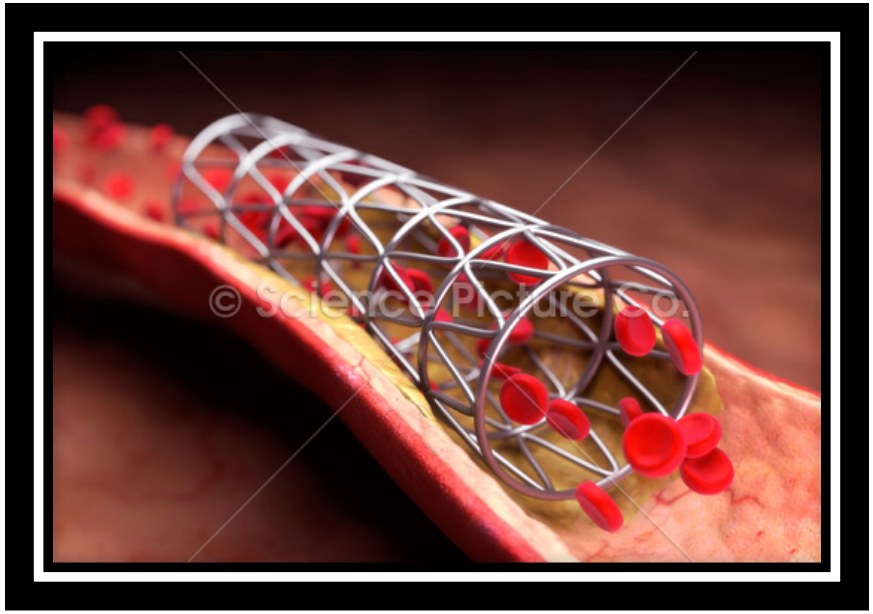
Student Researcher: Jackie Model
Mentor: Dr. Steven A. Jones

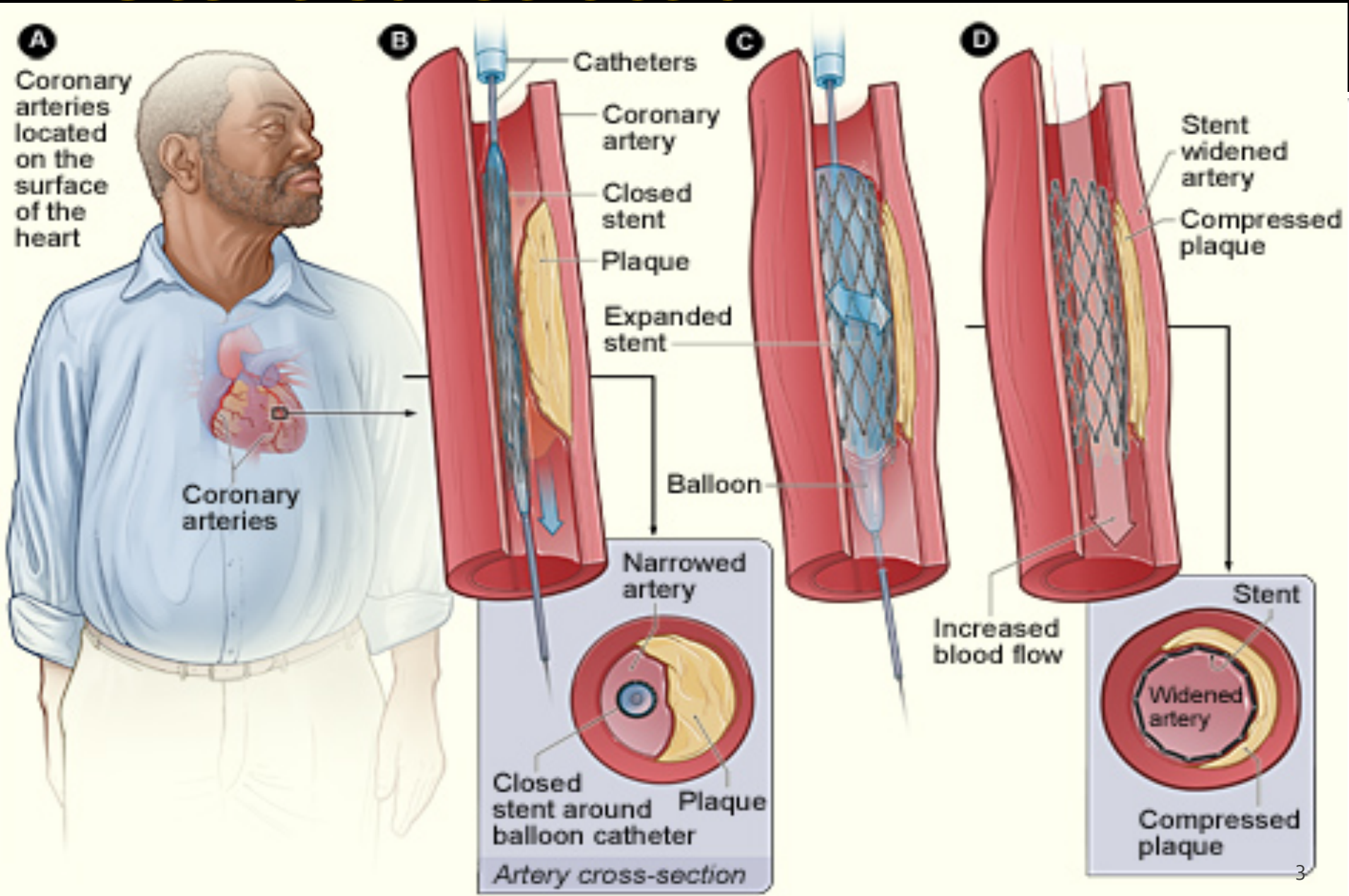
Platelet Adhesion Studies

Introduction

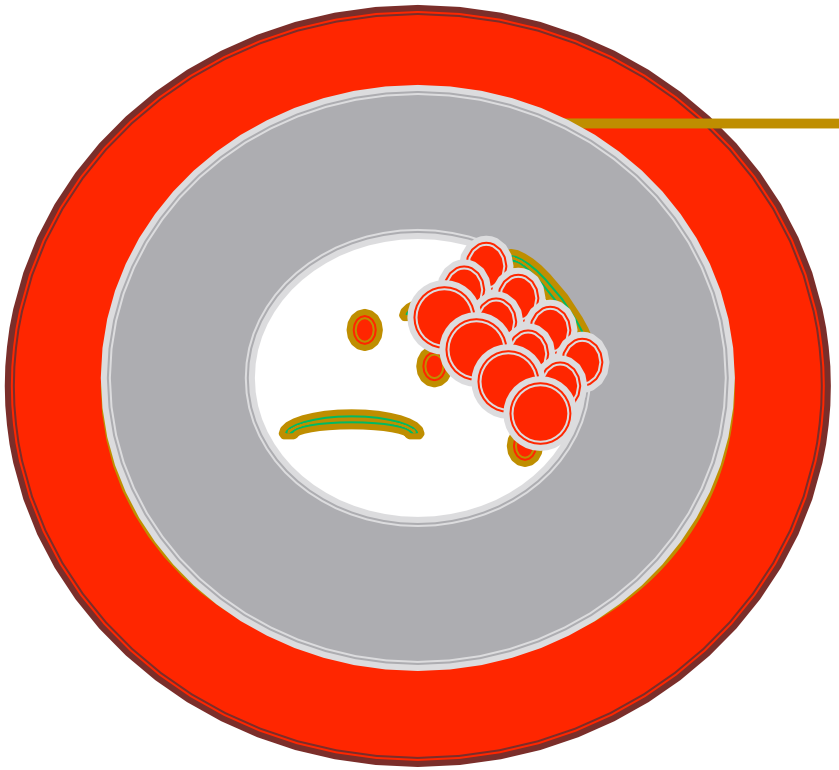
- Currently, after major arterial damage arterial stents are placed inside blocked or damaged arteries
- Platelets in blood lead to cell growth that can re-clog arteries
- Study the use of fibrinogen to prevent stent complications
- Platelet adhesion is to be studied on or near the fibrinogen stripe



Stent Construction



Demo of Stents with fibrinogen

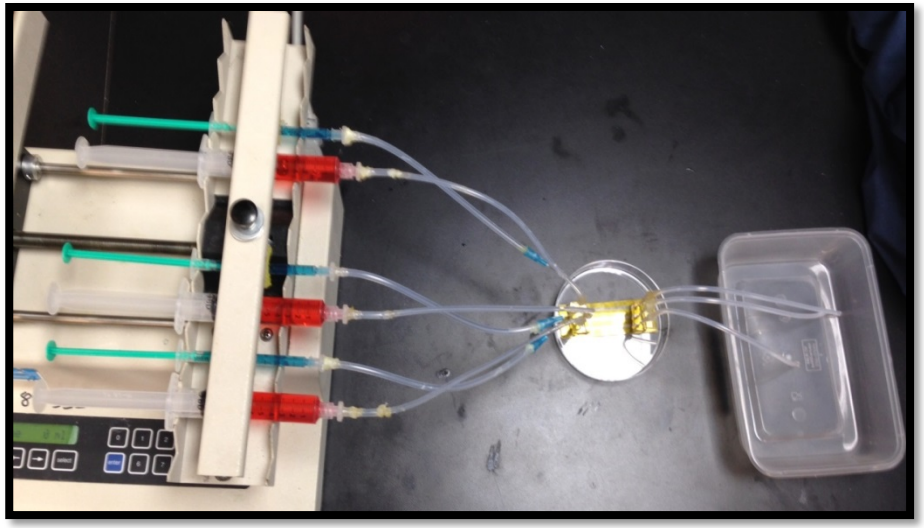


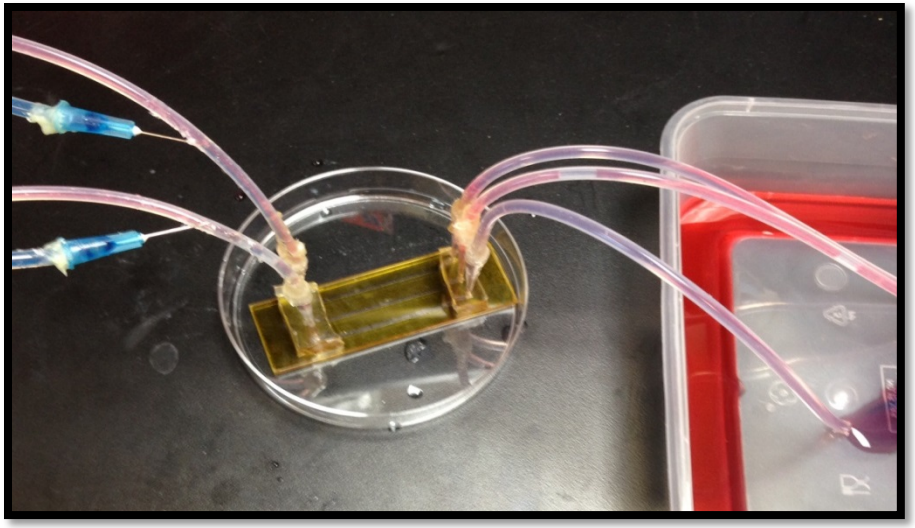
Background information

- Platelets are cells found in blood that help with clotting (thrombus)
- Fibrinogen is a muscle fiber that is produced by the liver
  - Main functions are overseeing blood clotting and inflammation
  - When bleeding occurs protein will become a fiber and act as a platform for platelets to attach
  - Use fibrinogen and platelets in this study to see how both would react in the body
- Collagen (being used in current study) is a protein abundant in the skin
  - Major structural proteins found in skin
  - In blood adhesion collagen fibers fall off the damaged vessel wall
  - The platelets have a specific activator on cell membrane to attach to the parts where collagen fell
  - Use collagen and platelets in study to see how both would react in the body

Experimental Methods

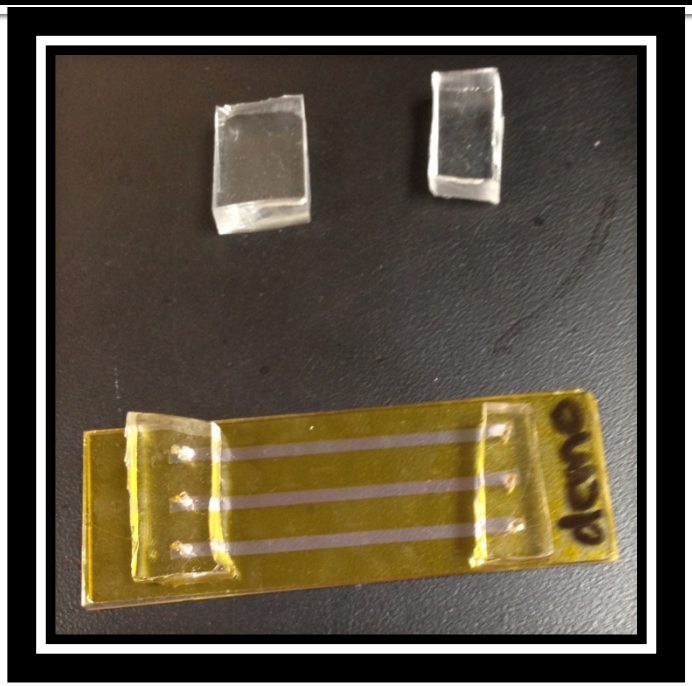
- Bovine whole blood perfused through micro-channels
- Micro-channels were coated through layer-by-layer assembly
- Single stripe of FITC-labeled fibrinogen was added across the width of each channel

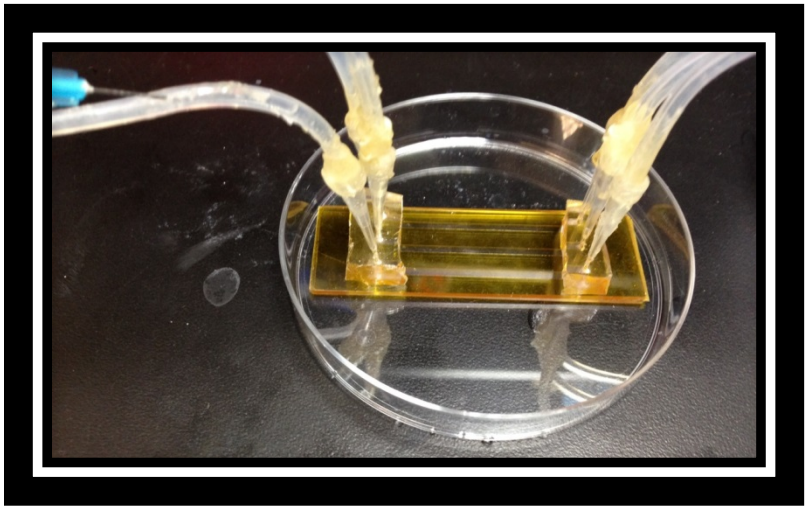




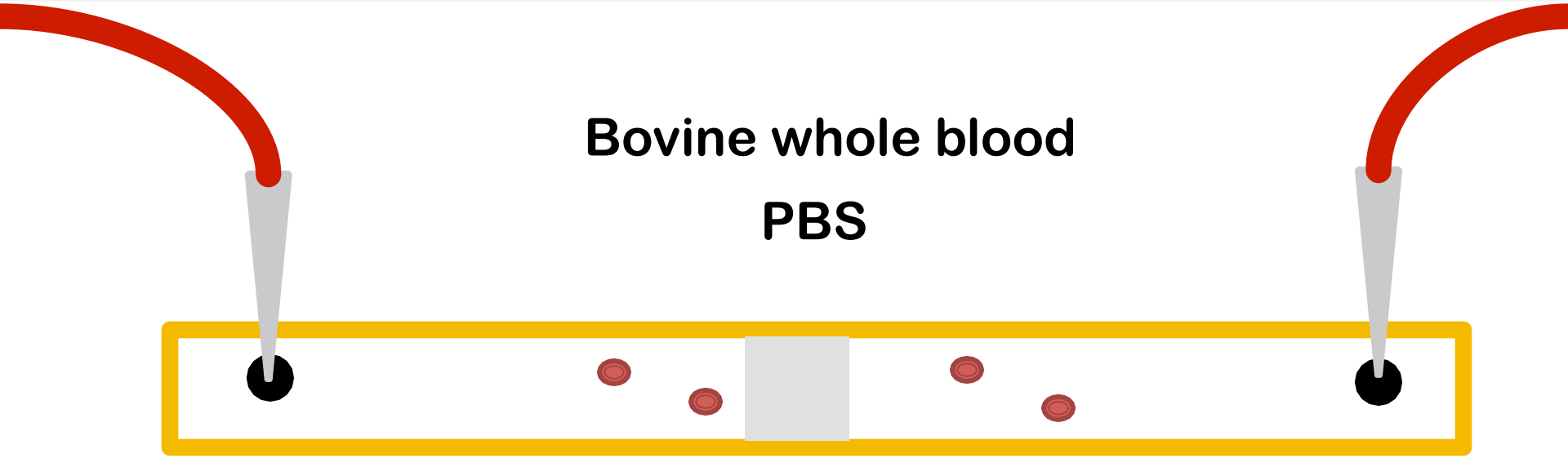
Experimental Methods cont.

- Channels were tagged with Acridine Orange (AO)
- Examined under a flouroscent microscope
  - FITC was used to examine fibrinogen stripe and TRITC was used to for platelets





Chemicals in channels



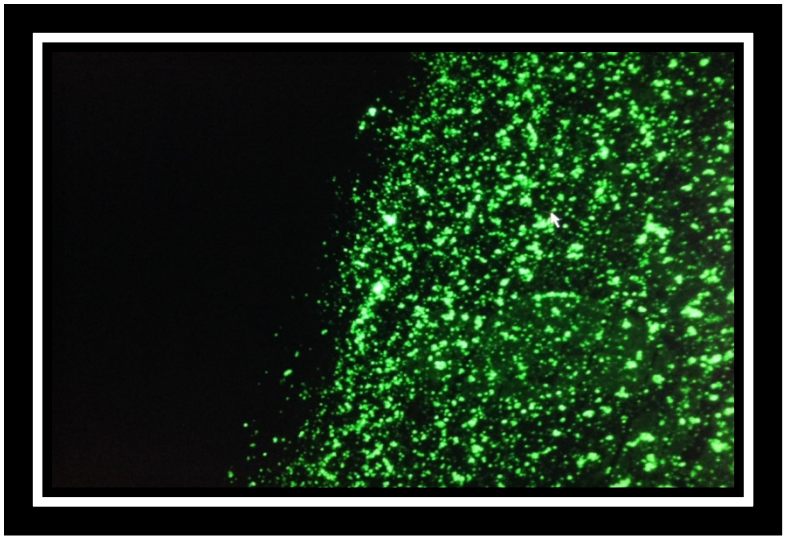
Glutaraldehyde

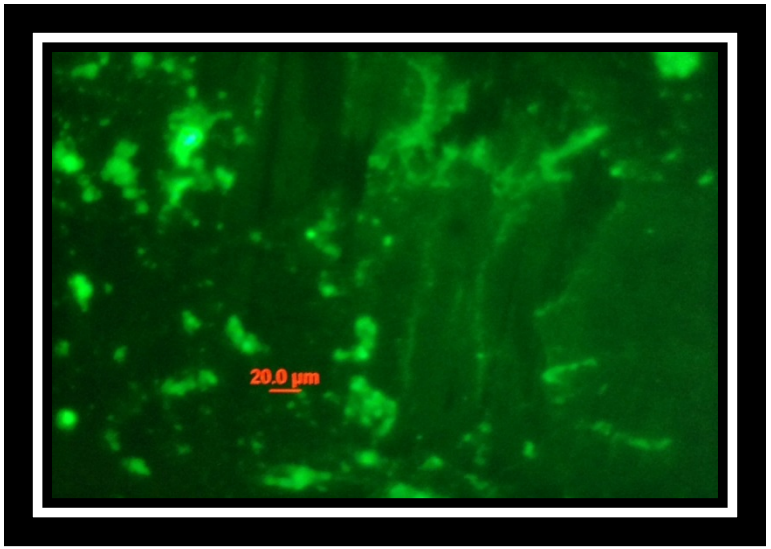
Isopropyl Alcohol

AO Stain

Results

- Expected to see platelet adhesion near stripe's edge
- Before testing, fibrinogen stripe was clear and distinct under FITC imaging
- After blood was perfused, fibrinogen stripe became indistinct



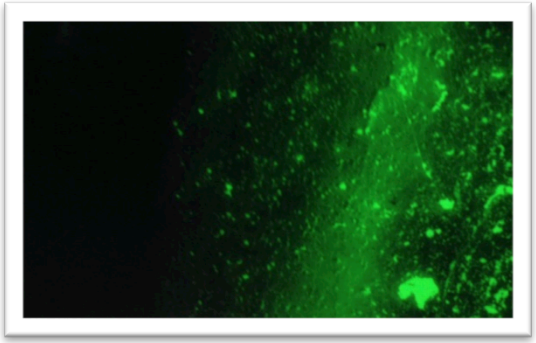


A change up

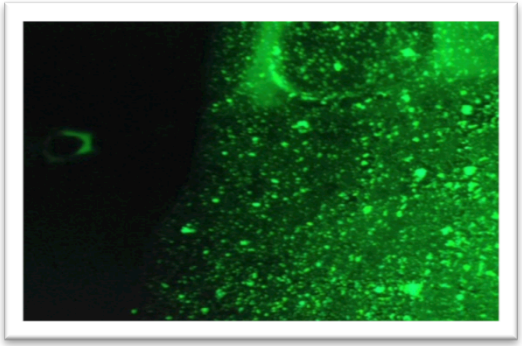
- Had to identify chemical causing fibrinogen stripe to disappear
- Each channel was perfused with only one of the following:
  - Bovine whole blood
  - Glutaldehyde
  - Isopropyl Alcohol
  - Phosphate Buffered Saline (PBS)
- Results showed fibrinogen stripe was still clear in all channels
- Suggests that either a combination of chemicals or untested chemical Acrindine Orange was responsible

New Results

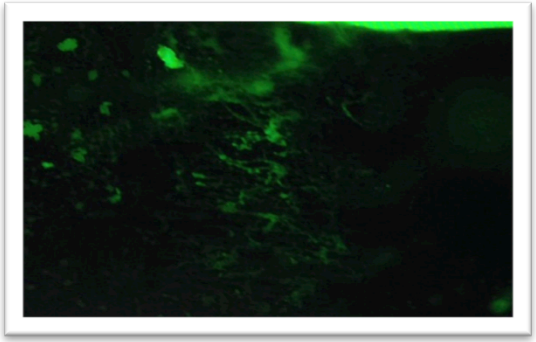
- Tests with multiple chemical combinations occurred
- Whenever Acridine Orange was added to the channels the fibrinogen became eroded



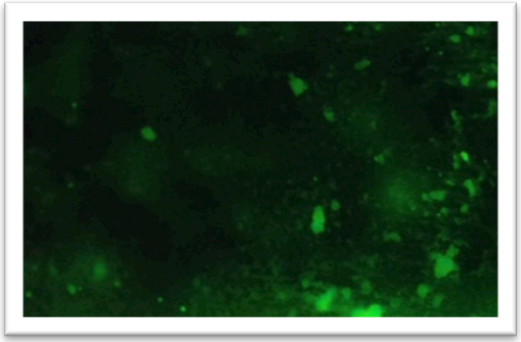
Before picture



Just Glutaraldehyde

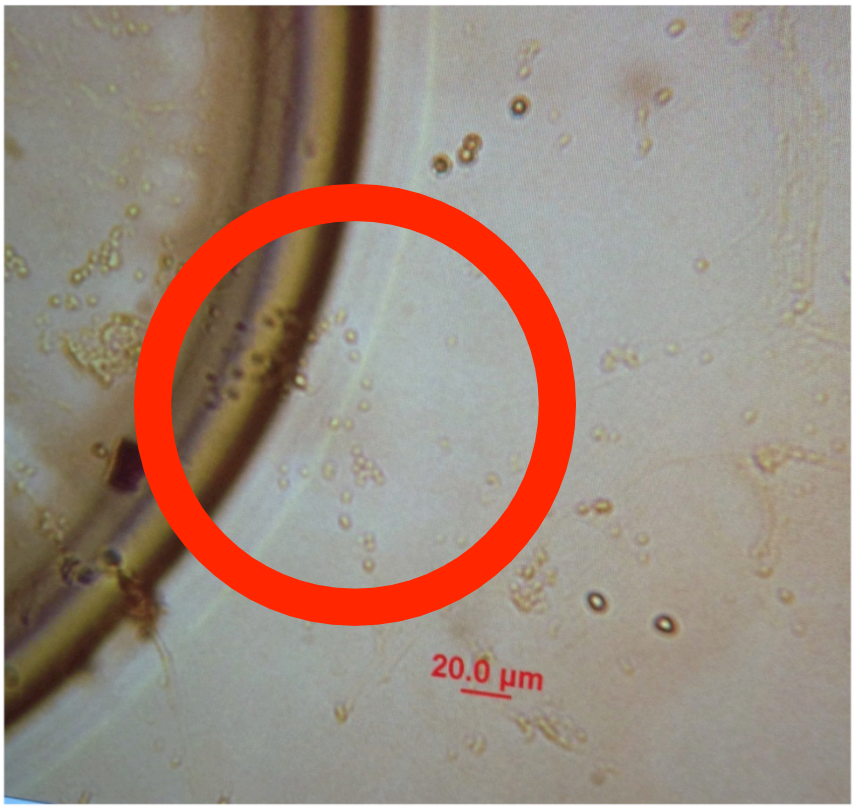


AO stain and glutaraldehyde

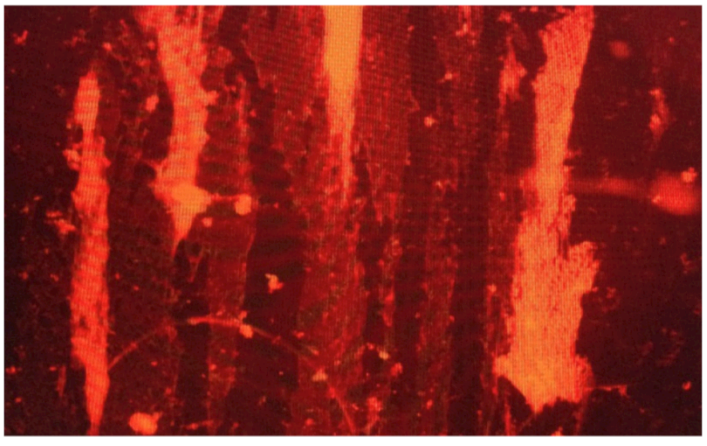


AO stain, glutaraldhyde, and isopropyl alcohol

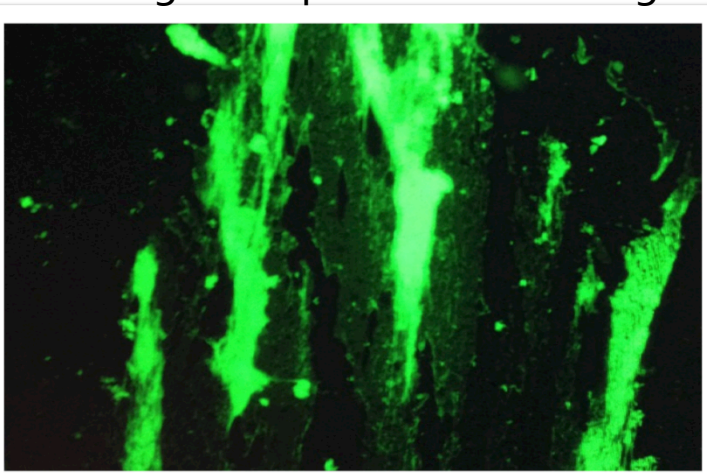
Under the Microscope



Platelets under bright light



Fibrinogen stripe under TRITC lighting



Fibrinogen stripe under FITC lighting

New Test methods

- After AO seemed to be the culprit different methods to keep the fibrinogen attached were developed
  - Make a solution of fibrinogen and PBS to make it more basic
  - Make a solution of fibrinogen and Tris Buffer solution to make stripe more basic
  - Flow AO through channels slower in order to prevent erosion
- After observing all three new methods results were the same
- Another experiment idea to use collagen instead of fibrinogen to see if the AO fibrinogen reaction is causing the erosion

Conclusion

- Acridine orange stain led to erosion of the fibrinogen protein
- Address this issue:
  - Use collagen to study whether or not AO or fibrinogen is the problem
  - Find an alternative way to stain the platelets
  - Find an alternative way to attach the fibrinogen

Acknowledgements

- Dr. Jones who was my advisor during the entirety of the project
- Doctoral student Varun for teaching me how to make micro-channels and advancing my knowledge in them

THANK YOU!
Any questions?