Modulating Sympathetic Nerve Regeneration for Vascular Remodeling in Peripheral Vascular Grafts

Taylor Brown[1,2], Jessica Li[2], Evi Liu[3], Ananya Shivakumar[1], Aurea del Carmen[3], Caitlyn Dang[3], Calvin L. Chao[3], Bin Jiang[1,2,3]

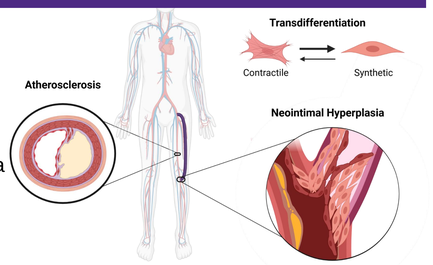
[1]Department of Biomedical Engineering, Northwestern University, Chicago, IL; [2]Center for Advanced Regenerative Engineering, Northwestern University, Evanston, IL;
[3]Department of Surgery, Northwestern University Feinberg School of Medicine, Chicago, IL

Northwestern CENTER FOR ADVANCED REGENERATIVE ENGINEERING

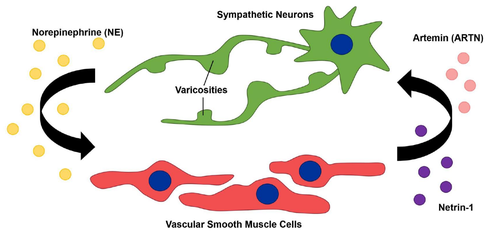
Northwestern Medicine® Feinberg School of Medicine

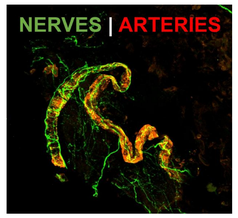
Introduction

- Vascular graft failure is an unmet need: 40% synthetic peripheral bypass grafts fail by 5 years, leading to amputation and mortality [**1**].
- Vascular smooth muscle cells (**VSMC**) switch from a contractile phenotype to a synthetic phenotype [**2**] to cause narrowing of the graft lumen at the anastomosis.



- Healthy arteries are innervated by the sympathetic nervous system to not only regulate VSMC contractility and tension, but also to regulate arterial maturation [**3**].

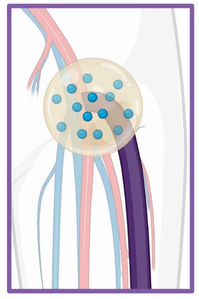




GAP: Vascular grafts used clinically lack innervation, a key regulator of vascular smooth muscle cells that drive remodeling

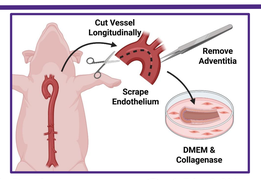
GOAL: Design perivascular therapies that modulate sympathetic nerve regeneration around vascular grafts to promote healthy remodeling.

DESIGN: Fibrin hydrogel containing nerve growth factors encapsulated in PLGA microspheres for extended release.



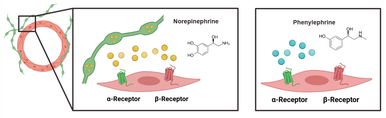
Methods

1. VSMC Isolation from Porcine Aorta Tissue



- Male Porcine Aorta Tissue
- 1.5 hr 0.2% Collagenase, 24 hr 0.1% Collagenase
- DMEM + FBS + P/S + 0.1 – 0.2% (w/v) Collagenase
- Differentiation & Immunocytochemistry

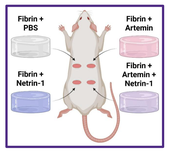
2. VSMC Culture with Norepinephrine (NE) & Phenylephrine (PE)



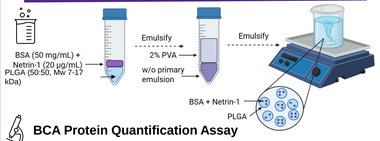
- Human Aortic Smooth Muscle Cells (HAoSMC) in Growth Media [0 nM], NE [100 nM, 100 uM, 1 mM], or PE [100 uM, 1 mM]
- 7 Days
- Resazurin Assay & Immunocytochemistry

3. Subcutaneous Implantation of Fibrin Gels with Nerve Growth Factors

- Male Sprague-Dawley Rats
- Fibrinogen: 20 mg/mL
  Thrombin: 2.5 U/mL
  Netrin-1: 1 µg/mL
  Artemin: 100 ng/mL
- 2 Weeks
- Histology & Immunofluorescence



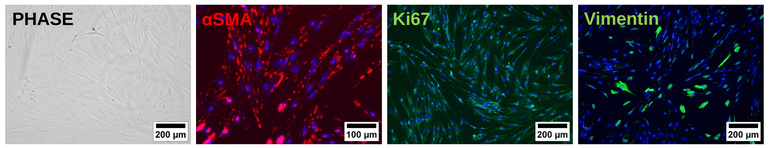
4. Bovine Serum Albumin + Netrin-1 Microsphere Characterization



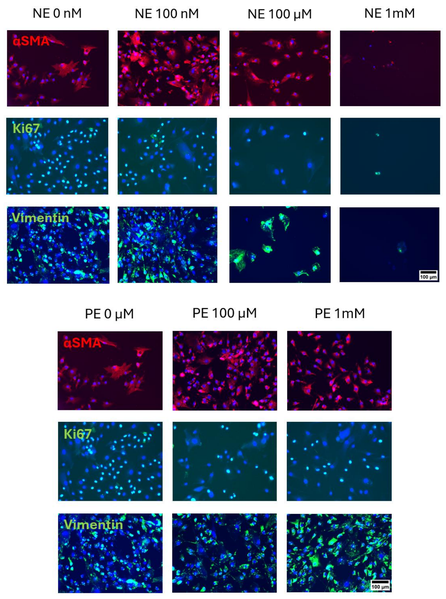
- BCA Protein Quantification Assay
- Human Umbilical Vein Endothelial Cells (HUVEC) in Basal Media with 0 – 1000 ng/mL Netrin-1 or 20 ng/mL VEGF
- 24 Hours
- Live/Dead, Resazurin, & DAF-FM DA

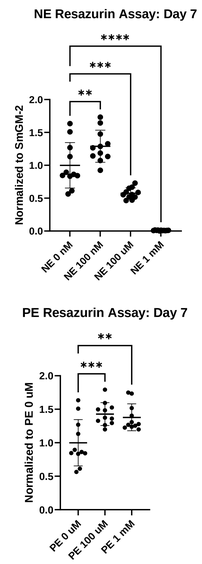
Results

1. Vascular smooth muscle cells can be isolated from aortic tissue using collagenase, a promising strategy to isolate patient cells in future studies.

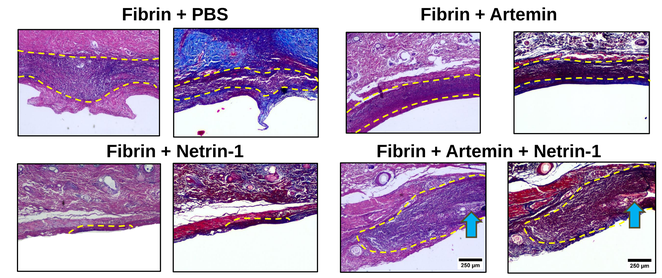


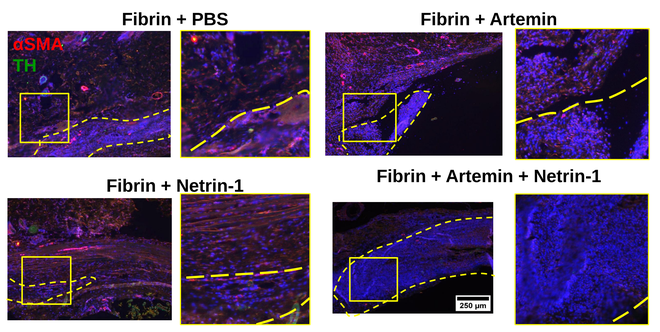
2. Norepinephrine treatment has dose-dependent metabolic effects on HAoSMCs, while α-adrenergic antagonist phenylephrine treatment yields consistent increases across concentrations.





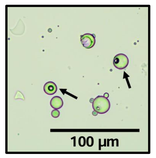
3. Subcutaneous fibrin gels with artemin and netrin-1 degraded within two weeks, and no differences in sympathetic nerve regeneration or vascular density were observed “within” the infiltrated fibrin gels.



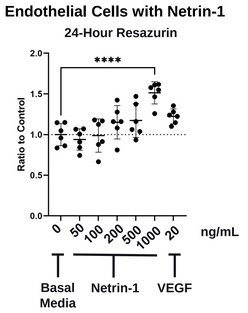


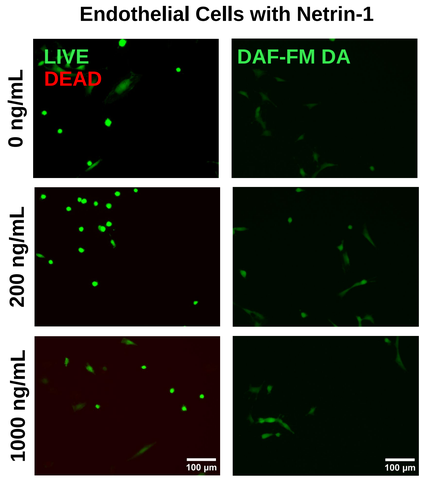
Results

4. PLGA microspheres with BSA and netrin-1 were formulated with ≤23% encapsulation efficiency but show burst release. Netrin-1 solution has a dose-dependent metabolic effect on endothelial cells.



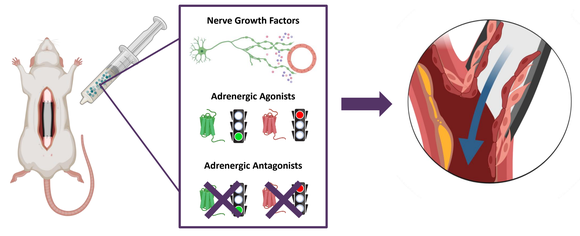
Drug Loading: **5%**
Encapsulation Efficiency: **23%**





Conclusions & Future Directions

1. **Mechanism:** Vascular smooth muscle cells respond to nerve signals in dose-dependent ways to regulate metabolism, with α-adrenergic signaling driving proliferation.
2. **Regeneration *in vivo*:** Though biocompatible, bulk delivery of neurotrophic factors in fibrin gels is insufficient to stimulate sympathetic nerve regeneration.
3. **Sustained Release:** Encapsulation of sympathetic growth factors, agonists, or antagonists in PLGA microspheres localized around the graft anastomosis in a hydrogel is a novel therapeutic strategy.



IMPACT: Engineering sympathetic regulation of vascular smooth muscle cells during vascular graft remodeling will inform the development of therapies that enable robust blood flow for a patient’s lifetime.

Acknowledgements & Funding

- Equipment & Services from the Analytical bioNanoTechnology Equipment Core (NSF ECCS-2025633 & Feinberg School of Medicine) & Mouse Histology and Phenotyping Laboratory (NCI P30-CA060553 for Robert H Lurie Comprehensive Cancer Center)
- Funding from Center for Advanced Regenerative Engineering RE-Training Program (NIH T32-EB031527) & American Heart Association Predoctoral Fellowship (24PRE1199686)

References

[**1**] Pashneh-Tala S, et al. Tissue Eng Part B 2016;22(1):68-100. DOI: 10.1089/ten.teb.2015.0100
[**2**] Frismantiene A, et al. Cell Signal 2018, 52, 48-64. DOI: 10.1016/j.cellsig.2018.08.019.
[**3**] Eichmann A, et al. Science Translational Medicine 2014, 6 (252), 1-4. DOI: 10.1126/scitranslmed.3008910.
[**4**] Andres R, et al. Development 2001;128(3685-3695). DOI: 10.1242/dev.128.19.3685
[**5**] Brunet I, et al. J Clin Invest 2014;124(7):3230-40. DOI:10.1172/JCI75181

bio RENDER