Nitric oxide-releasing polyacrylic acid/polyvinyl alcohol nanofibers

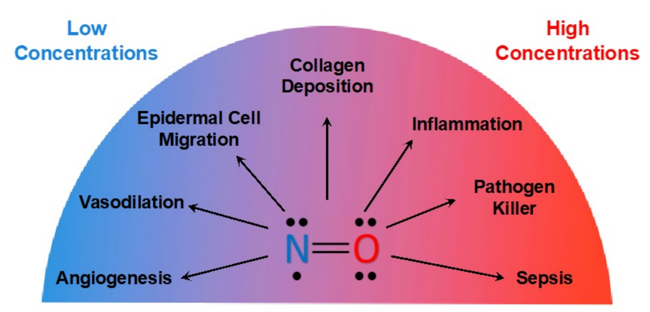
Darci E. Anderson,[1] Taron M. Bradshaw,[1] and Mark H. Schoenfisch[1,2]
[1]Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC
[2]Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC

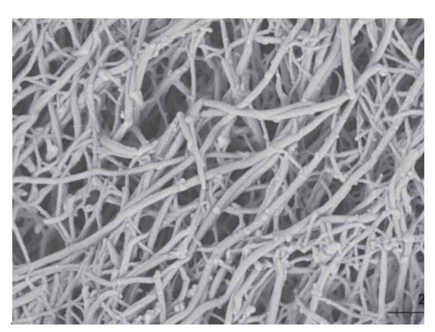
THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

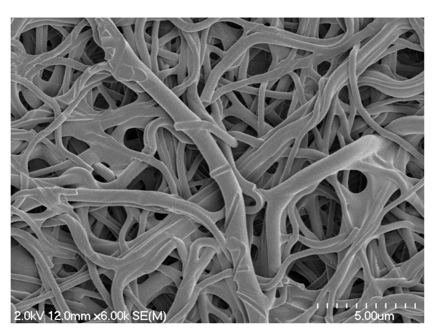
Background

Nitric oxide (NO) is an endogenous gas used as a signaling molecule for many processes in the body. It is known to play important roles in regulating vasodilation, blood pressure, hemodynamics, and other processes. In high concentrations, NO can effectively kill pathogens with no known development of resistance.[1] The presenting challenge with administration of NO as a therapeutic agent is targeted and effective delivery as NO presents in a highly reactive, gaseous state. Diazeniumdiolates are commonly used NO donor molecules used to overcome this challenge by releasing NO through a proton initiating mechanism. They can also be reacted with larger molecules for incorporation into delivery platforms.

Electrospun nanofibers are an emerging platform within drug delivery due to their morphological similarities with the extracellular matrix (ECM) in both size and structure.[2] They have been fabricated with both natural and synthetic polymers and loaded with different therapeutic molecules for targeted delivery.

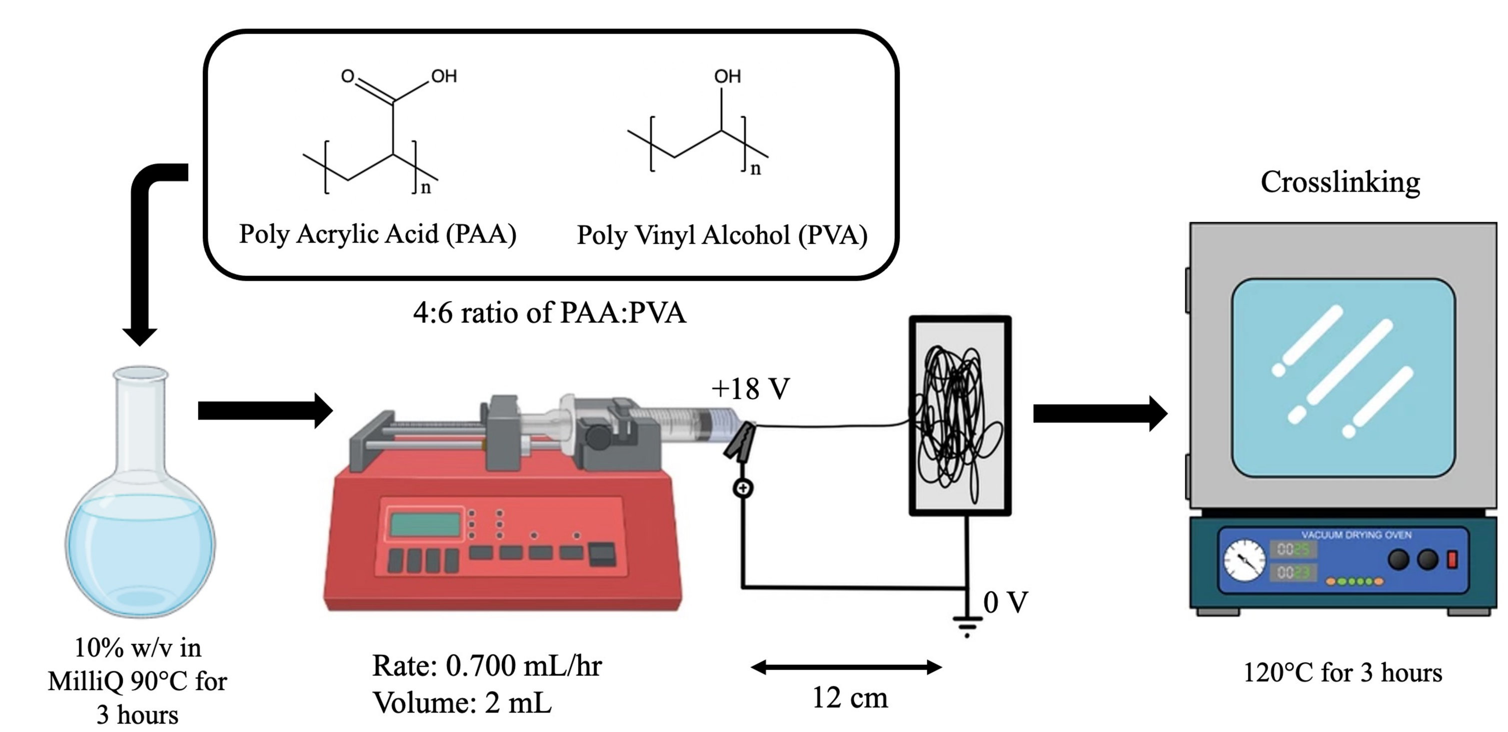



Extracellular Matrix [2]

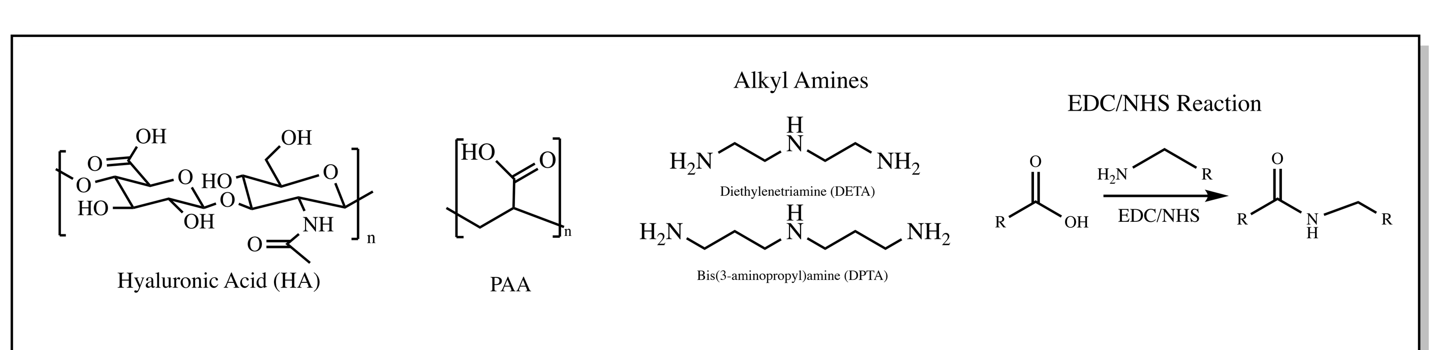

Electrospun Nanofibers

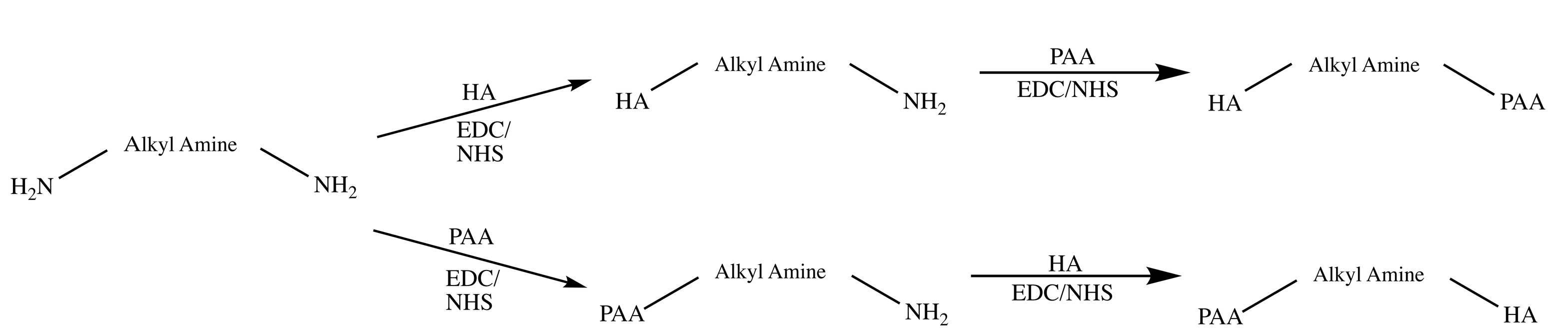
Polyacrylic acid (PAA)/Polyvinyl alcohol (PVA) electrospun nanofibers[3] were surface modified with a combination of the alkylamines Bis(3-aminopropyl)amine (DPTA) or Diethylenetriamine (DETA) and hyaluronic acid (HA) for the development of a macromolecular scaffold to facilitate targeted NO delivery.

Nanofiber Fabrication



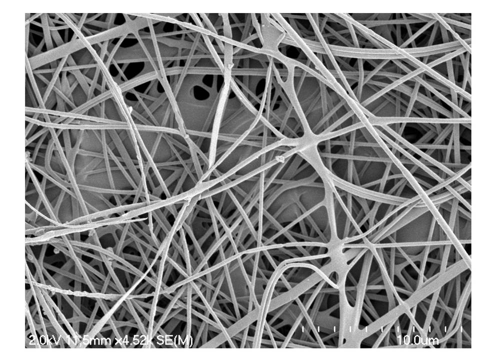
Incorporating Hyaluronic Acid

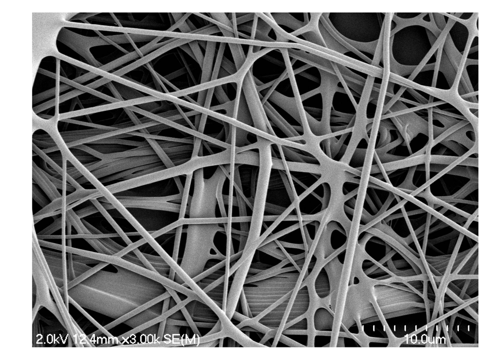




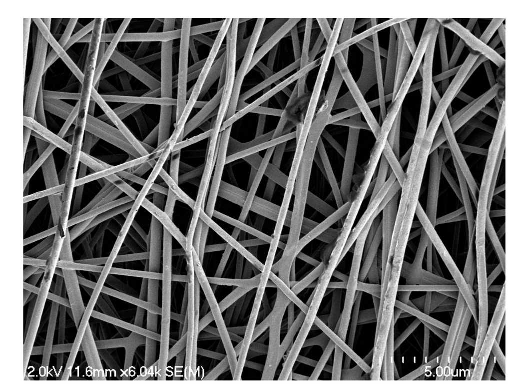
- Constituent of the ECM
- Involved in cell proliferation, migration, and tissue repair [4]

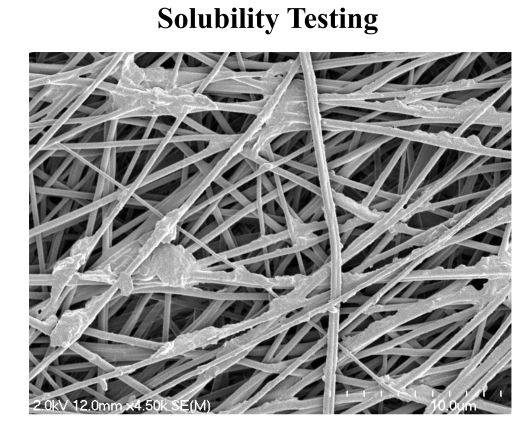
Fiber Characterization

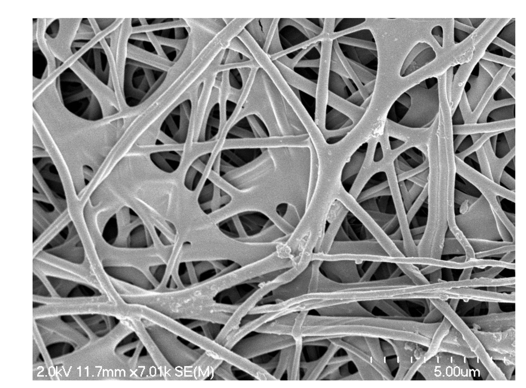

Pre-Crosslinking PAA/PVA Electrospun Fibers
375 ± 50 nm


Post-Crosslinking PAA/PVA Electrospun Fibers
550 ± 264 nm

Solubility Testing

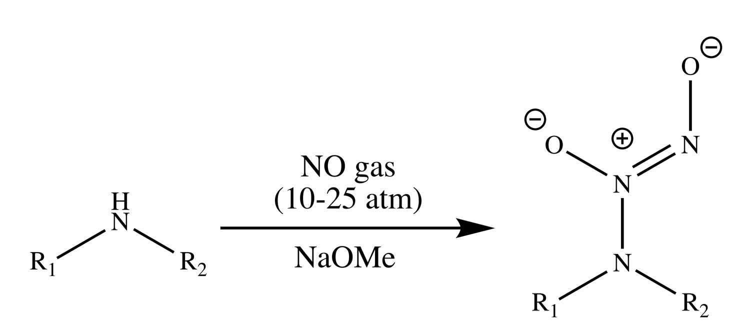

PAA/PVA Fibers
24 hours in Water

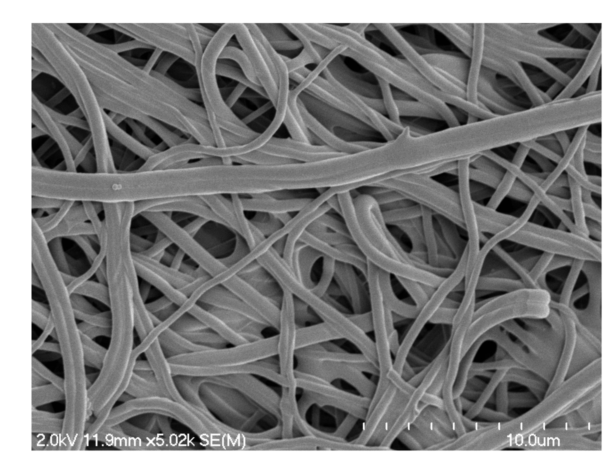

PAA/PVA Fibers
24 hours in Ethanol


PAA/PVA Fibers
24 hours in Acetone

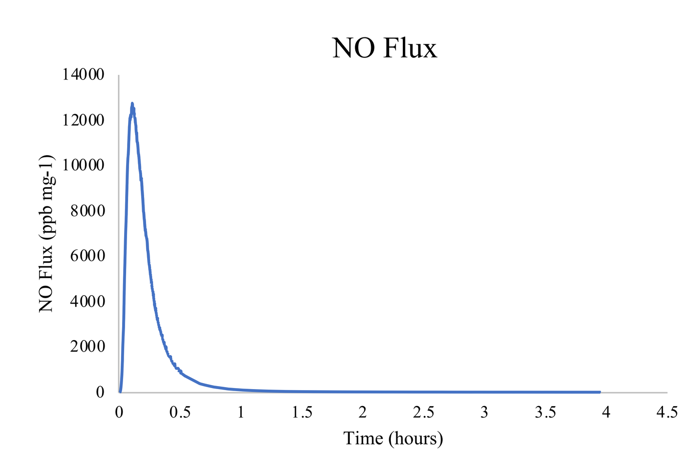
Loading Nitric Oxide

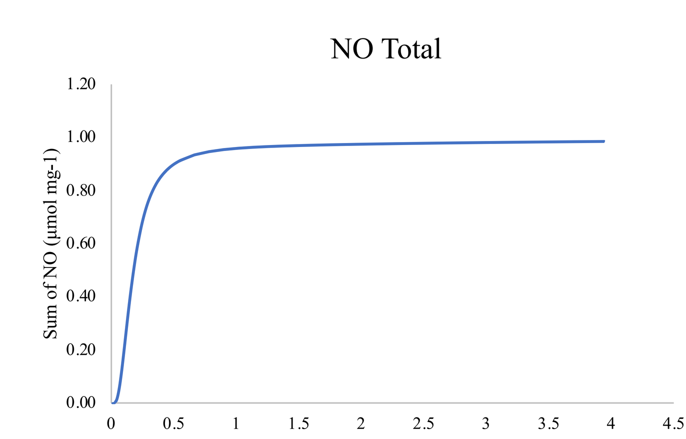
Formation of *N*-diazeniumdiolates




P-HA6-DPTA Fibers Post NO Loading

Nitric Oxide Analyzer – 30 mL PBS, pH 7.4, 37 °C



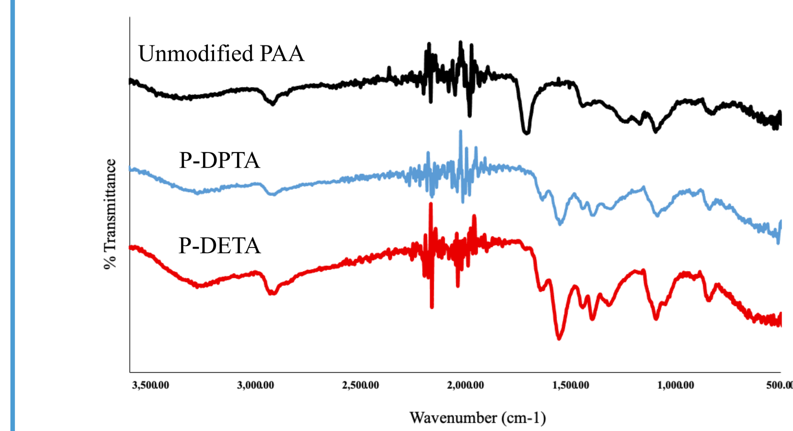


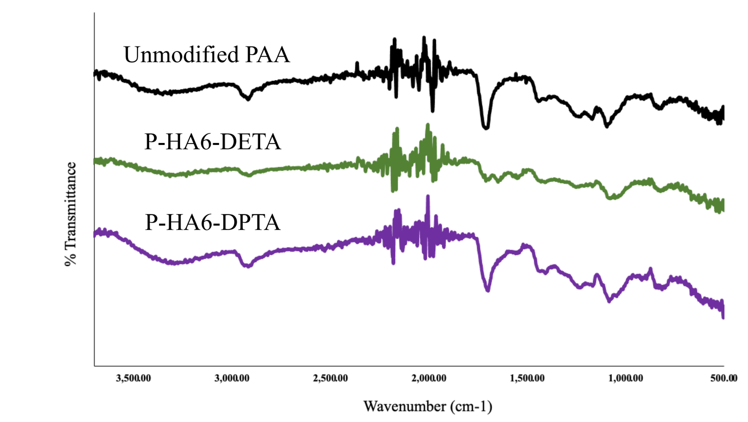
Material	$[NO]_{max}$ (pmol mg^{-1})	$[NO]_{total}$ (μmol mg^{-1})	t_d (h)	$t_{1/2}$(h)
P-DPTA	499.60 ± 3.11	1.20 ± 0.23	7.09 ± 0.03	0.57 ± 0.21
P-DPTA-HA6	769.17 ± 392.30	1.32 ± 0.30	6.36 ± 2.11	0.56 ± 0.33
P-HA6-DPTA	348.18 ± 231.21	0.18 ± 0.21	1.92 ± 1.35	0.11 ± 0.04
P-DETA	186.69 ± 38.74	0.65 ± 0.11	8.83 ± 0.90	0.71 ± 0.14
P-DETA-HA6	124.83 ± 22.39	0.45 ± 0.29	6.15 ± 3.23	0.82 ± 0.51
P-HA6-DETA	146.65 ± 41.95	0.10 ± 0.06	2.07 ± 1.70	0.15 ± 0.09

NO can successfully be loaded onto PAA/PVA nanofibers

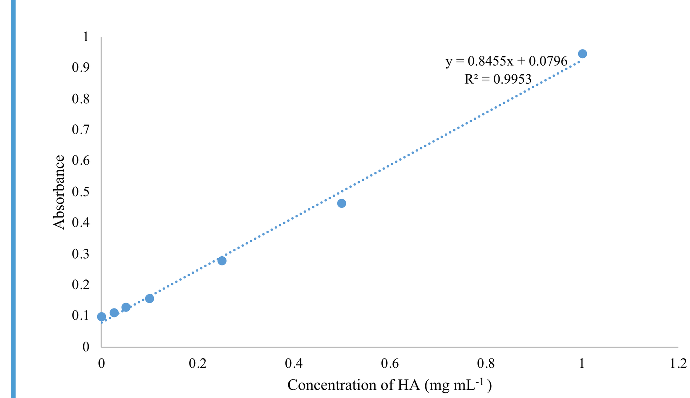
Effective HA Modification

FTIR





Carbazole Assay



Sample Type	Absorbance	HA Concentration (wt%)
P-DPTA	0.158 ± 0.005	< LOQ
P-DPTA-HA6	0.391 ± 0.004	14.4 ± 1.5
P-HA6-DPTA	0.240 ± 0.072	6.9 ± 2.9
P-DETA	0.150 ± 0.020	< LOQ
P-DETA-HA6	0.280 ± 0.027	8.9 ± 1.7
P-HA6-DETA	0.304 ± 0.007	10.6 ± 2.5

PAA/PVA electrospun mats can successfully be surface modified with HA

Conclusions and Future Directions

Conclusions

- PAA/PVA can be electrospun and crosslinked to form non-water soluble nanofiber mats
- Surface functionalization of PAA/PVA nanofiber mats can be successfully achieved through carbodiimide chemistry
- Amine surface modified PAA/PVA mats can be loaded with NO in sufficient amounts for potential therapeutic applications, specifically combinations P-DPTA and P-DPTA-HA6
- HA was successfully incorporated onto the nanofiber structure

Future Directions

- Tensile testing will be performed to determine the mechanical strength of the electrospun PAA/PVA nanofibers
- Cytotoxicity testing will be performed to evaluate cell response to the electrospun PAA/PVA nanofibers
- Bactericidal properties will be evaluated through MBC/MIC assays

Acknowledgements

This work was performed in part at the Chapel Hill Analytical and Nanofabrication Laboratory, CHANL, a member of the North Carolina Research Triangle Nanotechnology Network, RTNN, which is supported by the National Science Foundation, Grant ECCS-2025064, as part of the National Nanotechnology Coordinated Infrastructure, NNCI.

References

(1) Bruckdorfer, R. The Basics about Nitric Oxide. *Molecular Aspects of Medicine* **2005**, *26* (1-2), 3–31.
(2) Lim, E.-H.; Sardinha, J. P.; Myers, S. Nanotechnology Biomimetic Cartilage Regenerative Scaffolds. *Archives of Plastic Surgery* **2014**, *41* (03), 231–240.
(3) Nyman, E.; Henricson, J.; Ghafouri, B.; Anderson, C. D.; Kratz, G. Hyaluronic Acid Accelerates Re-Epithelialization and Alters Protein Expression in a Human Wound Model. *Plastic and Reconstructive Surgery - Global Open* **2019**, *7* (5).
(4) Park, J.-C.; Ito, T.; Kim, K.-O.; Kim, K.-W.; Kim, B.-S.; Khil, M.-S.; Kim, H.-Y.; Kim, I.-S. Electrospun Poly(Vinyl Alcohol) Nanofibers: Effects of Degree of Hydrolysis and Enhanced Water Stability. *Polymer Journal* **2010**, *42* (3), 273–276.