Mechanical Characterization and Neutrophil Extracellular Traps Response of a Novel Hybrid Geometry Polydioxanone Near-Field Electrospun Template

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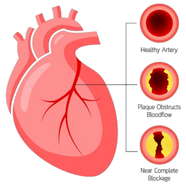
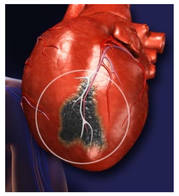
Cardiovascular Disease

- Cardiovascular disease (CVD) is a growing condition caused by narrowing blood vessels in a process termed atherosclerosis.
- This process results in angina, heart attack, stroke, and limb amputation due to ischemic tissues.
- In the United States CVD has accounted for nearly 20% of all deaths in 2015 and is projected to rise to nearly 24% of all deaths in 2030.
- End-stage treatment for CVD seeks to restoring blood flow to ischemic tissues through a grafted bypass conduit.
- Autologous vessel are frequently used to bypass blockages and is exclusively used in coronary bypasses
- Extensive harvesting time, limited availability.
- Synthetic grafts above 6 mm in inner diameter (ID) are off-the-shelf available and work adequately.
- However, current synthetic vascular grafts < 6 mm ID perform poorly and most frequently fail by graft occlusion via thrombosis and/or hyperplasia.
- Grafts also, but to a lesser extent, fail by dilation.



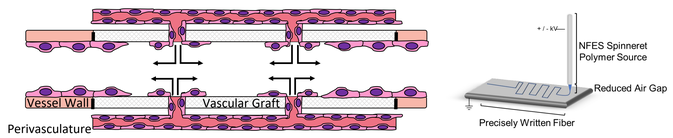
Minneapolis Heart Institute, 2021



WebMD, 2021

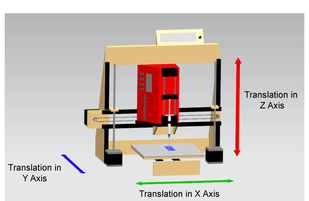
Statement of Purposee

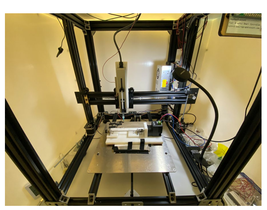
The ideal "off the shelf" tissue engineered, small-diameter (< 6 mm inner diameter (ID)) vascular graft hinges on designing a graft structure to act as a template that facilitates transmural ingrowth of perivascular capillaries to regenerate a neointimal surface. The purpose of this study was to design the next generation, small diameter, bioresorbable vascular graft to support vascular regeneration. The approach in this study utilized a NFES device to semi-stable write novel, polydioxanone (PDO) microfiber-based vascular conduit templates. The polymer spinneret was programmed to translate in a stacking grid pattern, which resulted in a scaffold with highly aligned grid fibers that were intercalated with low density, random fibers (hybrid structure design). Because of this semi-stable process, increasing the grid dimensions resulted in both a lower density of fibers in the center of each grid in the template as well as a lower density of "rebar-like" stacked fibers per unit area. Upon fabrication, the acute neutrophil response to the templates was evaluated using standard assays of neutrophil extracellular traps (NETs) upon template interaction due to the innate immune responses, neutrophil function, upon rapid and robust response to such an implant.



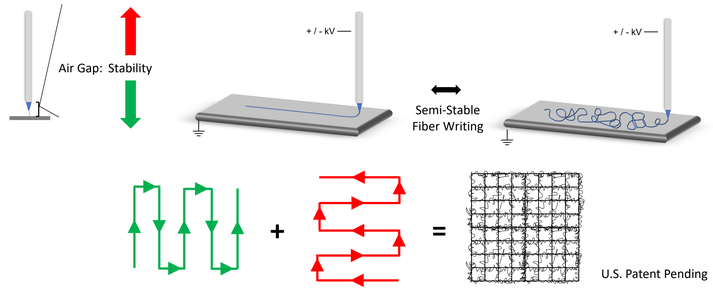
Methods Overview

A consumer 3D printer (Prusa 12" Basic Pegasus, Maker Farm, South Jordan, UT, USA) was modified by replacing the filament extrusion print head to accommodate a custom NFES print head, modified from our previous published NFES work [1]. Polydioxanone (DIOXOMAXX 100, Inherent viscosity 2.13 dL/g, Bezwada Biomedical, LLC, Hillsborough, NJ, USA) solutions used were at a concentration of 112 mg/mL for all templates. NFES grids were programmed with X- and Y- grid spacing of 200 x 200 µm^2 (NFES 200^2), 300 x 300 µm^2 (NFES 300^2), 400 x 400 µm^2 (NFES 400^2), and 500 x 500 µm^2 (NFES 500^2), with constant template thickness. Traditional electrospun templates of same composition with 1-2 and 0.3-0.5 µm fiber diameter served as controls. Scanning electron microscopy was used to characterize fiber diameter and pore spacing. Fluorescent microsphere (9.9 and 97 µm diameters (Cat No. G100, 35-11, Fisher Scientific, Waltham, MA, USA)) filtration was used to ascertain the effective restriction size of an object transiting the NFES and TES templates [2]. Templates were mechanically evaluated using uniaxial tensile testing. Finally, fresh human neutrophils were seeded on templates and NETs quantified after 3- and 6-hours using protocols previously published [3]. Statistical differences were tested between groups using an ANOVA with Holm-Sidak's multiple comparisons at a significance of $p < 0.05$.

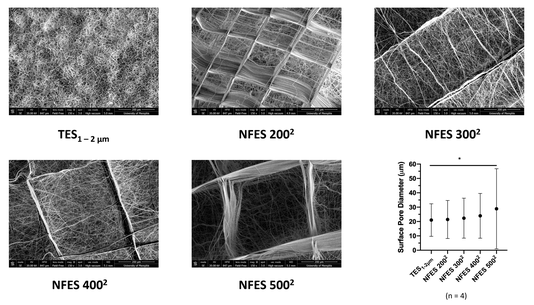




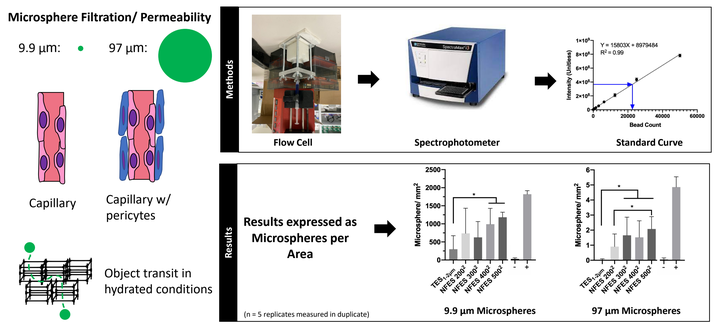
Semi-stable Fiber Writing



Fabrication Results

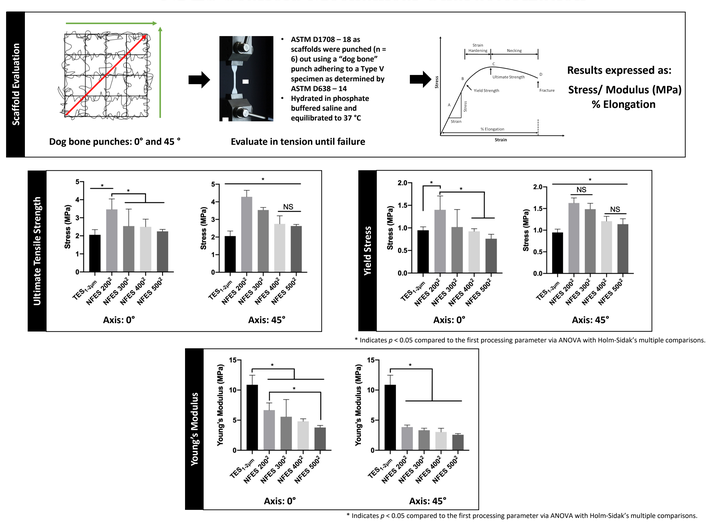


NFES Hybrid Architecture Effective Pore Size



* Indicates $p < 0.05$ compared to the first processing parameter via ANOVA with Holm-Sidak's multiple comparisons.

NFES Scaffold Mechanical Characterization



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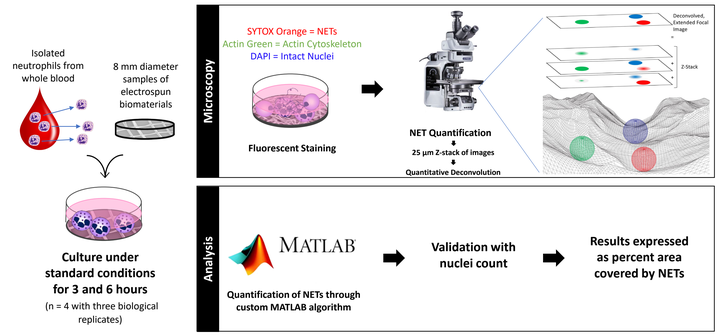
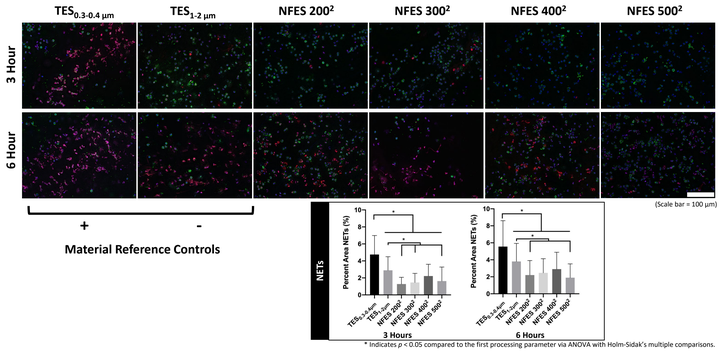
Neutrophil *In vitro* Isolation



NETs on NFES and TES PDO Scaffolds



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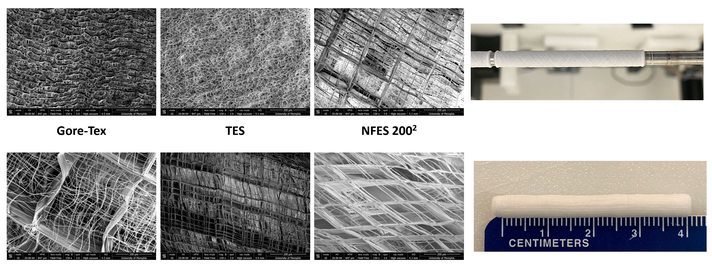
NFES Vascular Graft Fiber Architectures



Conclusion

Overall, the results of this study suggest that this novel NFES template architecture and properties of PDO can be highly tailored as a function of programming for small diameter vascular graft templates. Future studies will continue to understand neutrophil functionality (e.g., angiogenic potential) of the NFES templates as well as platelet activity upon blood interaction.

Literature Cited

1. King, W.E., III, et al. Polymers (Basel), 2019. 12(1).
2. Duke, S. Particulate Science and Technology, 1989. 7(3).
3. Fetz, A.E., et al., Tissue Engineering Part A, 2017. 23(19-20): p. 1054-1063.

Acknowledgements

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