Electrospinning With a Porogen to Create a Layered Scaffold For a Tissue Engineered Vascular Graft

Jayne Wolfe and Brandon J. Tefft, PhD

Marquette University and Medical College of Wisconsin Joint Department of Biomedical Engineering

INTRODUCTION

There is a strong clinical need for a small-diameter vascular graft that remains patent

- The "gold standard" treatment for coronary artery disease is bypass grafting with an autologous vessel. However, not all patients have healthy vessels that can be used as bypass grafts[1]
- While medium- and large-diameter synthetic grafts have performed well clinically, there are no small-diameter (<6mm) grafts due to unacceptable patency rates[1]
- Developing a small-diameter tissue engineered vascular graft (TEVG) incorporated with interstitial cells and a stable endothelial cell (EC) monolayer on the inner surface may offer patients an alternative to autologous vessel grafts

Nanofiber scaffolds generally suffer from poor cell infiltration

- Electrospun nanofiber scaffolds are advantageous for tissue engineering applications as the fibrous structure mimics native extracellular matrix, but the dense fiber architecture generally limits cell infiltration[2]
- Inducing porosity throughout a nanofiber scaffold can encourage cell and growth factor infiltration
- Previous groups have deposited NaCl during electrospinning by manual dropping or with vibration as a method to combine salt leaching and electrospinning to create nanofiber scaffolds with induced porosity[3]

OBJECTIVES

- Develop a method to improve cell infiltration into a nanofiber scaffold
- Explore NaCl as a porogen to induce porosity in a nanofiber scaffold
- Fabricate a layered scaffold for a small-diameter TEVG

METHODS

- A biodegradable polyester-urethane (DegraPol DP15, Ab Medica) was mixed with chloroform to obtain a 20%wt/v DP15/chloroform solution without NaCl
- Sieved NaCl (38-106µm) was added to a 5% DP15/chloroform solution to obtain a 70% wt/v NaCl/DP15 solution
- Electrospinning parameters were: pressure of 0.2barr (no NaCl) or 0.06barr (with NaCl), applied voltage of 13kV (no NaCl) or 7kV (with NaCl), distance of 15cm from emitter to mandrel, 4mm diameter mandrel, and mandrel rotation of 430rpm (SprayBase) (**Figure 1A,B**)
- Electrospinning of the inner and outer layers was performed using the solution without NaCl until 0.1mm thickness was achieved. The loose middle layer was electrospun using the solution with NaCl until 0.5mm thickness was achieved. The average total graft wall thickness was 0.7mm (**Figure 1C**). Grafts were soaked overnight in water to leach out the NaCl
- Scanning electron microscopy (SEM) was used to assess fiber diameter, morphology, and porosity with Matlab programs[4,5,6,]

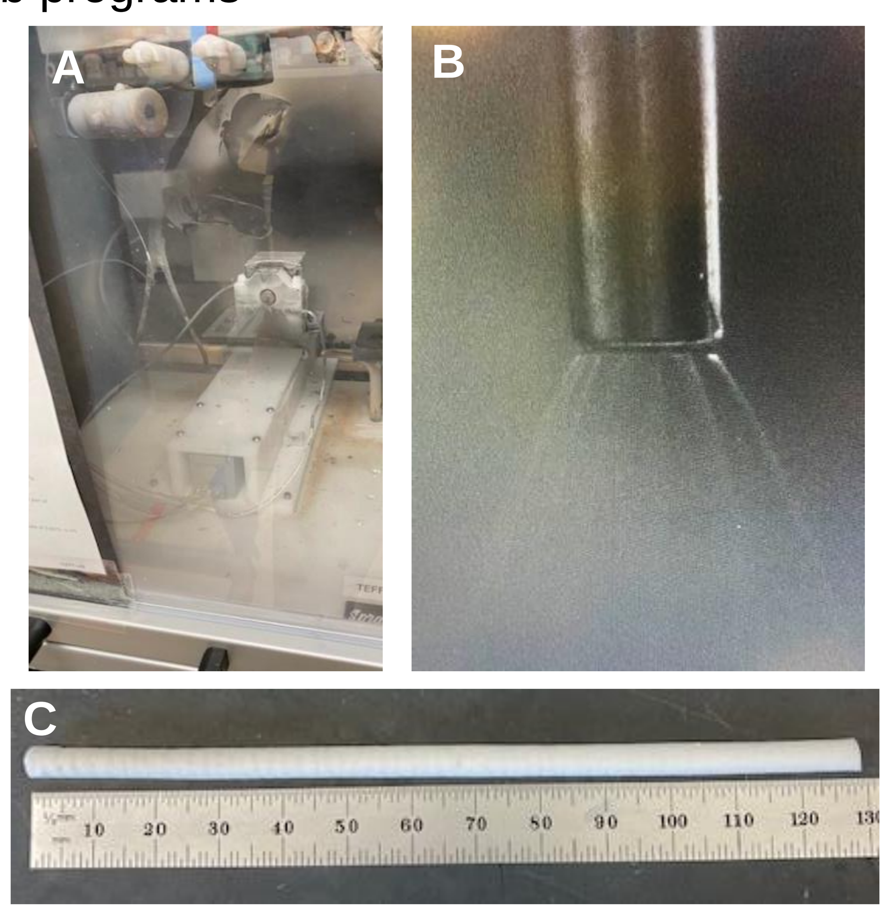

Figure 1. Electrospinning a layered vascular graft scaffold (A) Spraybase system used in our lab **(B)** Multiple jets formed with the DP15/chloroform solution **(C)** Electrospun 4mm inner diameter tubular scaffold

RESULTS

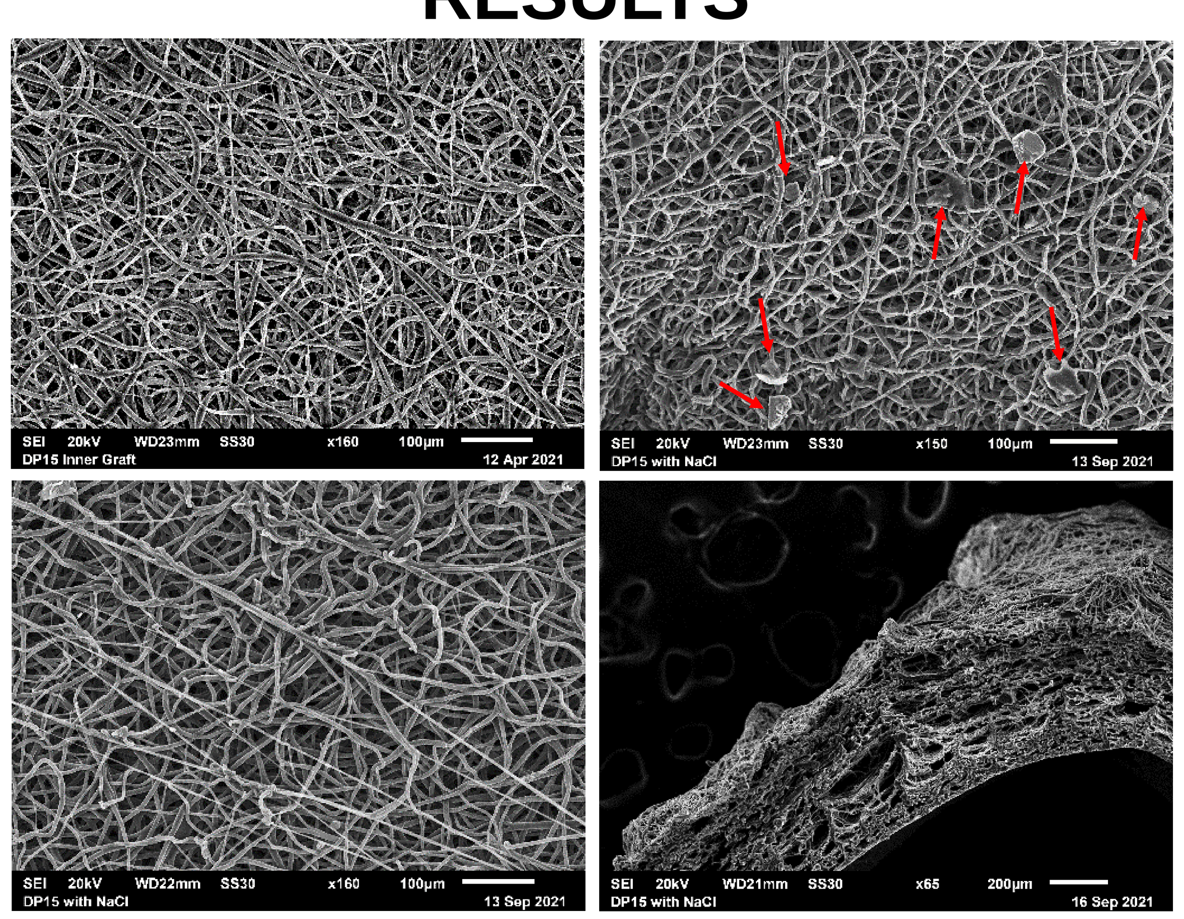

Figure 2. SEM images (A) Inner layer nanofibers without NaCl **(B)** Middle layer nanofibers with NaCl. Red arrows pointing to NaCl **(C)** Middle layer nanofibers after NaCl leaching **(D)** Cross-section of layered graft

RESULTS

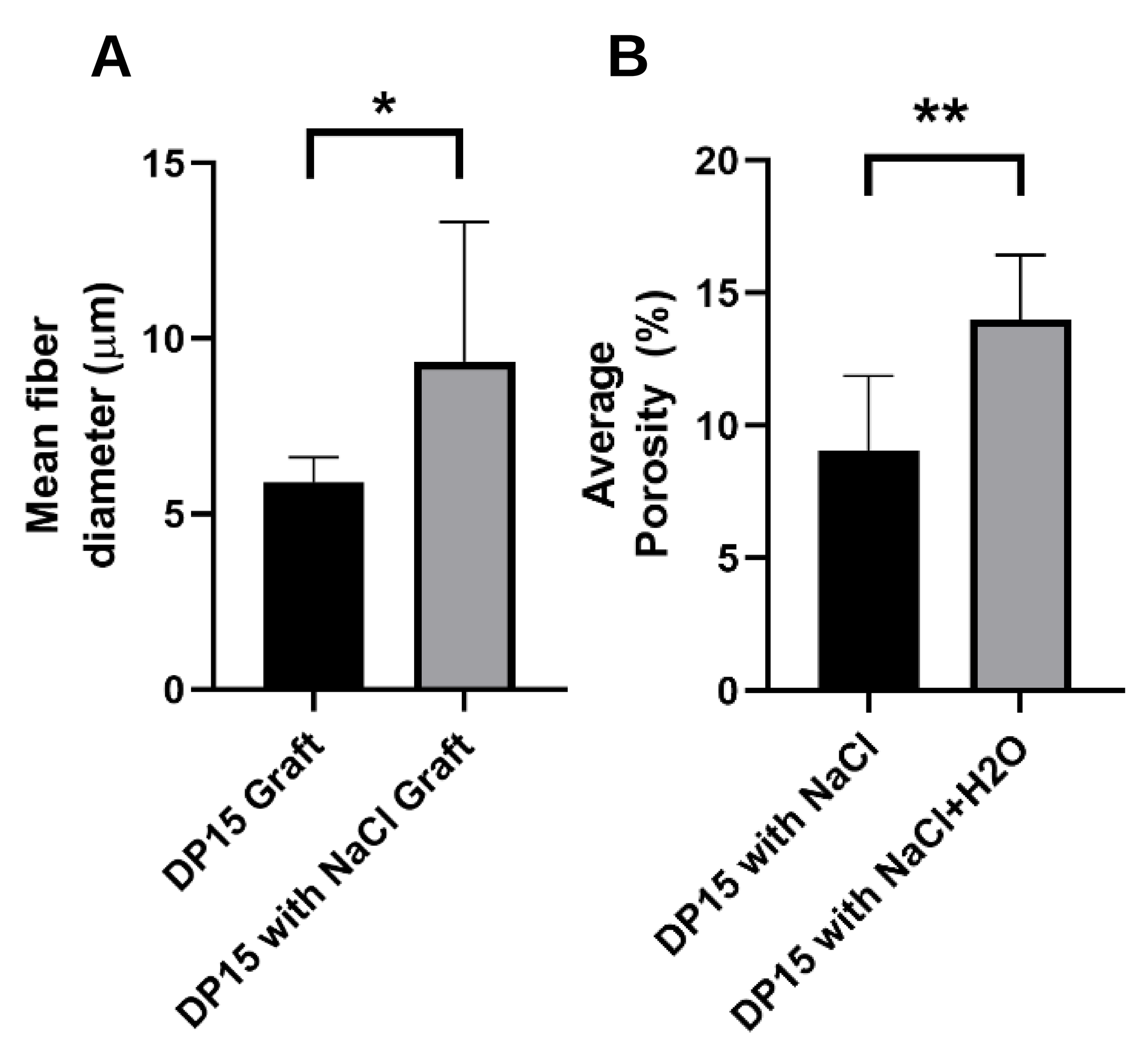

Figure 3. Increased porosity and fiber diameter with addition of NaCl to DP15. (A) The mean diameter of 9.3±4.4µm for the fibers electrospun with NaCl was significantly greater than the average diameter of 5.9±3.2µm for DP15 fibers electrospun without NaCl. **(B)** Leaching out the NaCl from the fibers led to a statistically significant increase in average porosity from 9.1% to 14.0%. Total of n=7 images analyzed per type of graft. Used a 2-sample t-test to compare group means. * indicates $p<0.05$ and ** $p<0.01$.

DISCUSSION

- The SEM images show successful deposition of NaCl among the nanofibers
- Unexpectedly, adding NaCl to the polymer solution led to an increase in fiber diameter compared to grafts electrospun without NaCl
- The porosity of a DP15 graft could be augmented using NaCl as a porogen
- These data indicate adding NaCl to DP15 during electrospinning leads to increased fiber diameter and porosity

FUTURE DIRECTIONS

- We will examine whole graft porosity with volume analysis using a hydrostatic weighing approach
- We are assessing the grafts according to ISO 7198 with dynamic radial compliance, pressurized burst strength, and tensile testing
- Cell infiltration studies are currently being conducted with human aortic adventitial fibroblasts (HAoAFs) and histology

REFERENCES AND ACKNOWLEDGMENTS

1. Mallis, P., Kostakis, A., Stavropoulos-Giokas, C., & Michalopoulos, E. (2020). Future perspectives in small-diameter vascular graft engineering. *Bioengineering*, *7*(4), 160.
2. Wu, J. & Hong, Y. J. B. m. Enhancing cell infiltration of electrospun fibrous scaffolds in tissue regeneration. 1, 56-64 (2016).
3. Nam, J., Huang, Y., Agarwal, S. & Lannutti, J. J. T. e. Improved cellular infiltration in electrospun fiber via engineered porosity. 13, 2249-2257 (2007).
4. Murphy, R., Turcott, A., Banuelos, L., Dowey, E., Goodwin, B., & Cardinal, K. O. H. (2020). SIMPoly: A Matlab-Based Image Analysis Tool to Measure Electrospun Polymer Scaffold Fiber Diameter. *Tissue Engineering Part C: Methods*, *26*(12), 628-636.
5. Rabbani, A., & Salehi, S. (2017). Dynamic modeling of the formation damage and mud cake deposition using filtration theories coupled with SEM image processing. *Journal of Natural Gas Science and Engineering*, *42*, 157-168.
6. Ezeakacha, C. P., Rabbani, A., Salehi, S., & Ghalambor, A. (2018, February). Integrated image processing and computational techniques to characterize formation damage. In *SPE International Conference and Exhibition on Formation Damage Control*. OnePetro.

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CONTACT INFORMATION

Please email Jayne Wolfe: jwolfe@mcw.edu

DEPARTMENT OF BIOMEDICAL ENGINEERING

MARQUETTE UNIVERSITY

MEDICAL COLLEGE OF WISCONSIN