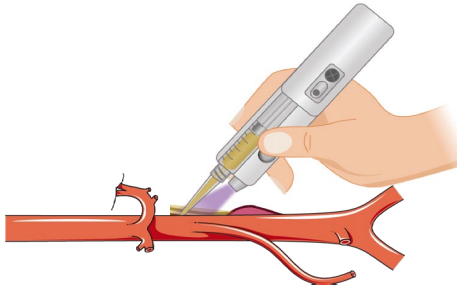
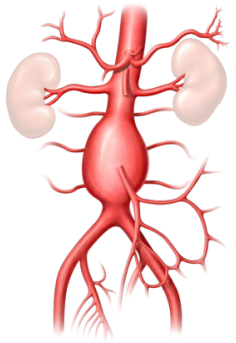
INTERLEUKIN-10 HYDROGEL-BASED "BIO-INK" THERAPY REDUCES ABDOMINAL AORTIC ANEURYSMS IN RODENT MODEL





Theresa Doiron, BS, Lab Director
Jennifer Stashevsky, MS, Histologist
Division of Vascular Surgery

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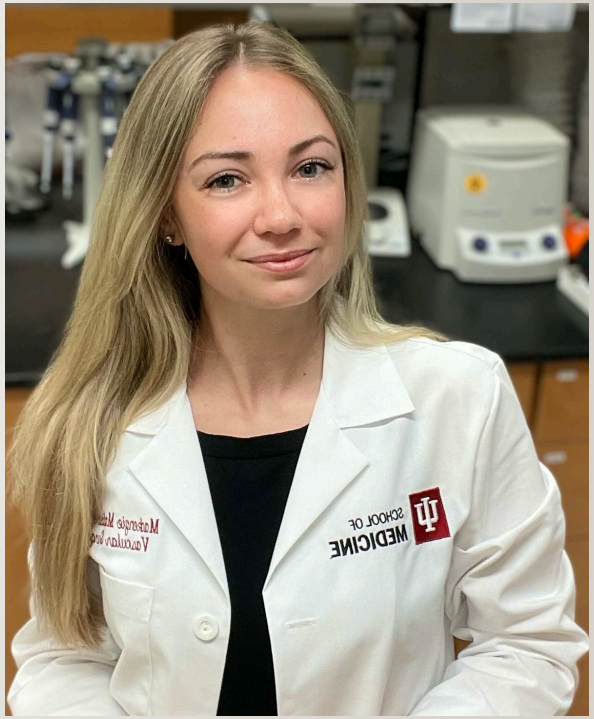

Dr. Mackenzie Madison

Introductions

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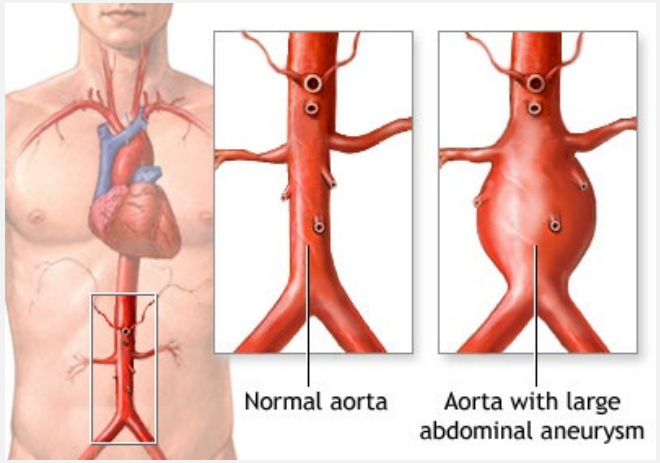
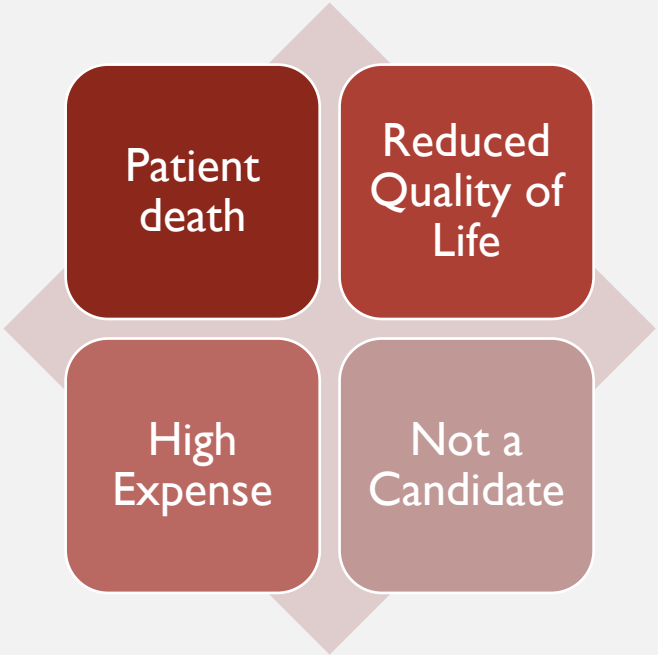


ABDOMINAL AORTIC ANEURYSM (AAA)

- Weakening and bulging of aortic wall due to tissue breakdown
- Increase in aortic diameter (>3 cm)
- 200,000 AAA cases in US/year
- Accounts for 15,000 deaths annually



Only medical intervention proven to reduce the risk of AAA rupture is surgical repair with risks.

90% of patients diagnosed with AAA have aneurysms that are below 5.5 cm threshold for surgical intervention.

Some patients cannot receive endovascular treatment due to aortic anatomy and are at high risk for open repair due to associated comorbidities.

INTERLEUKIN-10 HYDROGEL & BIOPENCIL

New Method of Treatment

Material:

Hydrogel (Gelatin-based "bioink")

↓

Interleukin 10 (IL-10) + Basic Fibroblast Growth Factor (bFGF)

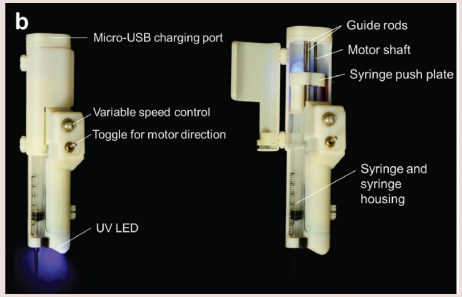
↓ ↓

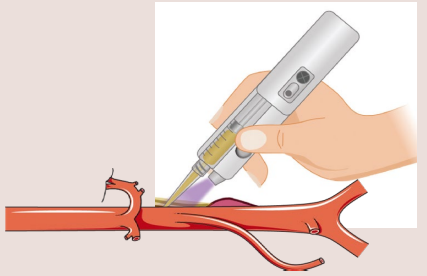
Anti-inflammatory molecule

Regulate cell proliferation and differentiation for wound healing

Delivery:

Biopencil: custom-made handheld hydrogel extruder





Hypothesis: IL-10 Bioink hydrogel will work to stop aneurysm expansion by…

Modulating immune responses to the aneurysm wall

Increasing wall thickness and structural integrity

Extracellular Matrix (EM) Degradation

Elastin breakdown

Elastin-Derived Peptides (EDPs) recruit inflammatory cell

Inflammatory Cell Invasion

M1 Macrophage: Proinflammatory

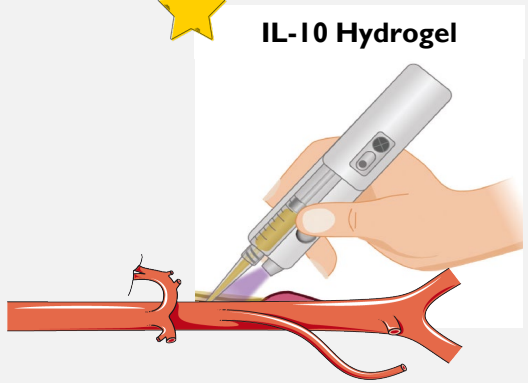
M2 Macrophage: Anti-inflammatory
- IL-10

KEY

Phenotypic switch

M1 → M2

Promotes wound healing

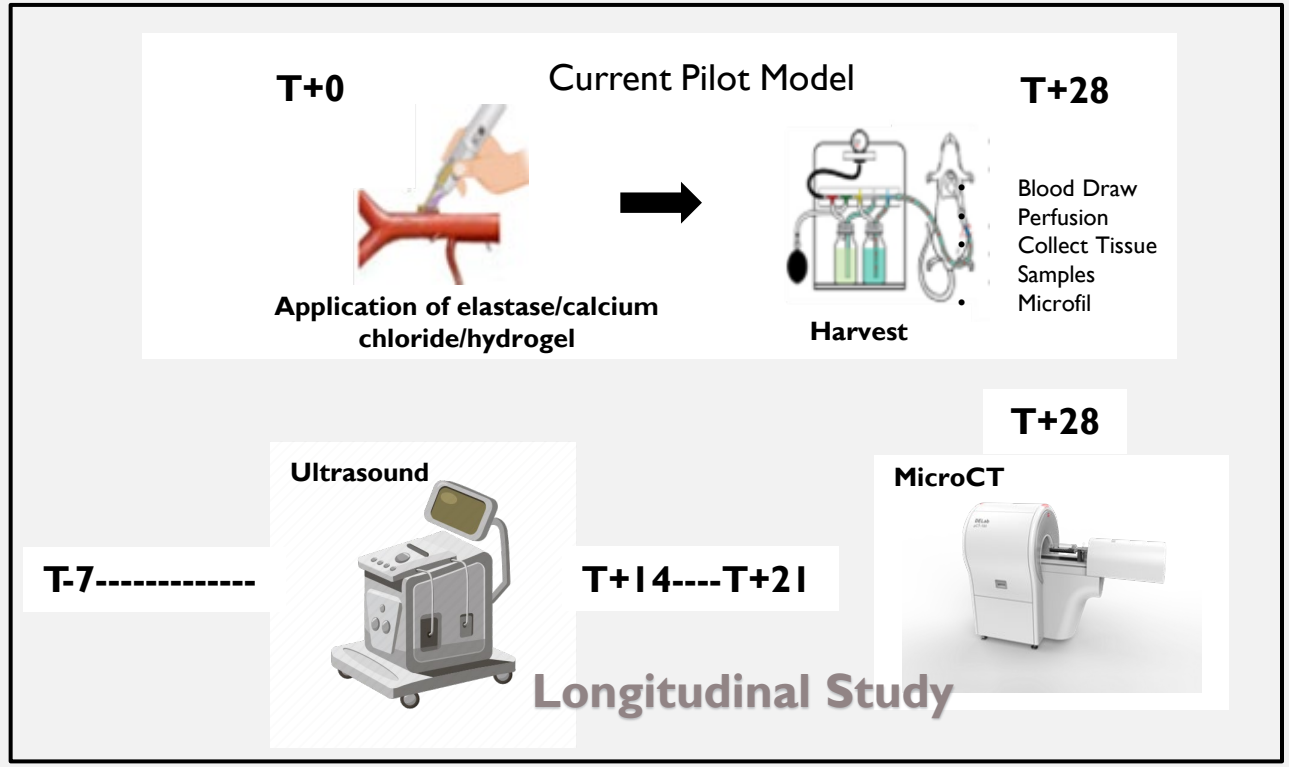


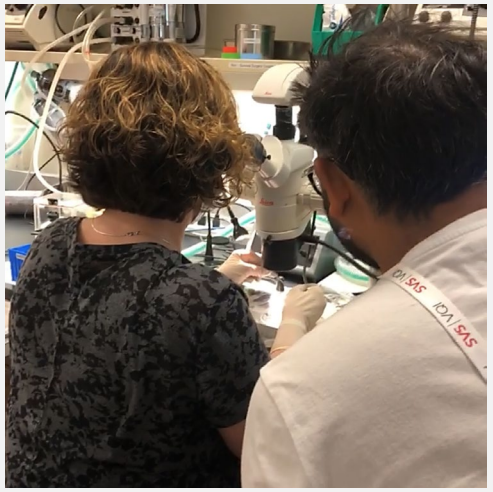
EXPERIMENTAL MODEL

- Pilot Study
  - N = 12
    - 8-week-old Sprague-Dawley (male & female)
  - Porcine pancreatic elastase bath (5 mins)
  - Calcium chloride bath (10 mins)
  - Hydrogel (7.5% agarose, gelatin-based hydrogel)

Control Group: **Sham Hydrogel**
Treatment Group: **IL-10 Hydrogel**

- Longitudinal Study
  - Ultrasound





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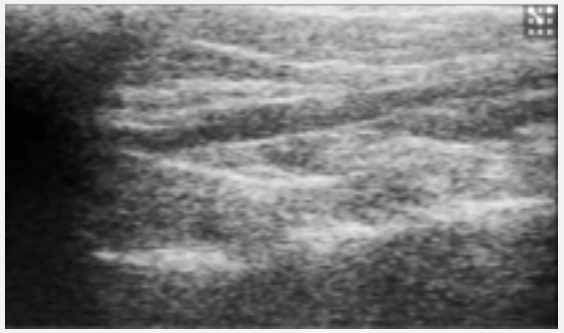
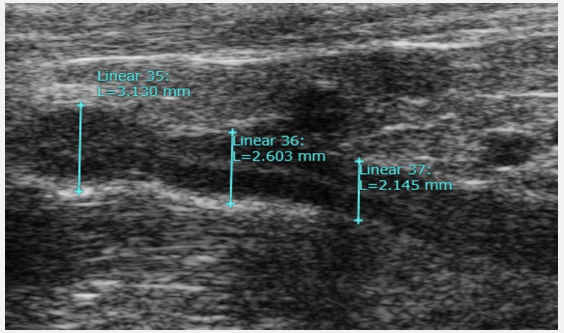
Ultrasound

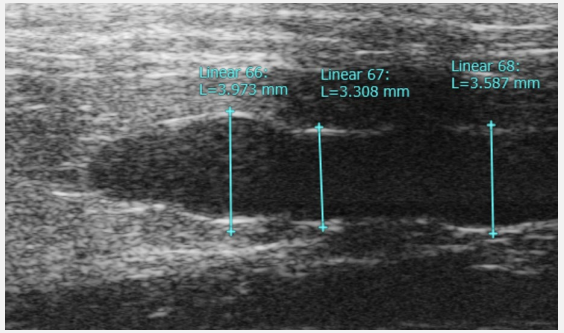
Baseline

T + 11 days

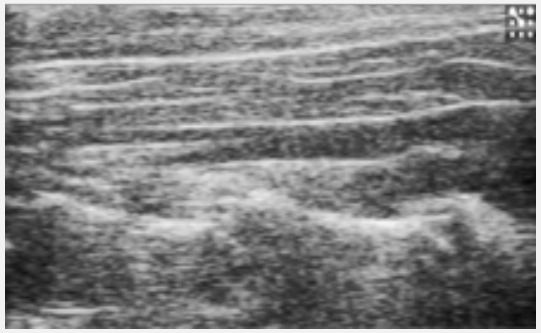
T + 17 days

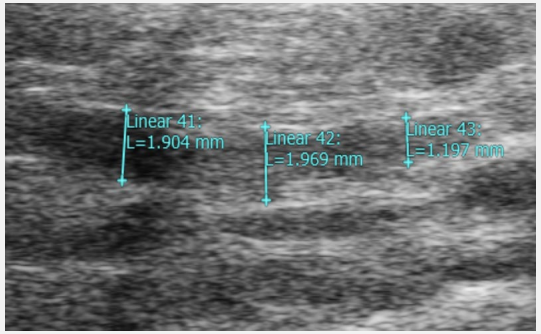


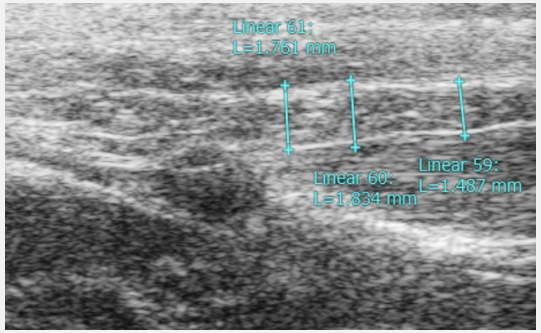




Sham Hydrogel



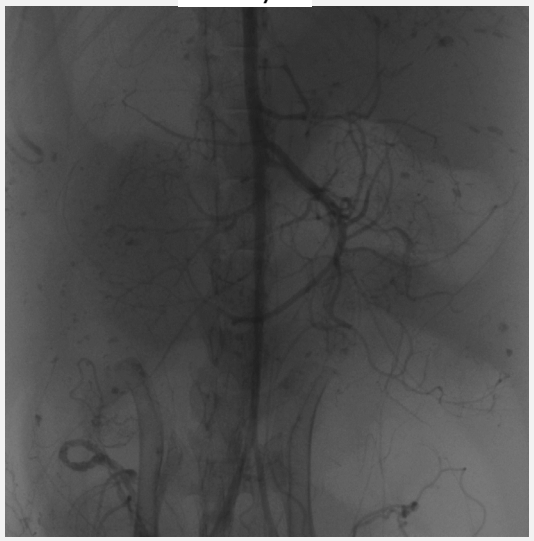




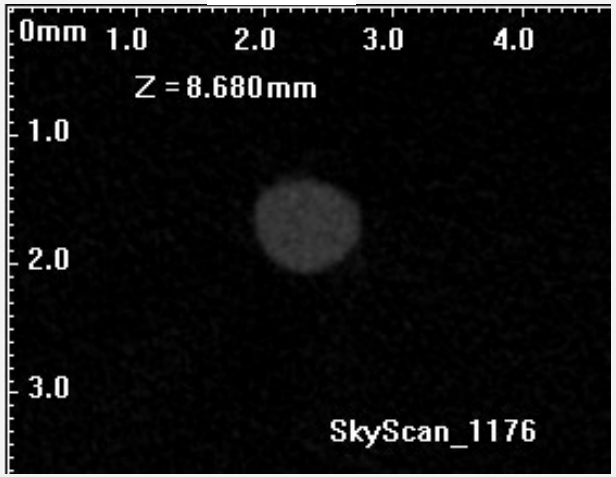
IL-10 Hydrogel

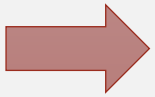
MicroCT

X-ray

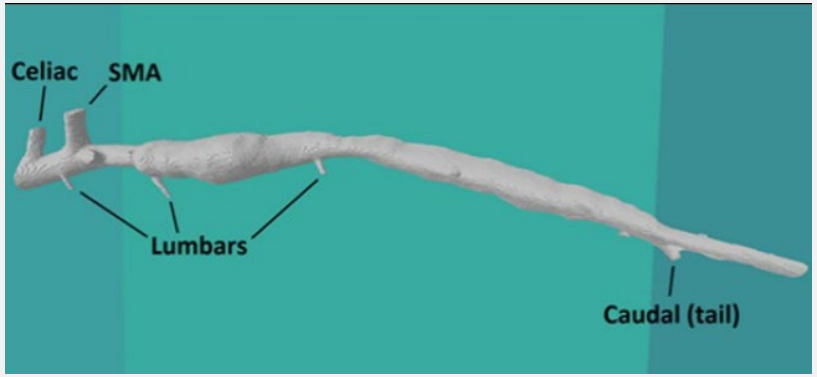


CT





3D Reconstruction



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ANALYSES

01

Histology and IHC

- Aorta and Spleen
- Morphology of tissue
- Macrophages

02

MicroCT Analysis

- Aortic Volume Analysis
- 3D Modeling

03

Flow Cytometry

- Aorta and Spleen
- M1-M2 macrophage ratio

Extracellular Matrix (EM) Degradation

Elastin breakdown

Elastin-Derived Peptides (EDPs) recruit inflammatory cell

Inflammatory Cell Invasion

M1 Macrophage: Proinflammatory

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- IL-10

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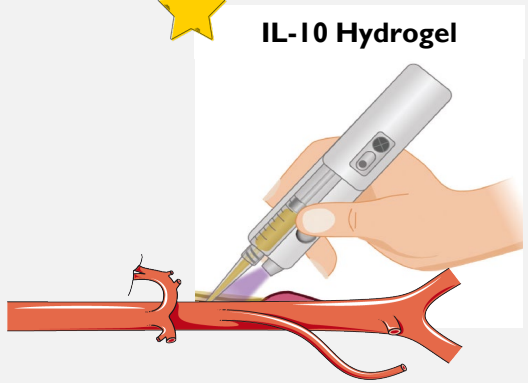
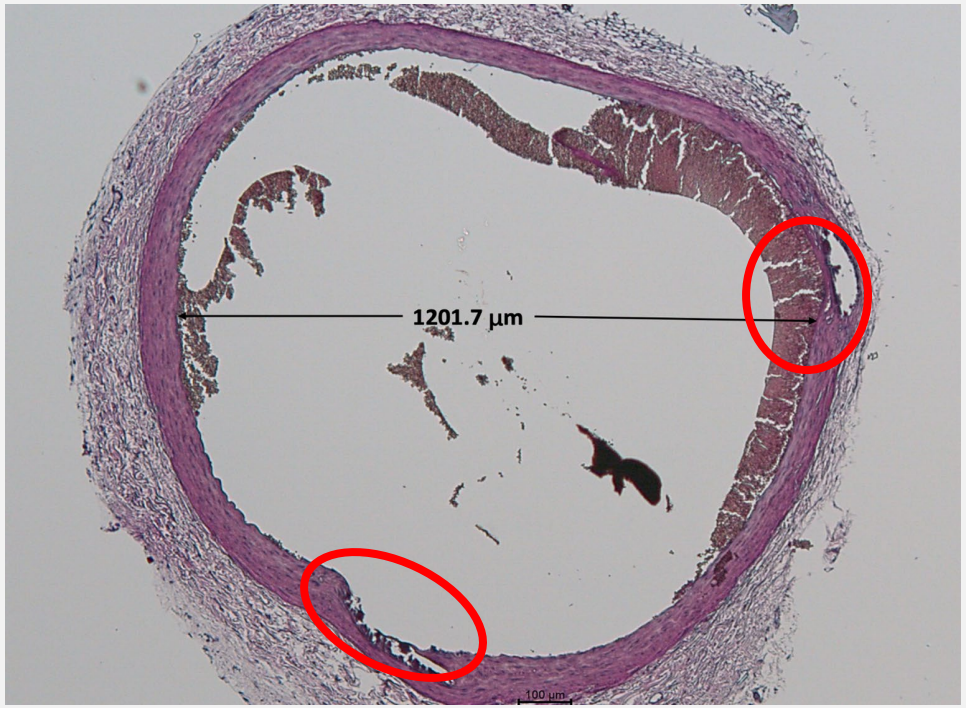
Promotes wound healing



IL-10 MAINTAINS AORTIC STRUCTURE AND INTEGRITY

Histology



Sham Hydrogel

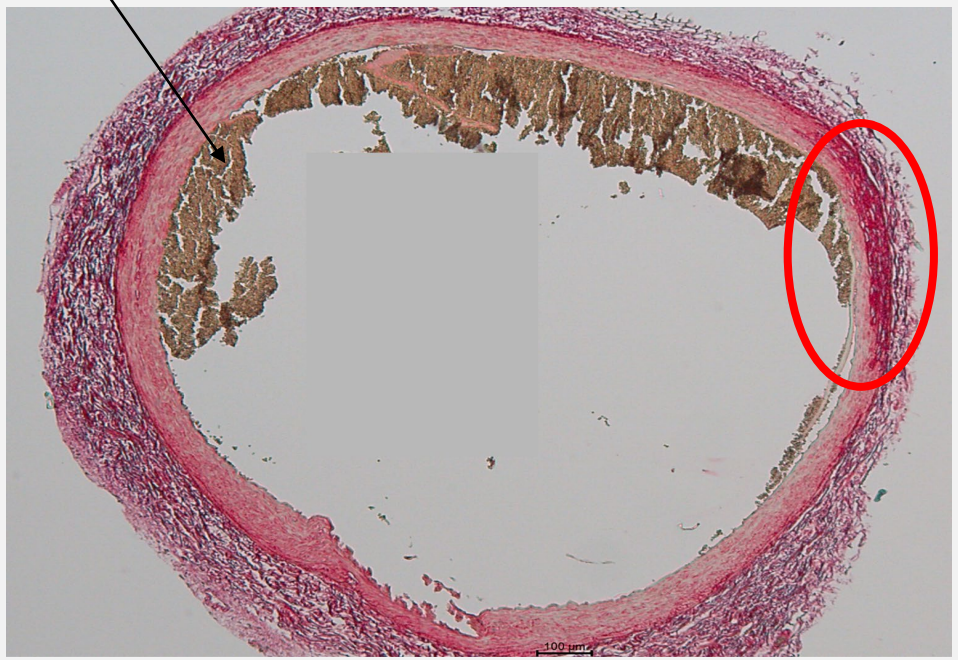
IL-10 Hydrogel

Hematoxylin & Eosin

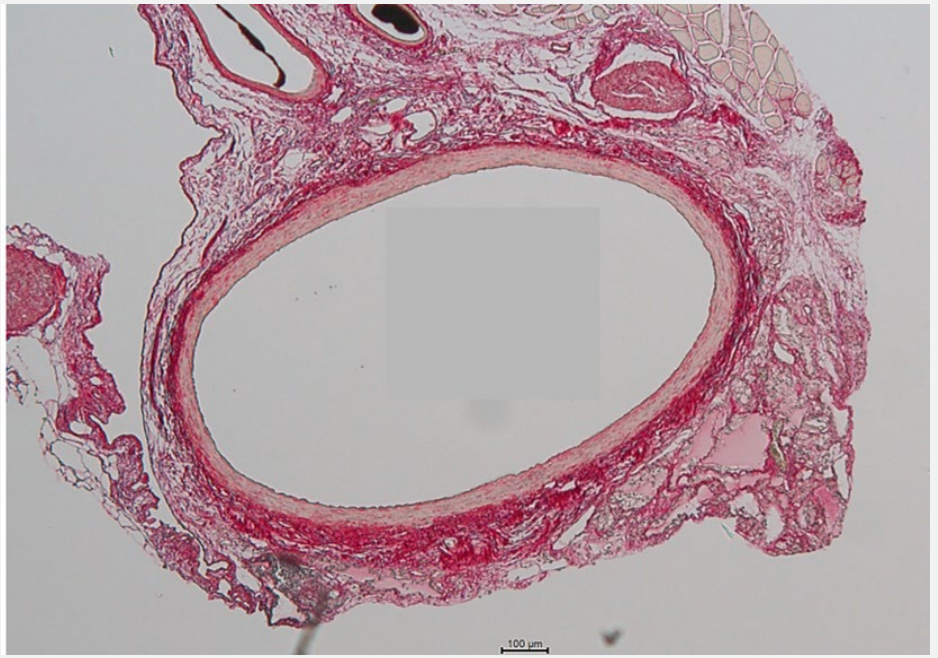
IL-10 REDUCES COLLAGEN DEPOSITION

TENSILE STRENGTH OF AORTIC WALL

Histology



Sham Hydrogel



IL-10 Hydrogel

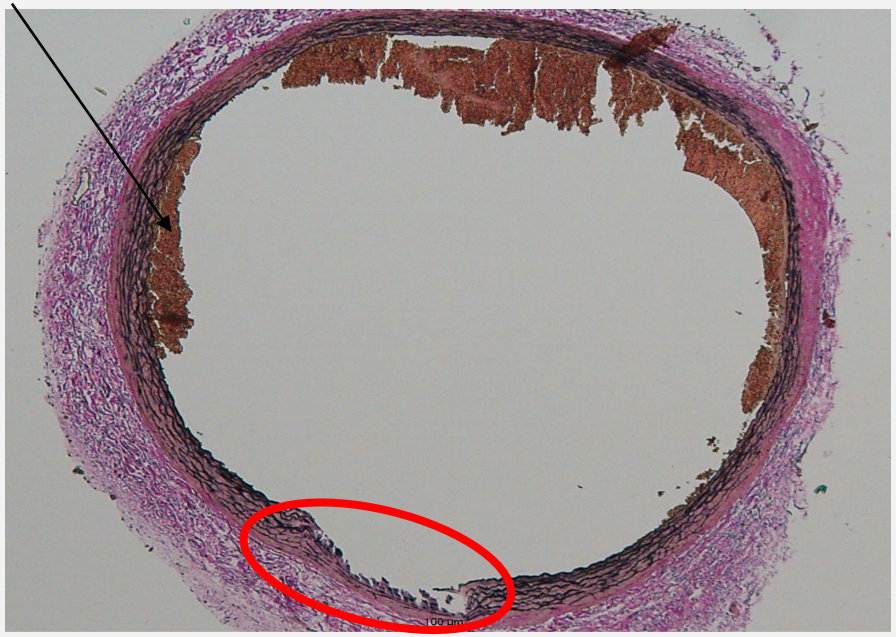
Picrosirius Red

IL-10 PREVENTS ELASTIN DEGRADATION

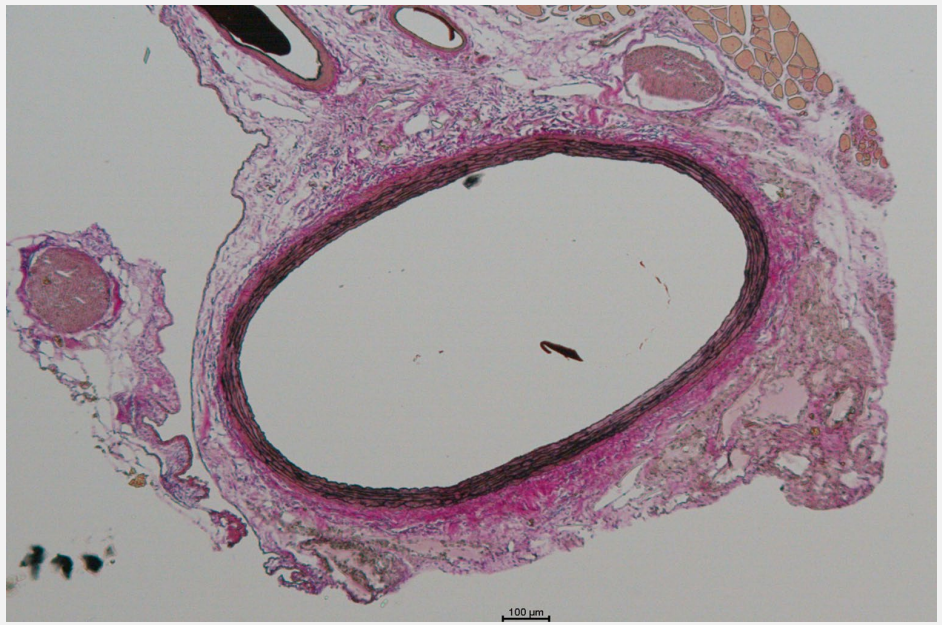
VASCULAR ELASTICITY AND STRUCTURAL INTEGRITY OF AORTIC WALL

Histology

Residual Blood



Sham Hydrogel



IL-10 Hydrogel

Verhoeff-Van Gieson

Extracellular Matrix (EM) Degradation

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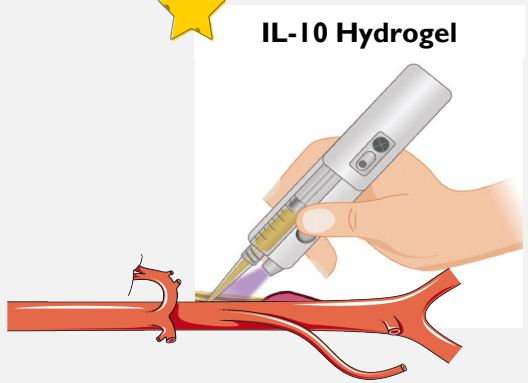
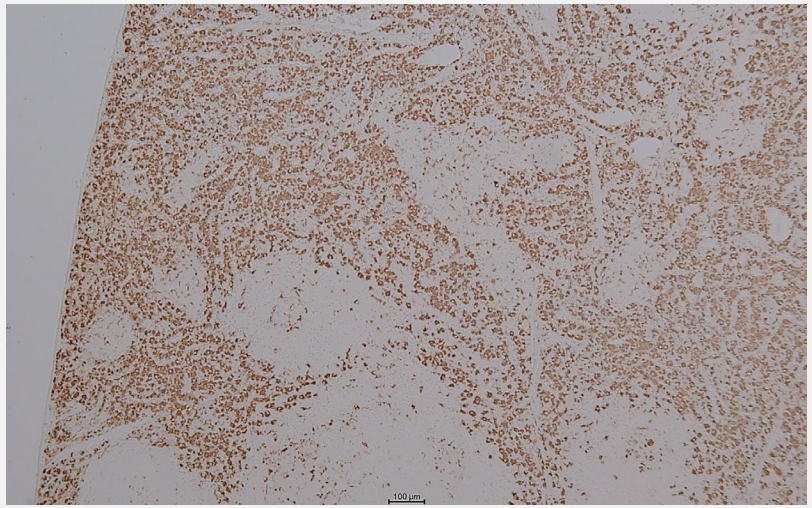
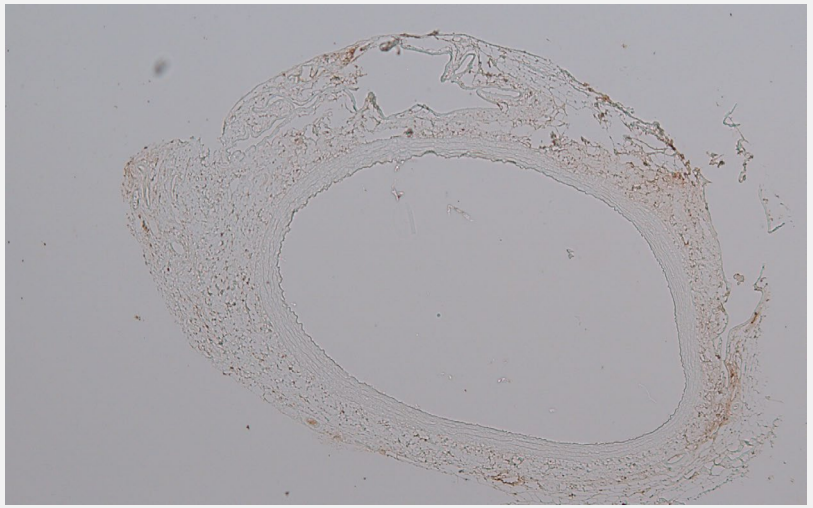


MACROPHAGES

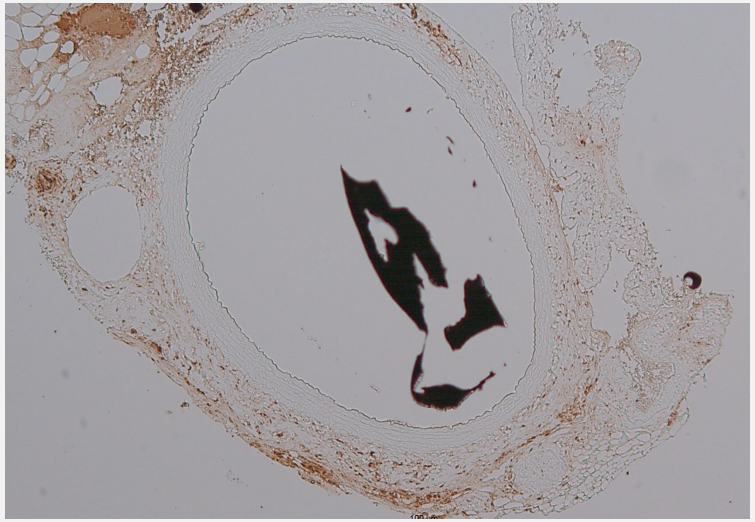
Immunohistochemistry



Spleen: Positive Control



Aorta: Sham Hydrogel

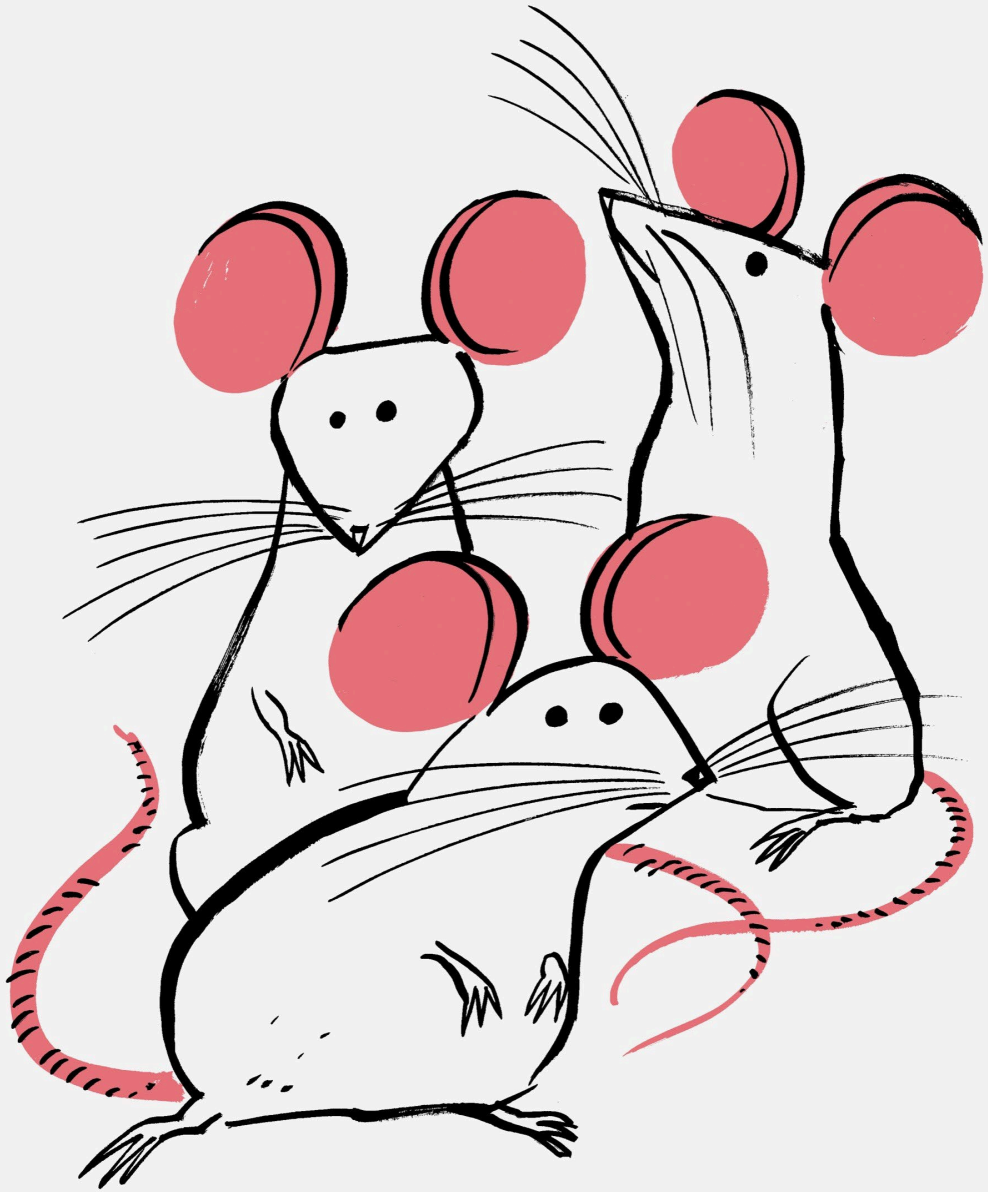


Aorta: IL-10 Hydrogel

ED1 (CD68)

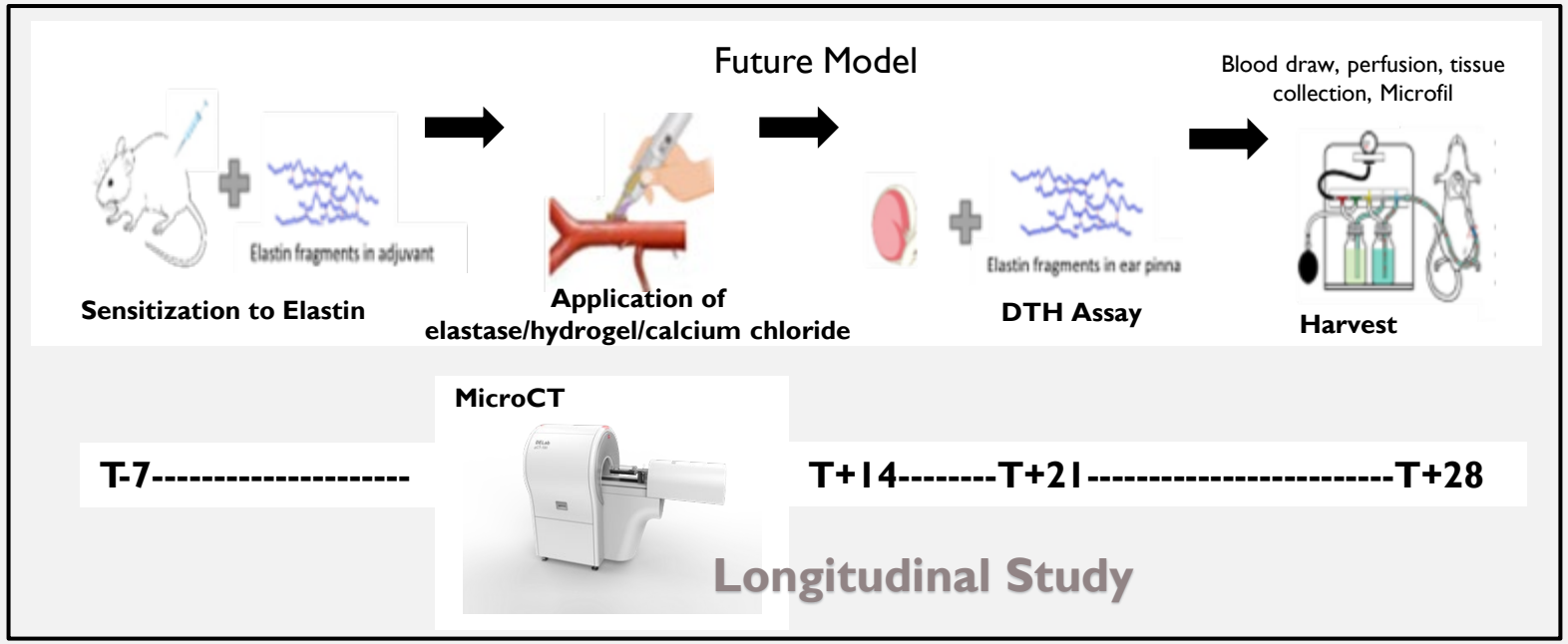
Stains all macrophages

FUTURE WORK



1.) Pilot Study → Working Protocol

- Elastin Sensitization
- Delay Type Hypersensitivity Assay (DTH)
- Continuous Contrast Agent (longitudinal analysis using MicroCT)

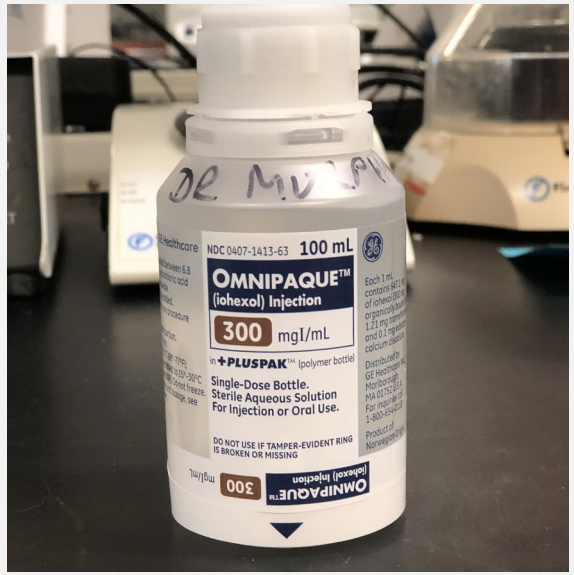


Ultrasound

- Subject to interuser variability
- Cannot make precise measurements of aortic diameter

MicroCT

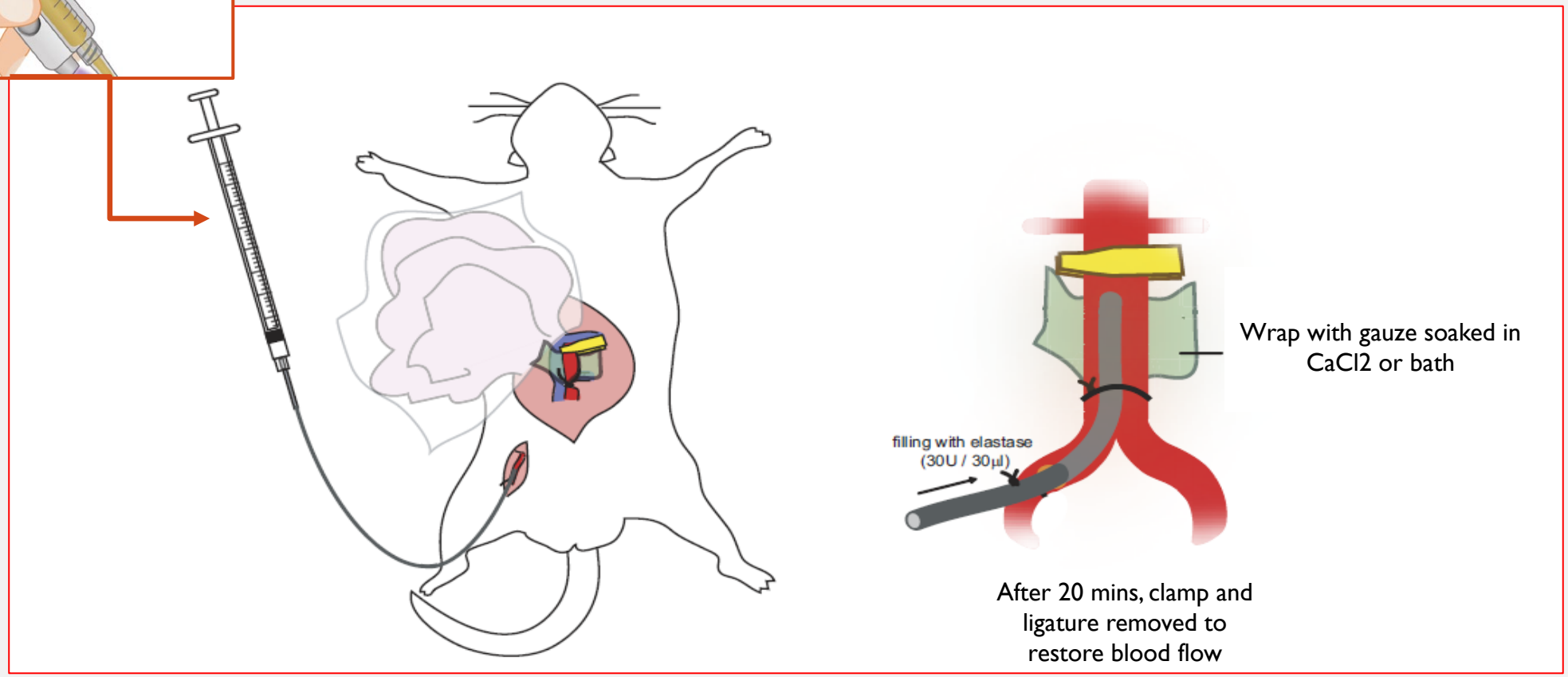
- High resolution technique
- 3D reconstruction to analyze aortic volume
- High repeatability



2.) Creation of Alternative Elastase AAA Model

Intralumenal route of elastase + Extraluminal route of calcium chloride

Report to have 93% success rate for creation of AAA with zero mortality



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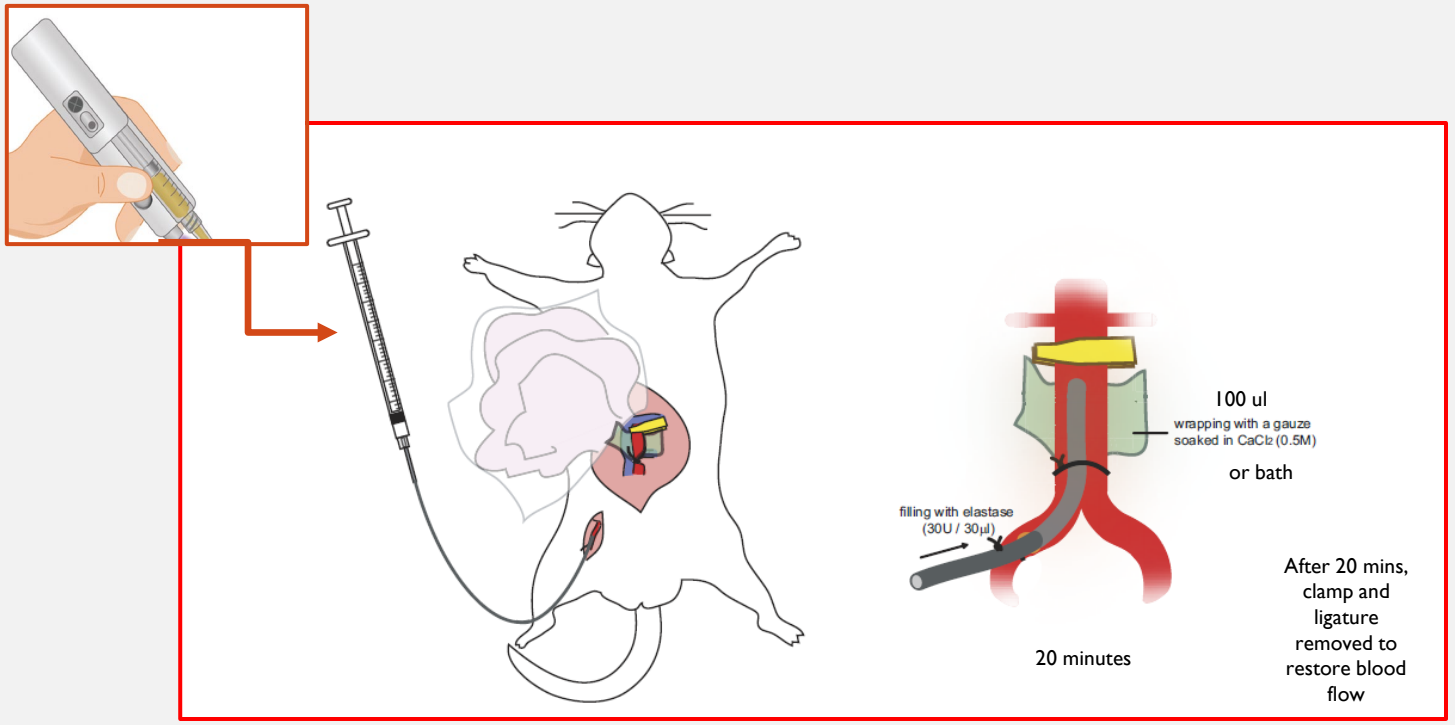
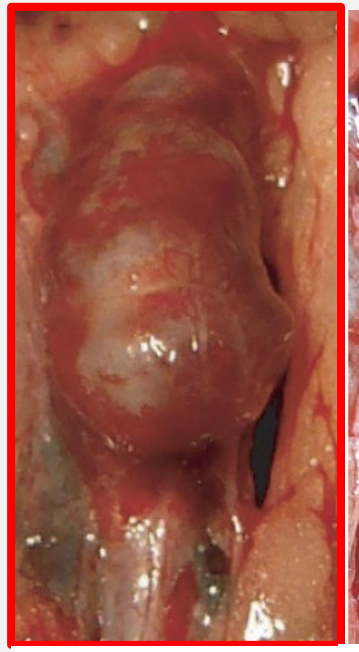
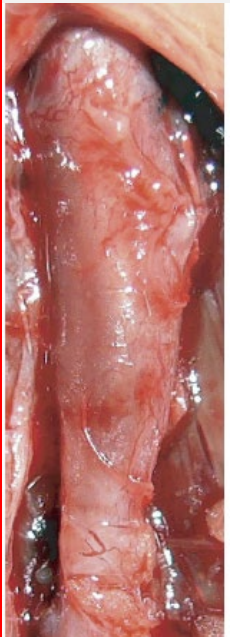


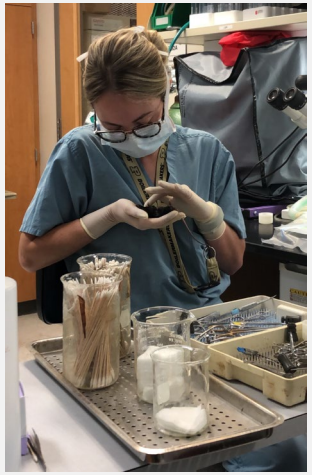


Intralumenal Application



Extralumenal Application

- More clinically applicable
- Stronger evidence-based outcomes
- More challenging



Amazing Team!

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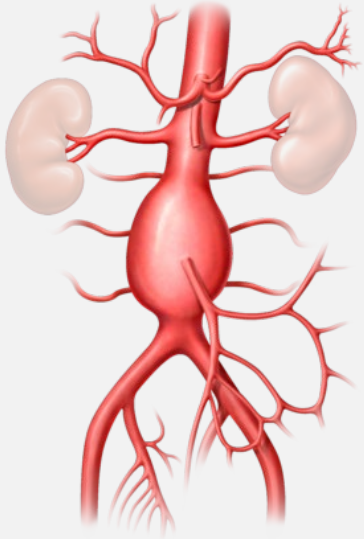
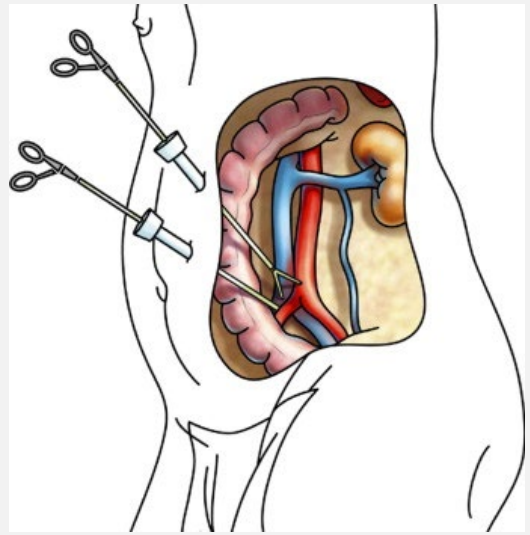
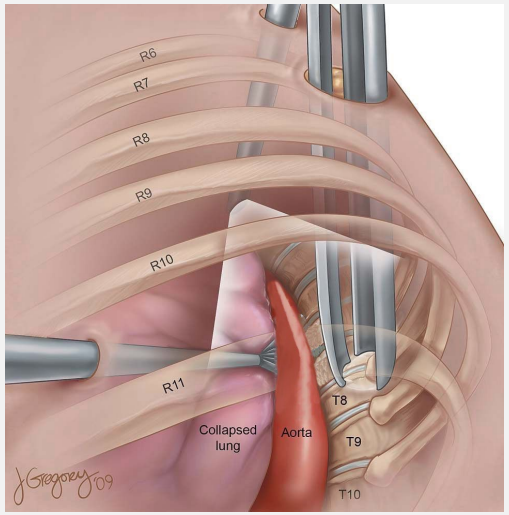
CLINICAL APPLICATION

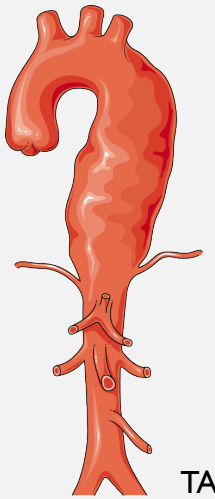


AAA



Laparoscopic Surgery





TAAA

Video Assisted Thoracoscopic Surgery (VATS)

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ACKNOWLEDGEMENT

Lab Personnel

- Michael P. Murphy, MD
- Steven J. Miller, PhD
- Mackenzie Madison, MD
- Chang-Hyun Gil, PhD
- Jennifer Stashevsky, MS
- Nic Moldovan, PhD
- Humraaz Samra, MD
- Leni Moldovan, PhD
- Lili Zhang

Students

- Olivia Jimenez (IMPRS)
- Stone Chen (IMPRS)
- Anush Motaganahalli, BS
- Kevin Silva, BS

Support

- Cryptic Masons Medical Research Foundation
- Society of Vascular Surgery

APPENDIX

3.) Alternative Rat Species – Assess Comorbidity of Aging and Hypertension

- **Spontaneous Hypertensive Rat (SHR)**
  Only Male SHR will be used because female SHR do not express a true hypertensive phenotype.

- **Normotensive Wistar Kyoto (WKY) Rat**