Mechanically Conditioned Tissue Engineered Blood Vessels Resistant to Diabetic Pathologies

Bethany Lefeber, Dan Simionescu, Agneta Simionescu

Cardiovascular Tissue Engineering and Regenerative Medicine Laboratory, Clemson University, Clemson, SC

Introduction

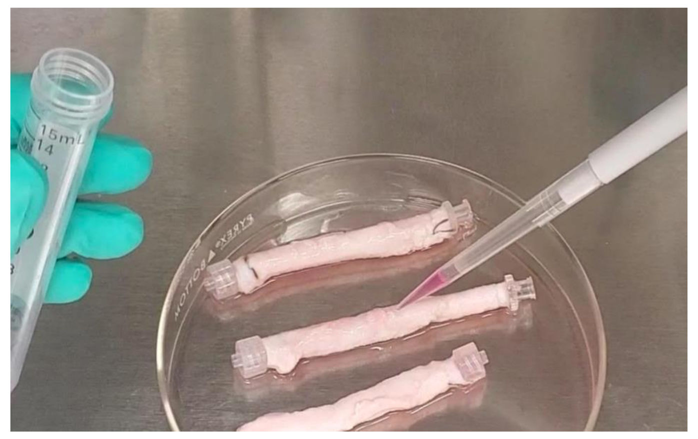
- Diabetes Mellitus is a major risk factor for cardiovascular disease, and currently affects millions of people worldwide.
- Hyperglycemia and insulin resistance, in combination with dyslipidemia, oxidative stress, and inflammation, accelerate vascular diseases.
- Tissue engineered blood vessels (TEBV) are a successful tool to repair damaged blood vessels.
- Penta-galloyl glucose (PGG), an antioxidant, stabilizes ECM proteins and prevents inflammation and slowing their degradation.
- **Our TEBV will be treated with antioxidant molecules, repopulated with human vascular cells, and conditioned *in vitro*, using a custom bioreactor, to reproduce the mechanical and biochemical environment of vasculature in diabetic environments.**

Methods

- Porcine carotid arteries were decellularized with Sodium Hydroxide and detergents in a pressurized perfusion decellularization system.
- One third of the scaffolds were treated with 0.1% PGG.
- All grafts were repopulated with human vascular cells using passive seeding techniques.
- Seeded scaffolds were rotated for 24 hours prior to being mounted in the vascular bioreactor.
- After cell seeding, arteries were carefully mounted into the vascular chamber and secured into place.
- Pressure and flow were ramped up within the first 24 hours.
- Vascular grafts were exposed to physiological pressure and flow for one week.
- Five grafts were exposed to each experimental condition.
- Diabetic Bioreactor Media = 5.5g/L glucose supplement.

Experiment #	Graft Treatment	Cell Seeding	Media Conditioning
1	None	hSMC	Standard Bioreactor Media
2	None	hSMC	Diabetic Bioreactor Media
3	PGG	hSMC	Diabetic Bioreactor Media
4	None	hEC	Standard Bioreactor Media
5	None	hEC	Diabetic Bioreactor Media
6	PGG	hEC	Diabetic Bioreactor Media
7	None	hFb	Standard Bioreactor Media
8	None	hFb	Diabetic Bioreactor Media
9	PGG	hFb	Diabetic Bioreactor Media
10	None	hEC + hSMC + hFb	Standard Bioreactor Media
11	None	hEC + hSMC + hFb	Diabetic Bioreactor Media
12	PGG	hEC + hSMC + hFb	Diabetic Bioreactor Media

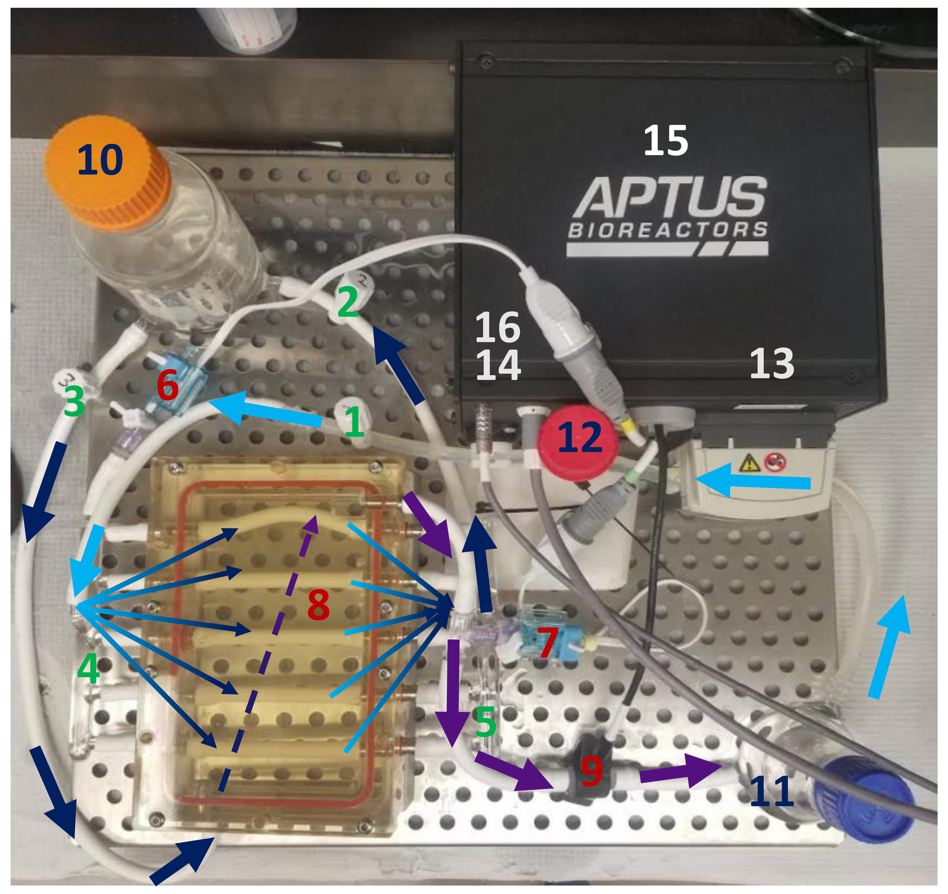
Perfusion Decellularization System



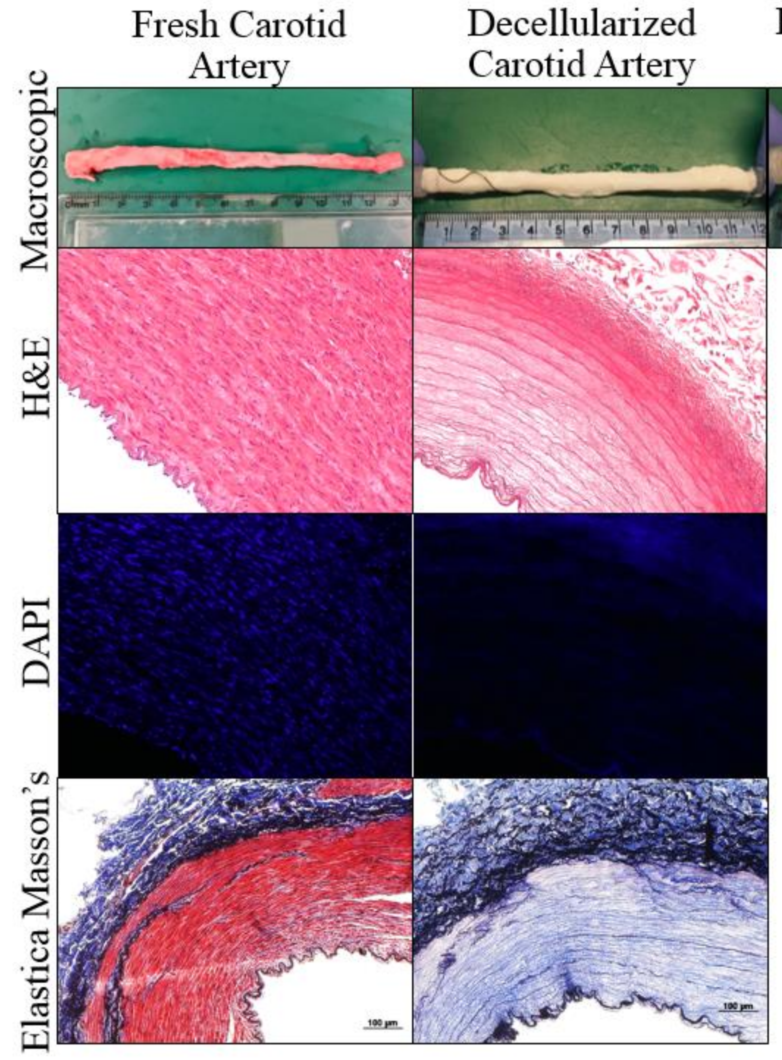
Human Endothelial Cells (hEC), human Smooth Muscle Cells (hSMC), and human Fibroblast (hFb) seeding.

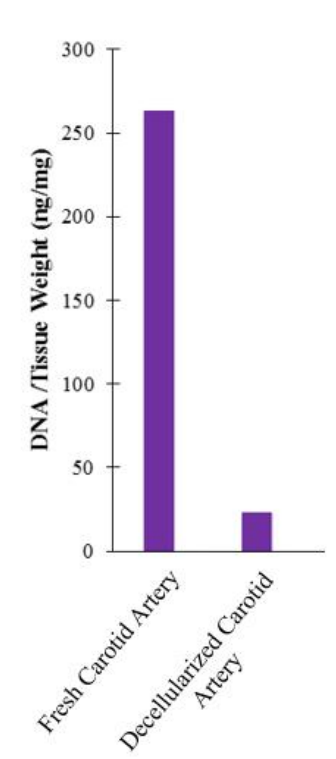
Vascular Bioreactor System

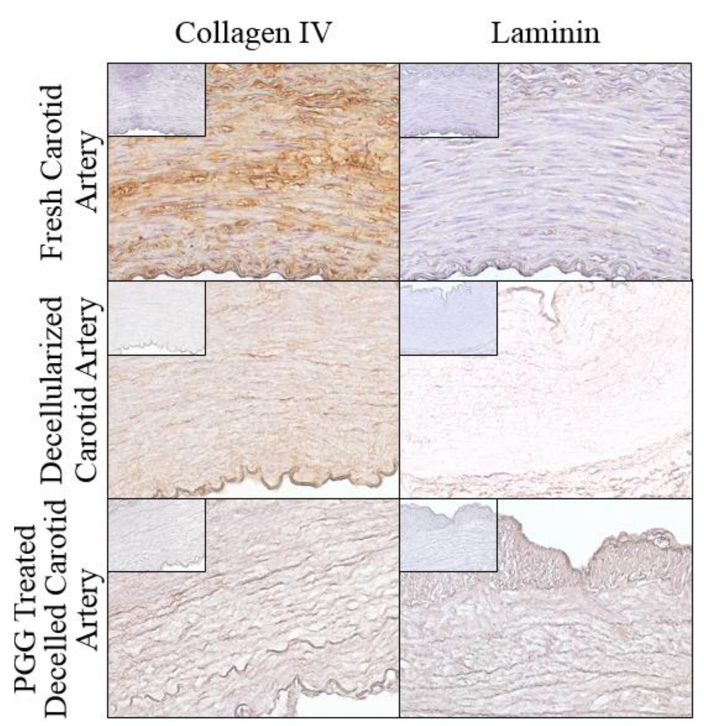
- Vascular bioreactor system includes:
  1-3) Pressure Controls
  4-5) Flow Manifolds
  6-7) Pressure Transducers
  8) Vascular Chamber
  9) Flow Meter
  10) Compliance Chamber
  11) Reservoir
  12) Pulse Dampener
  13) Peristaltic Pump
  14) Waveform Generating Pinch Valve
  15) Vascular Module Station
  16) Connections to LabVIEW.
- LabVIEW software is utilized for real-time pressure and flow control.



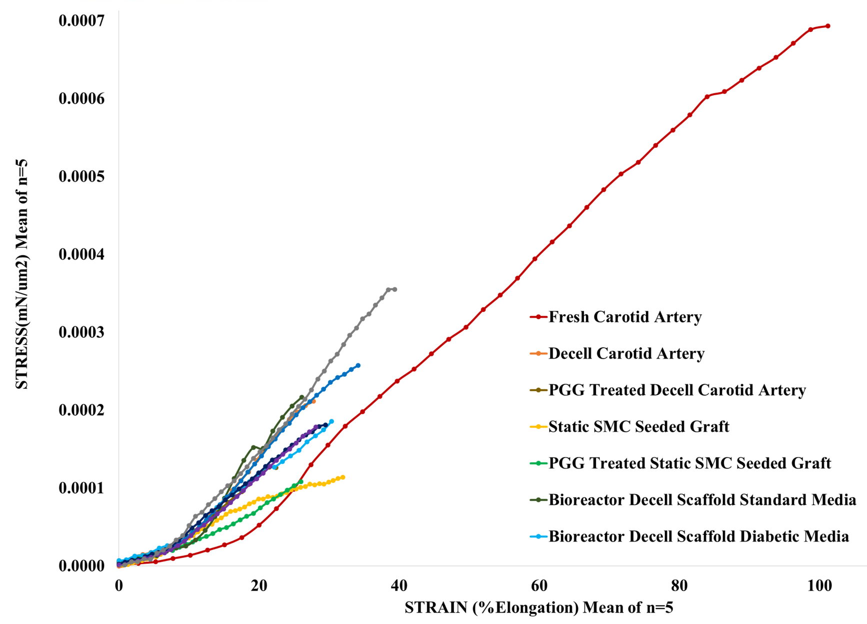
Results







- Macroscopic views of fresh, decellularized, and PGG-treated (yellow tint) carotid arteries.
- Complete removal of nuclear material was observed with H&E (purple) and DAPI (blue).

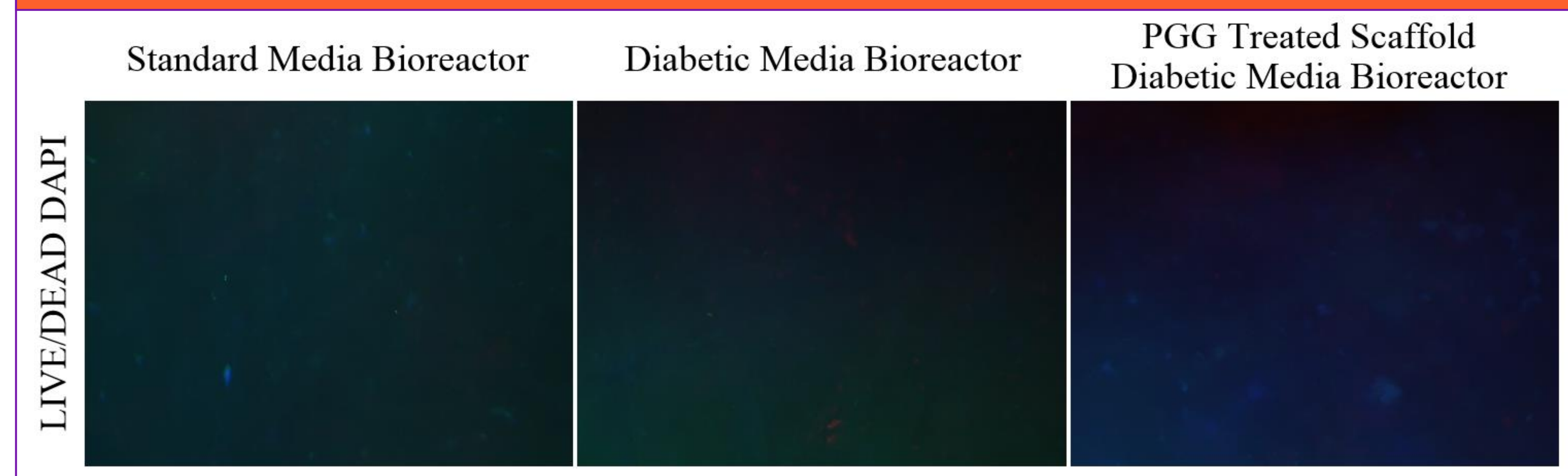


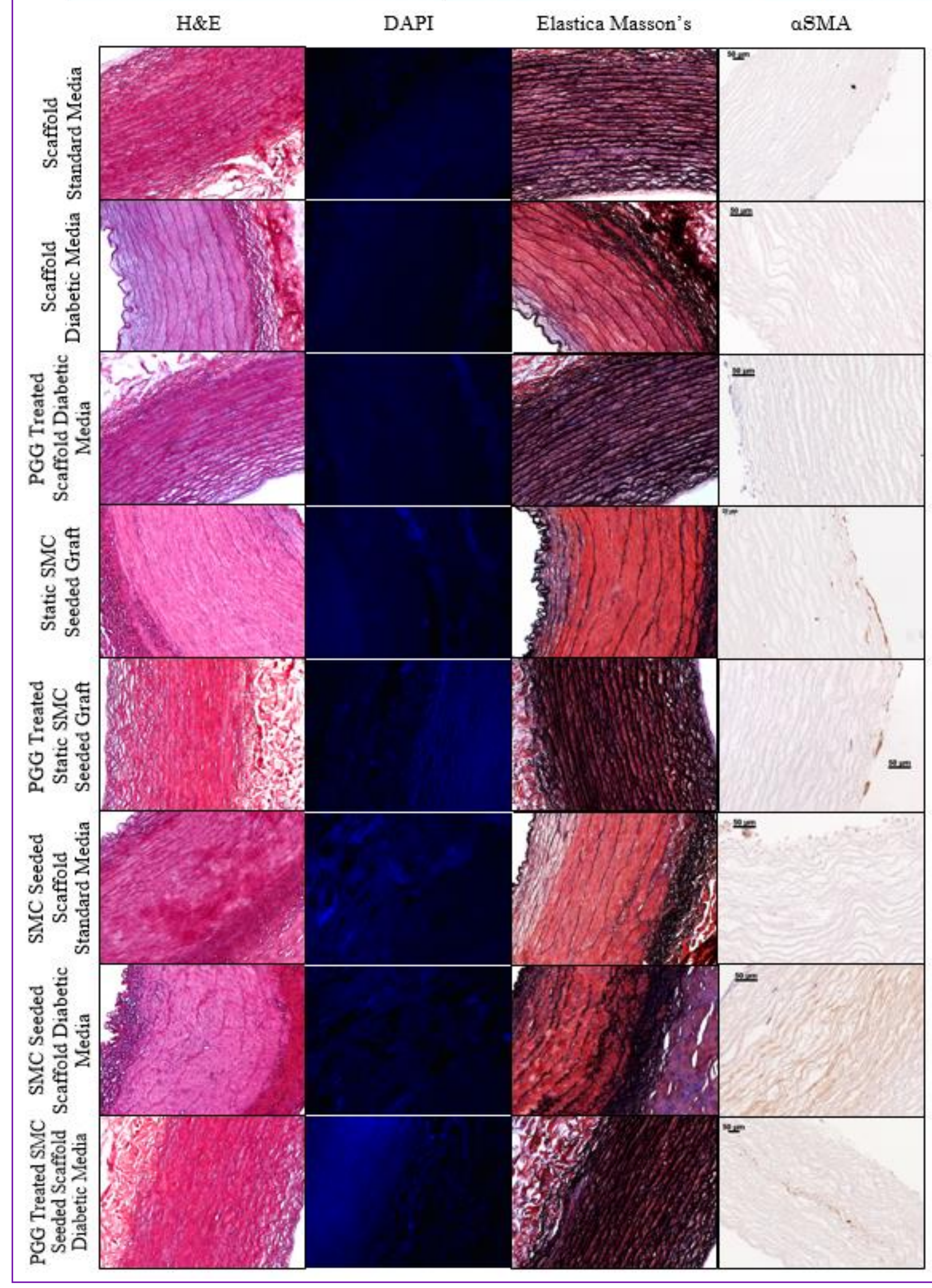
Sample	M_{Toe}	UTS	$\%E_F$
Fresh Carotid Artery	8.59×10^{-6}	6.93×10^{-4}	101.20
Decell Carotid Artery	1.00×10^{-5}	2.11×10^{-4}	27.79
PGG Treated Decell Carotid Artery	7.84×10^{-6}	1.26×10^{-4}	22.40
Static SMC Seeded Graft	2.42×10^{-6}	1.14×10^{-4}	31.94
PGG Treated Static SMC Seeded Graft	5.51×10^{-6}	1.08×10^{-4}	25.96
Bioreactor Decell Scaffold Standard Media	1.29×10^{-5}	2.17×10^{-4}	26.11
Bioreactor Decell Scaffold Diabetic Media	6.82×10^{-6}	1.86×10^{-4}	30.38
Bioreactor PGG Treated Decell Scaffold Diabetic Media	9.57×10^{-6}	2.57×10^{-4}	34.15
Bioreactor SMC Seeded Scaffold Standard Media	7.43×10^{-6}	1.81×10^{-4}	29.52
Bioreactor SMC Seeded Scaffold Diabetic Media	8.28×10^{-6}	1.78×10^{-4}	28.13
Bioreactor PGG Treated SMC Seeded Scaffold Diabetic Media	1.21×10^{-5}	3.55×10^{-4}	39.39

- Maintenance of extracellular matrix proteins, elastin (black) and collagen (blue), was observed with Elastica Masson's.
- DNA content in fresh and decellularized carotid arteries was quantified and compared.
- Immunohistochemistry (IHC) staining observed basement membrane protein preservation (positive = brown) of collagen IV and laminin after decellularization.
- Fresh, decellularized, PGG-treated, and mechanically conditioned grafts were exposed to tensile forces; elastic modulus of the toe (physiological) region (M_{Toe}), Ultimate Tensile Strength (UTS), and percent elongation at failure ($\%E_F$) results are shown.

All Images 20X Magnification

Results





- LIVE/DEAD DAPI staining of vascular grafts after bioreactor conditioning.
  Green = Live cells
  Red = Dead cells
  Blue = Cell Nuclei
- H&E staining observed cell nuclei (purple) of SMC seeding on static and dynamic grafts.
- Seeded SMCs were visualized with DAPI (blue) on static and dynamic grafts.
- Elastin (black) and collagen (blue) were observed with Elastica Masson's after bioreactor conditioning.
- IHC was used for visualization of SMC expression (brown) with αSMA.

All Images 20X Magnification

Conclusions

- Based on the results of this study, it can be confirmed that our vascular bioreactor system models physiological flow and pressure in carotid arteries.
- Future work will observe the impact diabetes has on vascular cells and ECM components; additionally, comparisons will be made between PGG treated grafts and non-PGG treated grafts.

Acknowledgements

This research was supported, in part, by the National Institute of Health (RO1 HL 133303).