Development of a Multifunctionalized Vascular Scaffolding System to Induce *In Situ* Endothelialization

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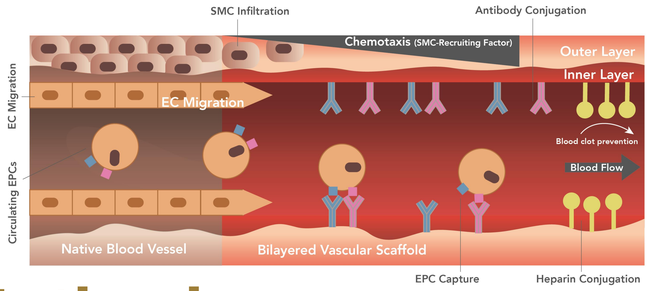
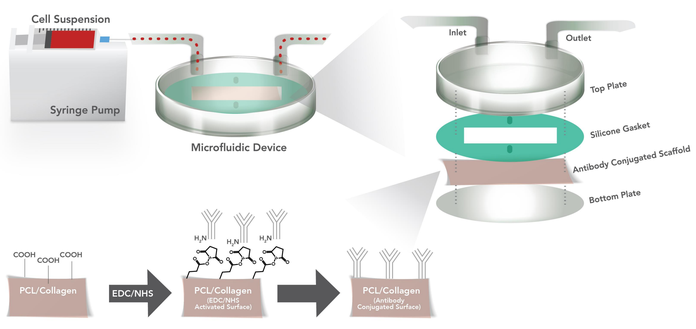
Objective

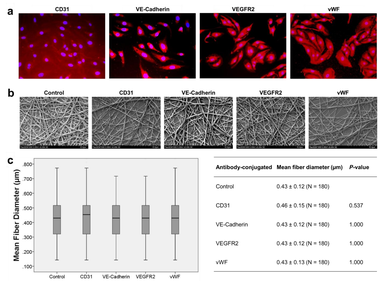
Tissue engineered vascular grafts (TEVGs) are promising alternatives to small-diameter prosthetic grafts. Previous methods of seeding tubular scaffolds with autologous vascular cells have been successful; however these methods require copious preparation time. Endothelial cell (EC) growth onto the luminal surface of the vascular scaffolds may be critical for integration of a TEVG to the host environment. An alternative approach for TEVGs includes the *in situ* endothelialization of acellular scaffolds by capturing circulating endothelial progenitor cells (EPCs) and ECs from the blood stream through biofunctionalization.



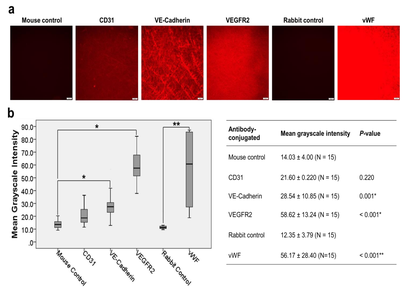
Methods

Fibrous scaffolds, analogous to the vascular lumen, were electrospun with a 1:1 poly(ε-caprolactone) (PCL)/collagen blend solution. Scaffolds were surface modified to immobilize EPC- and EC-specific antibodies, and compared to unmodified controls. The antibodies tested were CD31, vascular endothelial cadherin (VE-Cadherin), vascular endothelial growth factor receptor 2 (VEGFR2), and Von Willebrand factor (vWF). Microfluidic cell capture experiments were performed for each antibody using previously optimized conditions. The most efficacious antibodies were paired to examine synergistic cell capturing capabilities.

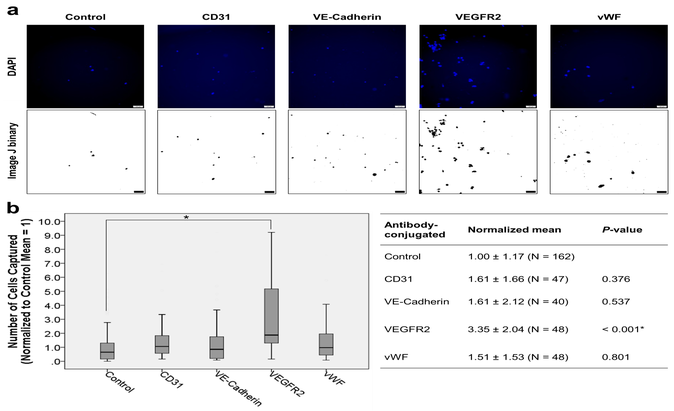




Antibody-conjugated	Mean fiber diameter (μm)	*P*-value
Control	0.43 ± 0.12 (N = 180)	
CD31	0.46 ± 0.15 (N = 180)	0.537
VE-Cadherin	0.43 ± 0.12 (N = 180)	1.000
VEGFR2	0.43 ± 0.12 (N = 180)	1.000
vWF	0.43 ± 0.13 (N = 180)	1.000

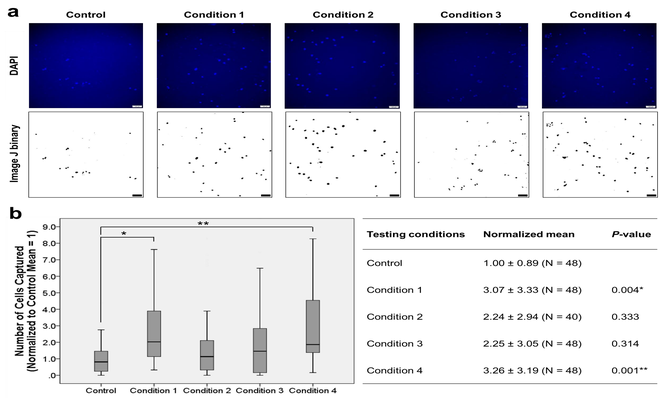


Antibody-conjugated	Mean grayscale intensity	*P*-value
Mouse control	14.03 ± 4.00 (N = 15)	
CD31	21.60 ± 0.220 (N = 15)	0.220
VE-Cadherin	28.54 ± 10.85 (N = 15)	0.001*
VEGFR2	58.62 ± 13.24 (N = 15)	< 0.001*
Rabbit control	12.35 ± 3.79 (N = 15)	
vWF	56.17 ± 28.40 (N=15)	< 0.001**



Antibody-conjugated	Normalized mean	*P*-value
Control	1.00 ± 1.17 (N = 162)	
CD31	1.61 ± 1.66 (N = 47)	0.376
VE-Cadherin	1.61 ± 2.12 (N = 40)	0.537
VEGFR2	3.35 ± 2.04 (N = 48)	< 0.001*
vWF	1.51 ± 1.53 (N = 48)	0.801

Testing conditions	Antibody-conjugated	Concentration of dilutions
Control	VEGFR2	100 μg/mL
Condition 1	VEGFR2 + CD31	100 μg/mL
Condition 2	VEGFR2 + VE-Cadherin	100 μg/mL
Condition 3	VEGFR2 + CD31	200 μg/mL
Condition 4	VEGFR2 + VE-Cadherin	200 μg/mL



Testing conditions	Normalized mean	*P*-value
Control	1.00 ± 0.89 (N = 48)	
Condition 1	3.07 ± 3.33 (N = 48)	0.004*
Condition 2	2.24 ± 2.94 (N = 40)	0.333
Condition 3	2.25 ± 3.05 (N = 48)	0.314
Condition 4	3.26 ± 3.19 (N = 48)	0.001**

Results

Antibody-conjugated scaffolds captured circulating cells at higher rates than unmodified scaffolds. Compared to controls, vWF-, CD31-, VE-Cadherin-, and VEGFR2-bound scaffolds captured more ECs than unmodified controls (1.00 ± 1.17; 1.51 ± 1.53; 1.61 ± 1.66; 1.61 ± 2.12; and 3.35 ± 3.04, $p < .001$, respectively). Scaffolds bioconjugated with two antibodies demonstrated synergistic capture efficacy compared to bioconjugation with a single antibody. Compared to scaffolds bioconjugated with 100 μg/mL of VEGFR2, scaffolds bioconjugated with 50 μg/mL of both VEGFR2 and CD31, 50 μg/mL of both VEGFR2 and VE-Cadherin, 100 μg/mL of both VEGFR2 and CD31, and 100 μg/mL of both VEGFR2 and VE-Cadherin all captured more circulating ECs (1.00 ± 0.89; 3.07 ± 3.33, $p < .05$; 2.24 ± 2.94; 2.25 ± 3.05; and 3.26 ± 3.19, $p < .05$, respectively).

Conclusions

Capture of circulating EPCs and ECs can be optimized with bioconjugation of one or more antibodies on the luminal surface of TEVGs. This project has the potential to vastly improve upon existing TEVG approaches. While the use of immobilized antibodies has previously been proven to be advantageous in capturing ECs, comparisons between the efficacy of distinct antibodies has yet to be. Furthermore, the use of multiple antibodies for a synergistic effect in honing EC attachment to the lumen of engineered vessels is a novel approach in the application of TEVGs. This project contributes necessary knowledge to the field of tissue engineering in regard to the comparison of existing modalities.

References

1. Pashneh-Tala, Samand, Sheila MacNeil, and Frederik Claeyssens. "The tissue-engineered vascular graft—past, present, and future." Tissue Engineering Part B: Reviews 22.1 (2015): 68-100.
2. Lee, Sang Jin, et al. "Development of a composite vascular scaffolding system that withstands physiological vascular conditions." Biomaterials 29.19 (2008): 2891-2898.