can be produced within 2 days with contralateral co-culture of ASCs and HAMVECs across a polyurethane scaffold [5].

Hypothesis and Aims

HAMVECs and ASC can be indirectly co-cultured across porous, electrospun, polyurethane scaffolds to rapidly produce autologous, non-activated, and non-thrombotic endothelia.



Methodology

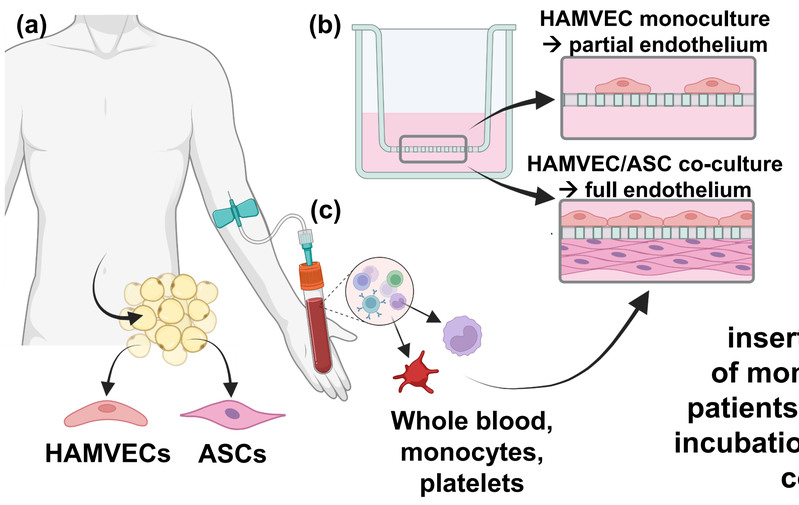
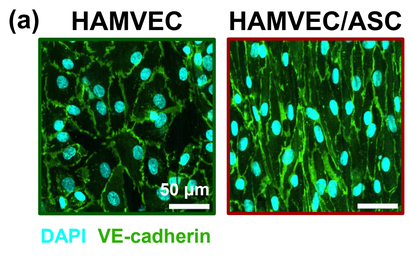


Figure 2. (a) HAMVEC and ASC isolation from patient adipose tissue; (b) Mono-culture of HAMVECs vs. co-culture of HAMVECs and ASCs across nanofibrous polyurethane scaffolds (adhered to Transwell inserts) for 7 days; (c) Isolation of monocytes and platelets from patients' whole blood, followed by incubation of whole blood or blood constituents on endothelia.

Results

Co-cultured HAMVECs display increased barrier function vs. monocultured HAMVECs at 14 days, and co-cultured HAMVECs are more elongated by 7 days of culture



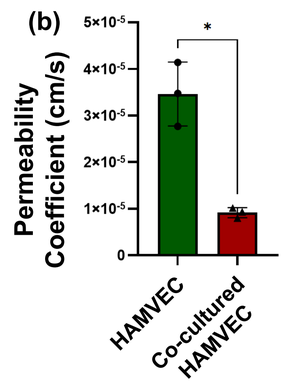


Figure 3. (a) *HAMVEC coverage in mono- and co-culture (7 days);* (b) *Permeability coefficients of mono- and co-cultured HAMVECs after 14 days, N=3, n=2, paired t-test used, * indicates p<0.05.*

Co-cultured HAMVECs express more endothelial nitric oxide (NO) synthase (eNOS) per cell than monocultured HAMVECs, and similar amounts of NO

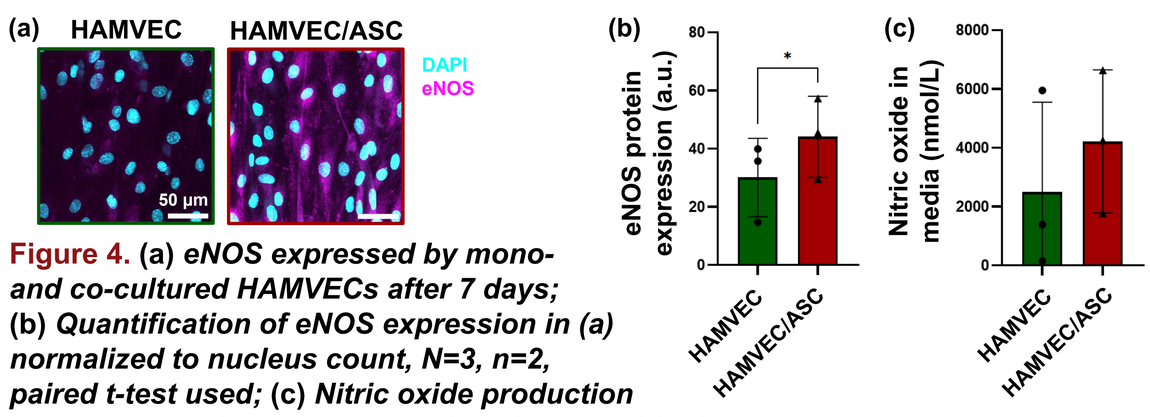


Figure 4. (a) *eNOS expressed by mono- and co-cultured HAMVECs after 7 days;* (b) *Quantification of eNOS expression in (a) normalized to nucleus count, N=3, n=2, paired t-test used;* (c) *Nitric oxide production measured via Griess assay, N=3, n=2, paired t-test used.*

Results

Co-cultured HAMVECs express less intercellular adhesion molecule1 (ICAM-1) with and without tumor necrosis factor-α (TNF-α) stimulation

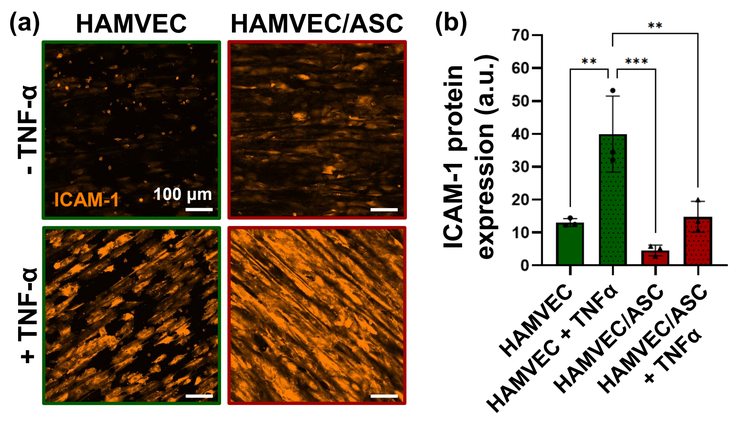


Figure 5. (a) *ICAM-1 expressed by mono- and co-cultured HAMVECs, with / without TNF-α at 7 days;* (b) *Quantification of ICAM-1 expression in (a) normalized to HAMVEC coverage, N=3, n=2, one-way ANOVA with Tukey's post hoc used, ** indicates p<0.01, *** indicates p<0.001.*

Monocultured and co-cultured endothelia both perform favourably in terms of hemolysis and complement cascade activation

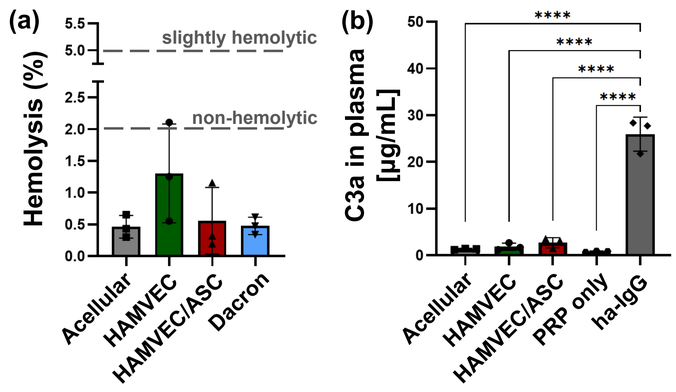


Figure 6. (a) *Hemolysis ratios of test groups vs. Dacron control after exposure to whole blood, N=3, n=2, one-way ANOVA with Tukey's post hoc used;* (b) *C3a concentrations measured in plasma after incubation of platelet-rich plasma (PRP) on test substrates vs. a negative control (PRP only) and a positive control (PRP incubated with heat aggregated immunoglobin G (ha-IgG)), N=3, n=2, one-way ANOVA with Tukey's post hoc used, **** indicates p<0.0001.*

Co-cultured endothelia display reduced white blood cell adhesion and reduced platelet adhesion and activation

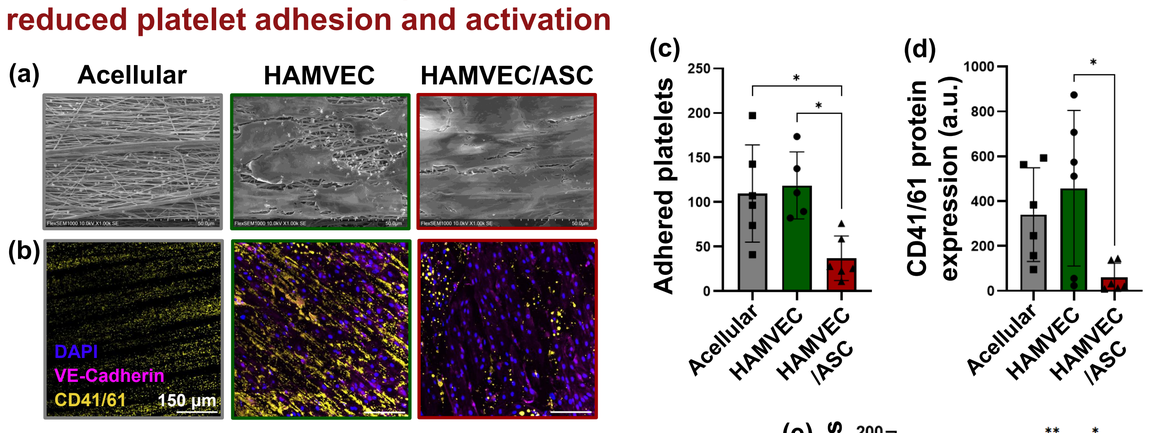


Figure 7. (a) *Scanning electron micrographs and* (b) *immunostained scaffolds showing platelet adhesion on acellular, partially endothelialized, and fully endothelialized scaffolds;* (c) *Numbers of platelets adhered to scaffolds from (a), N=3, n=2, one-way ANOVA with Tukey's post hoc used;* (d) *CD41/61 expression by platelets from (b), N=3, n=2, one-way ANOVA with Tukey's post hoc used;* (e) *Numbers of monocytes adhered to acellular, partially endothelialized, and fully endothelialized scaffolds with and without TNF-α incubation, N=3, n=2, one-way ANOVA with Tukey's post hoc used.*

Conclusions

- Co-culture of HAMVECs with ASCs reduces the permeability of the engineered endothelial monolayer after 14 days
- Co-culturing HAMVECs and ASCs for 7 days produces a fully endothelialized scaffold with elongated morphology, increased vasodilator expression, and decreased inflammatory marker expression
- Partial and full endothelia do not damage red blood cells or activate the complement cascade
- Full endothelia reduce monocyte and platelet adhesion and activation
- Co-culture accelerates confluent and blood compatible endothelization, potentially enhancing clinical adoption of tissue-engineered grafts

References and Acknowledgements

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