# Evaluation of Potential Blood Contacting Surfaces for Implantable Medical Devices

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**Multi-scale Thrombosis Modeling Seminar Series**
**Experiment Studies Subgroup**

**April 16, 2010**

# Grant Experimental Research Plan

| Experiment | Purpose |
|---|---|
| Microchannel – 1 (parallel plate) | determine surface reaction (deposition) rates for candidate materials and finish [SA-2] |
| Microchannel +side ports (+coarctation) | identification of agonist synergy (+shear) [SA-2] |
| Microchannel +step +crevice +"obstacles" | validation of micro-scale and mixture theory models for RBC and platelet transport [SA-1] |
| | validation of model of deposition [SA-3] |
| Radial impeller (blade tip) | validation of model of plt. activation and deposition [SA-3] |
| Blood immersed bearing | |

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# Specific Aim 2: Material Evaluation

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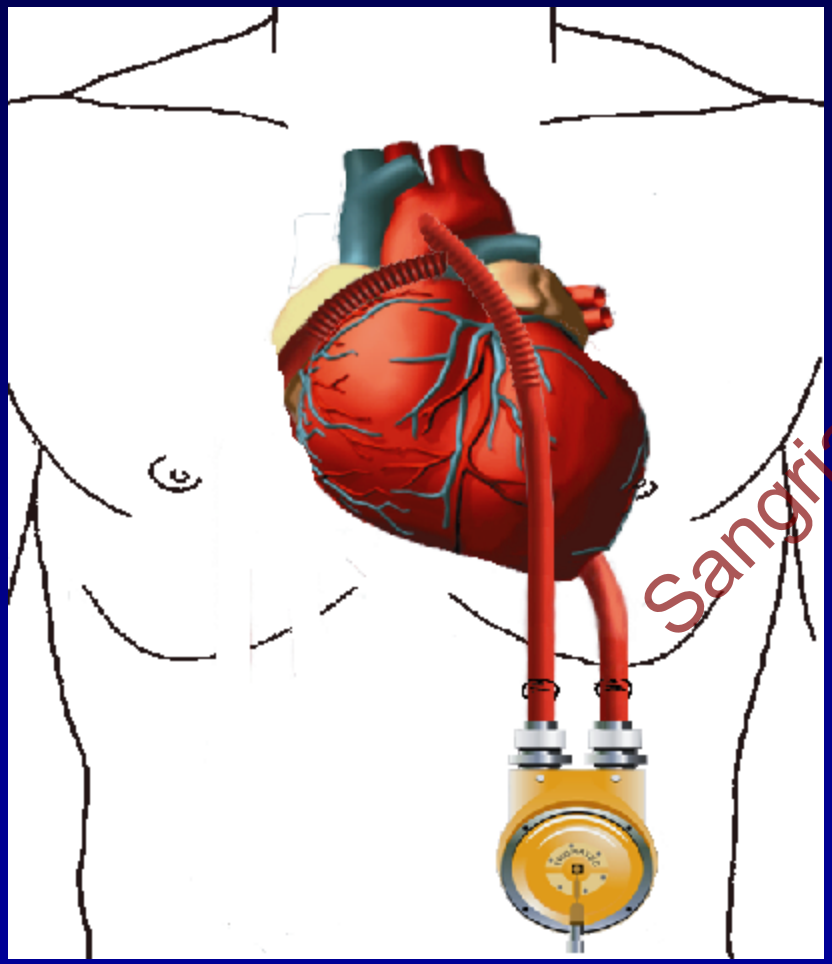
# Left Ventricular Assist Device (LVAD)



Thoratec Inc.

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- Intended to perform the majority of the work of the left ventricle
- VAD inflow cannulated at the apex of the LV
- VAD outflow anastomosed at the aortic root

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BODY MASTERS

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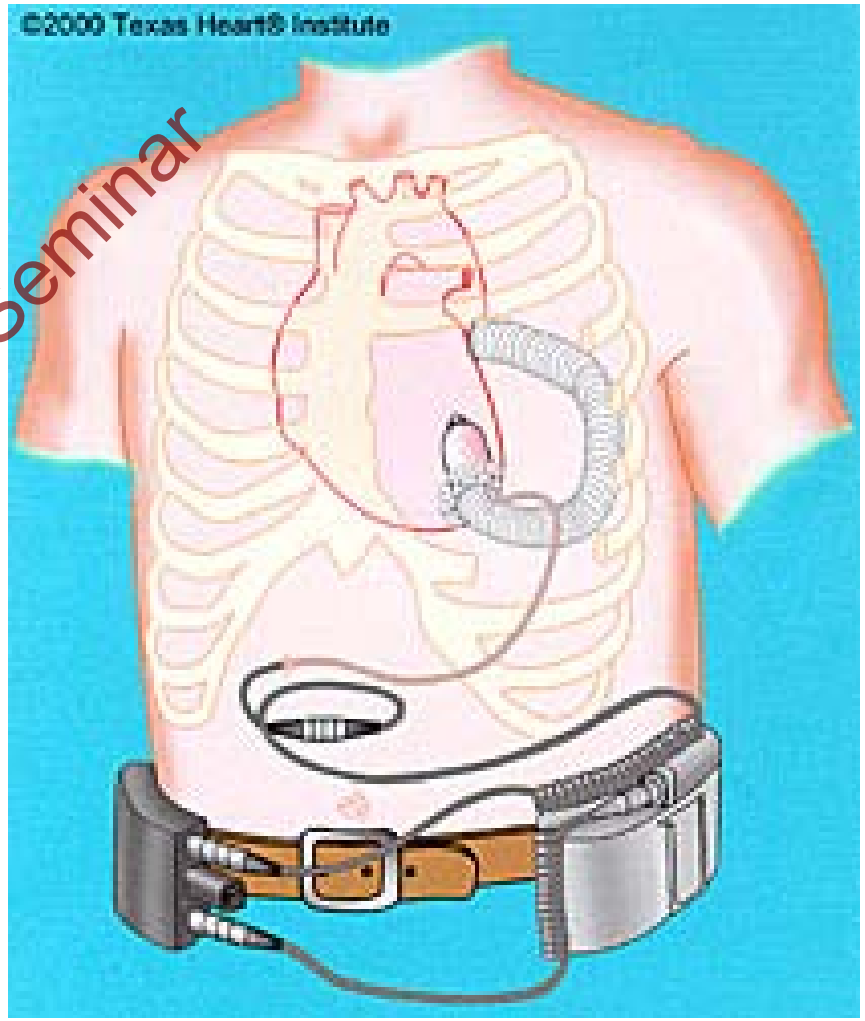


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# Introduction – Example from VADs

- Advancements in rotary VADs through the use of magnets, computational fluid dynamics and battery technology have improved pump performance and the quality of life for patients as compared to pulsatile VADs
  - Smaller pump size
  - Expanded size of eligible patient population
  - One moving part
  - Magnetic levitation – no bearings or valves
  - Longer battery life
  - Theoretically longer pump life
  - Thinner drive line
  - Quieter operation
- Despite these developments, all VAD patients are still plagued by hemostasis problems such as bleeding, thrombosis and infection

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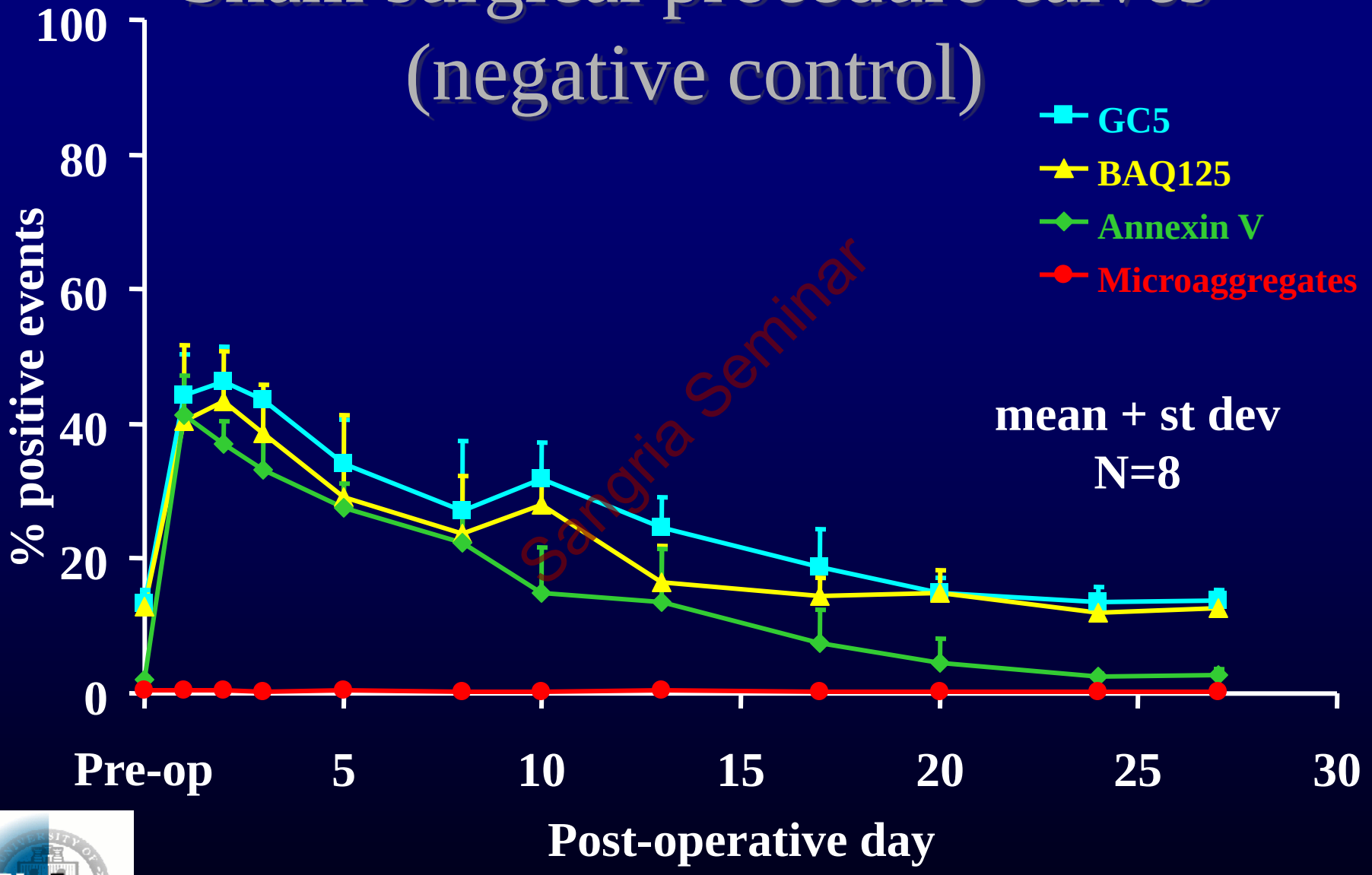
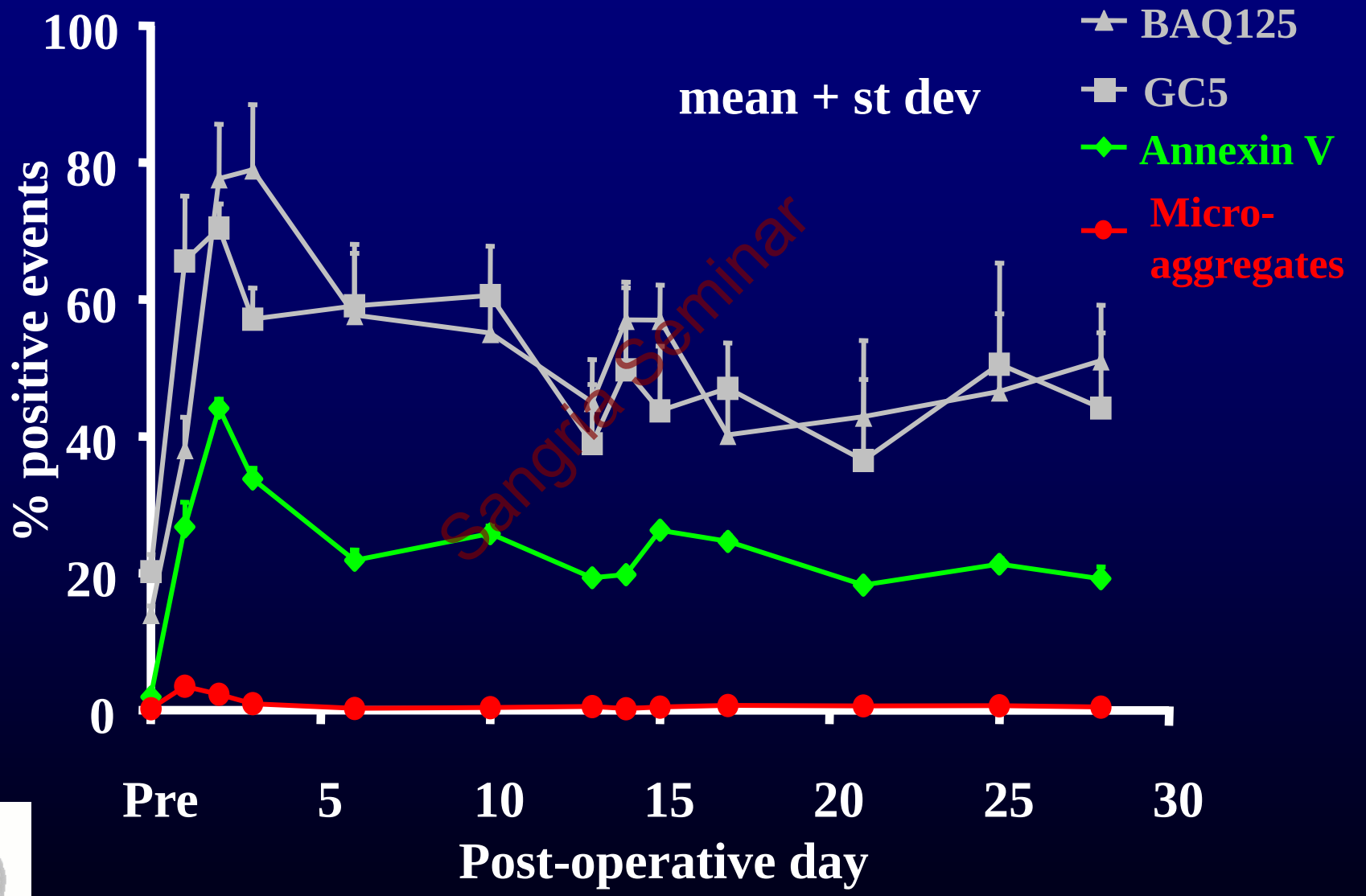
# Sham surgical procedure calves (negative control)



# Calf with Heartmate II VAD

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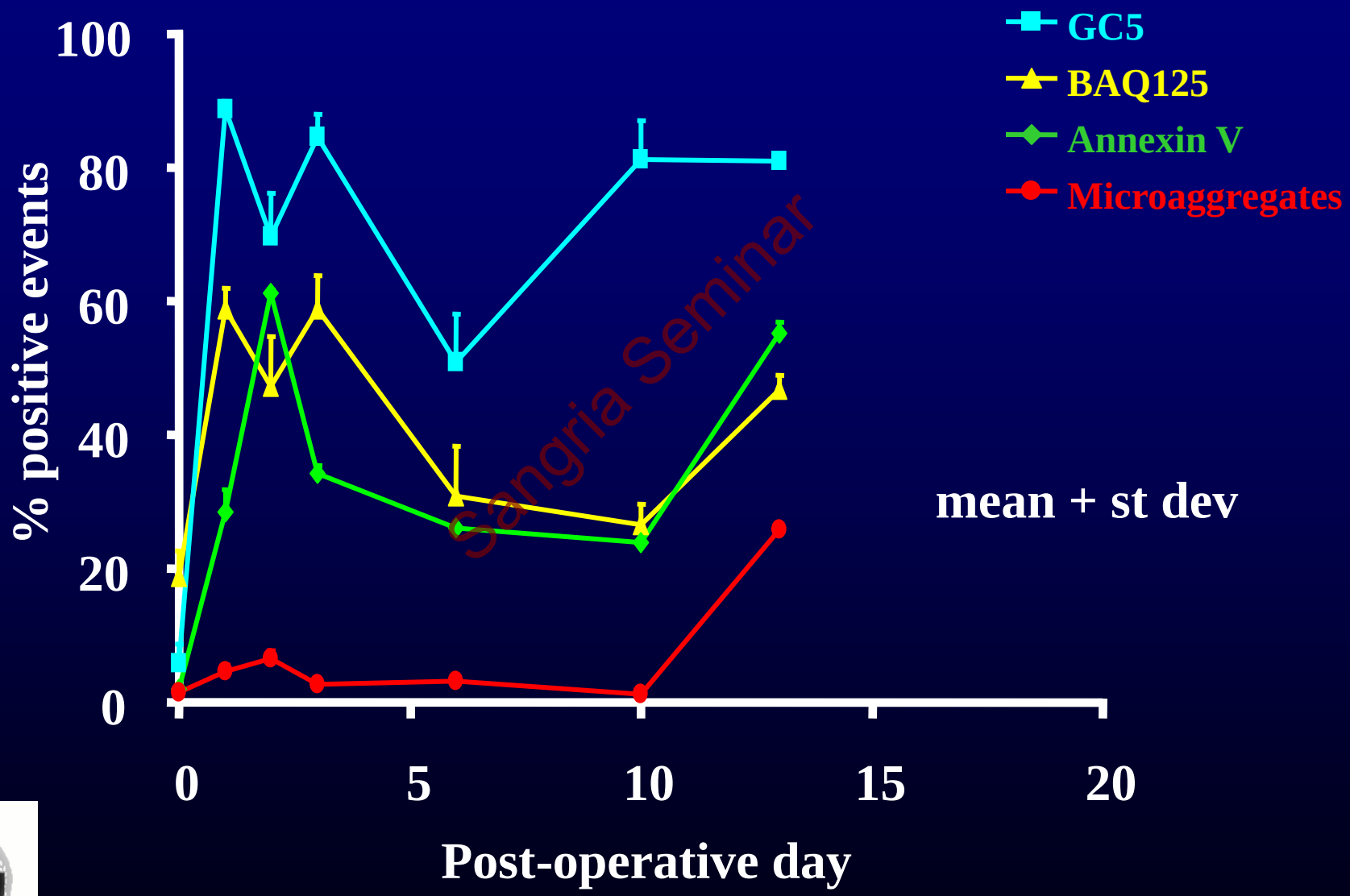
Bio Eng

# Calf with occluded outflow




Bio Eng

# Introduction – Example from VADs

- One area where progress has been slow with rotary VADs is the biocompatibility of the blood – contacting surfaces
- Most use polished titanium alloys (HeartMate II, Jarvik 2000, HeartWare, etc – $TiAl_6V_4$)
- Utilization of different materials and/or coatings may be able to decrease hemostasis complications while maintaining many of the material properties that make titanium alloys attractive

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# Hypothesis

Alternative materials will be more biocompatible than $TiAl_6V_4$ for use as the blood contacting surfaces of medical devices when evaluated by platelet adhesion and bulk phase platelet activation.

We will use the outcomes of these studies for the thrombosis model inputs

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# Materials to be Evaluated

Candidate materials/coatings already acquired:

1. TiAl6V4
2. Alumina
3. Zirconia Toughened Alumina (ZTA)
4. Yttria Tetragonal Zirconia Polycrystal (YTZP)
5. Magnesia paritally stabilized Zirconia (DuraZ™)
6. Polyetheretherketone (PEEK)
7. 30% Carbon Fiber PEEK (CF-PEEK)
8. Implantable Grade Polyetherketoneketone (IG-PEKK)
9. Silicon Carbide
10. MPC polymer coating (per Dr. Ye)
11. PMA co-polymer coating (per Dr. Ye)

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# Materials to be Evaluated

- Additionally, most of the materials above have been polished to varying (defined) Ra and lay finishes
  - Ra of 16, 12-10, 8, 4 and mirror finish
  - Lay orientation parallel to flow, perpendicular to flow, and random
- Shear rates of 100 $s^{-1}$ and 1000 $s^{-1}$ will also be tested
  - Different adsorbed plasma proteins affect platelet deposition at different shear rates
    - Fibrinogen at low shear rates
    - vWF and fibronectin at high shear rates
- Selection Criteria
  - The materials are already FDA approved for implantation
  - Similar or improved material properties to titanium
    - Toughness, strength, non-magnetic, easily machined

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# Preliminary Data

Blood – contacting rocker study performed by Dr. Snyder before acquiring candidate materials:

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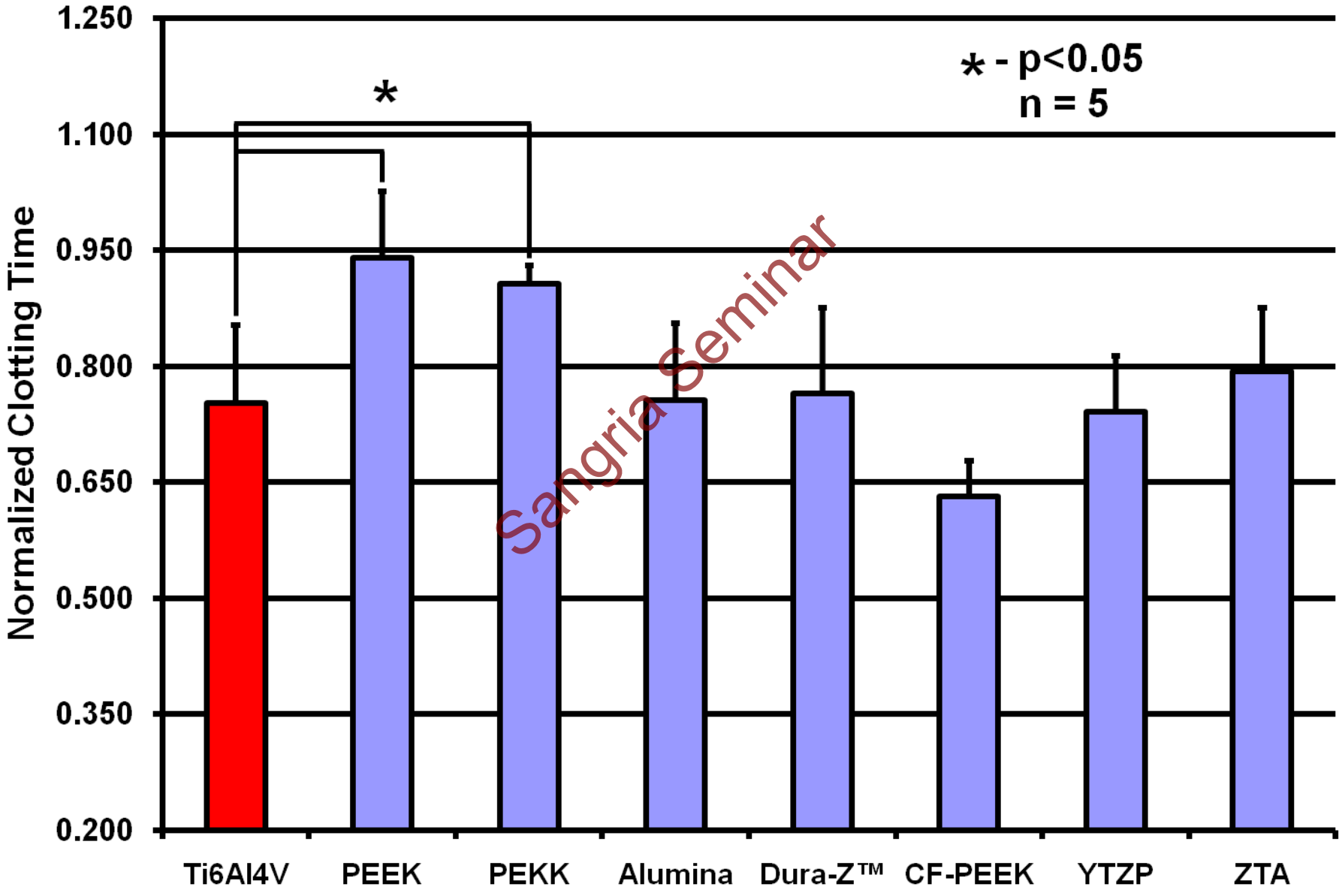
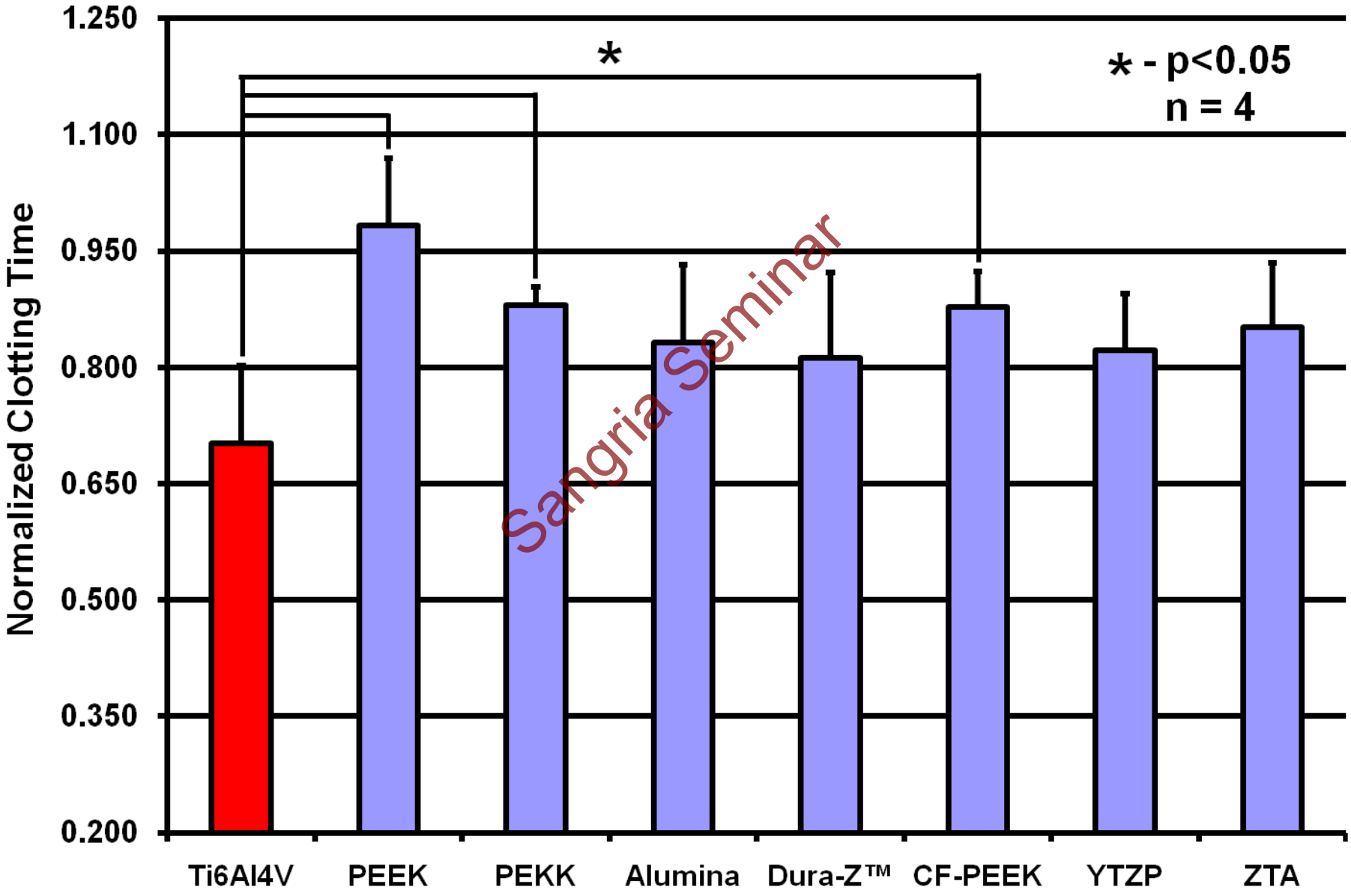
Thrombus deposition on different materials following rocker tests. Sample rods of Ti-6Al-4V ELI (top), cpTi (middle), and YTZP (bottom) from the same rocker test are shown. The red lines below each sample indicate millimeters. The current industry standard Ti alloys are nearly completely covered in thrombus, while the zirconia ceramic has desposition on approximately 16% of its surface.

# Human Blood, Normalized Clotting Time Studies



# Sheep Blood, Normalized Clotting Time Studies

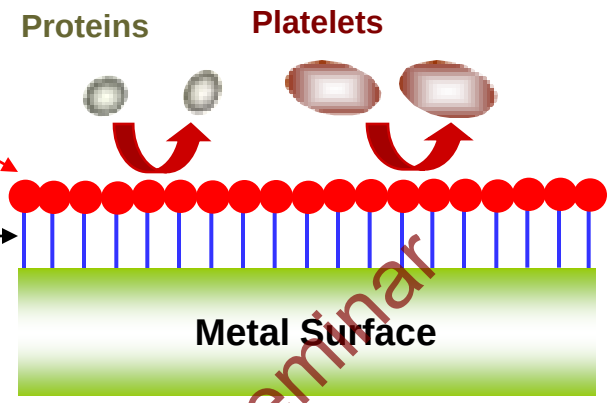


## Our Objective:

# Construction of Stable Hemocompatible Interface on a Metallic Surface

**Blood compatible interface with biomimetic and zwitterionic phosphorylcholine (PC) or sulfobetaine (SB) groups**

**Chemical (Covalent) bonding between a metal surface and the blood compatible moieties**




**Highly blood compatible & Non-thrombogenic Surface**

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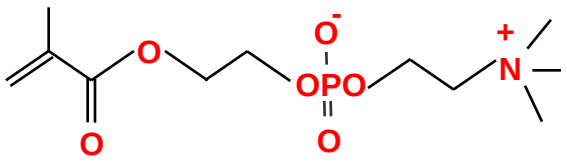
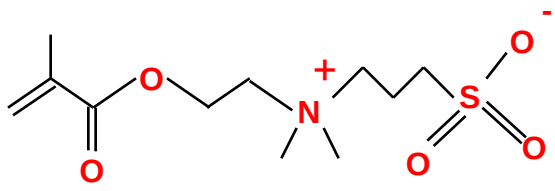
➢ **Chemical structure of PC or SB groups containing monomers**

**2-Methacryloyloxyethyl Phosphorylcholine (MPC)**

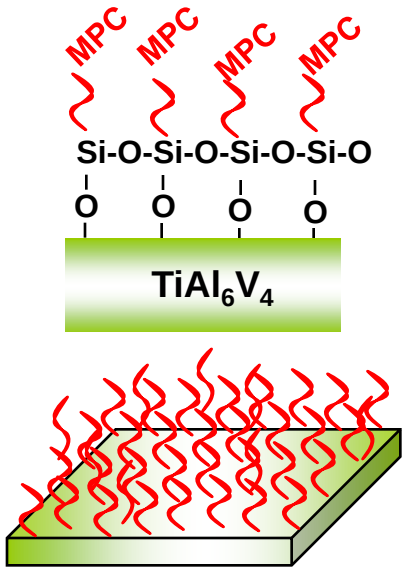


**N-(3-Sulfopropyl)-N-(methacryloxyethyl)-N,N-dimethylammonium betaine (SMDAB)**



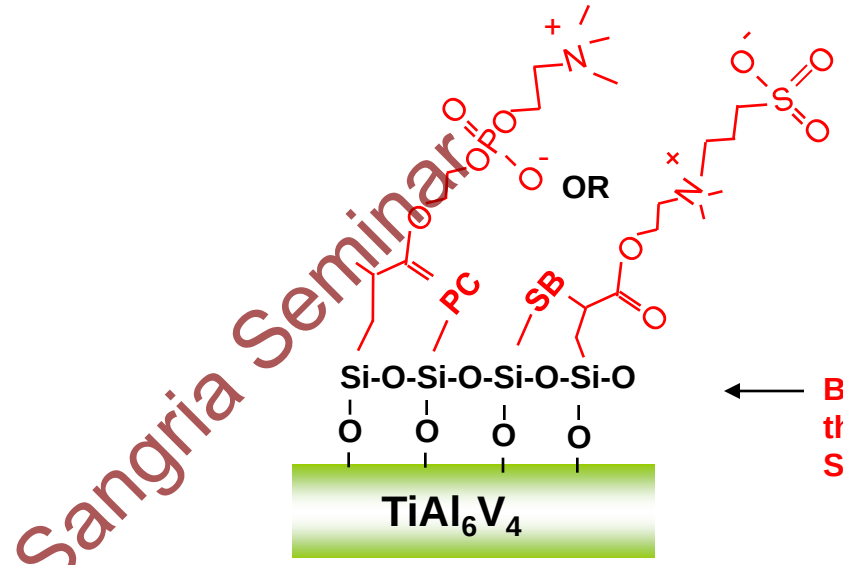
# Our Previous Studies on a Titanium Alloy ($TiAl_6V_4$) Surface

**1) Plasma induced grafting polymerization of MPC***




**Ti-MPS-MPCgraft**

**2) Single-step modification with a silanated PC or SB modifiers (MPCSi or SBSi)***




**Ti-MPCSi or Ti-SBSi**

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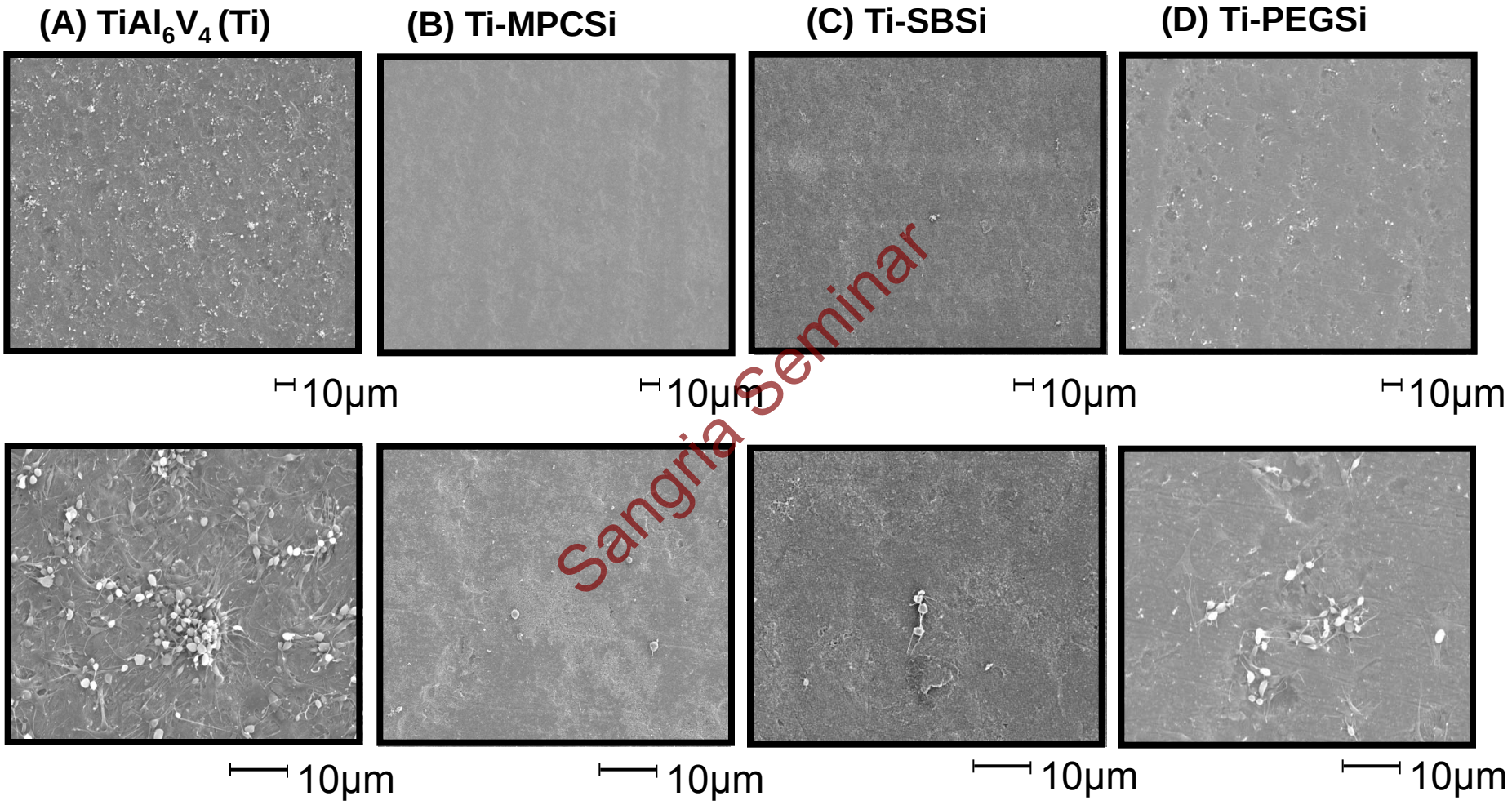
- **Exhibited significant decreases in acute platelet adhesion and bulk phase activation in comparison with unmodified titanium surfaces in vitro blood contact test.**
- **Needs comparison studies between different modification techniques and different moieties modified surfaces in terms of the surface blood compatibility.**

*1) S.H. YE et. al, Colloid Surf B. Biointerfaces 2009 In press
*2) S.H. YE et. al, ASAIO & BMES 2008 Annual Meeting

# Platelet Deposition after Contacting with Ovine Blood in Vitro

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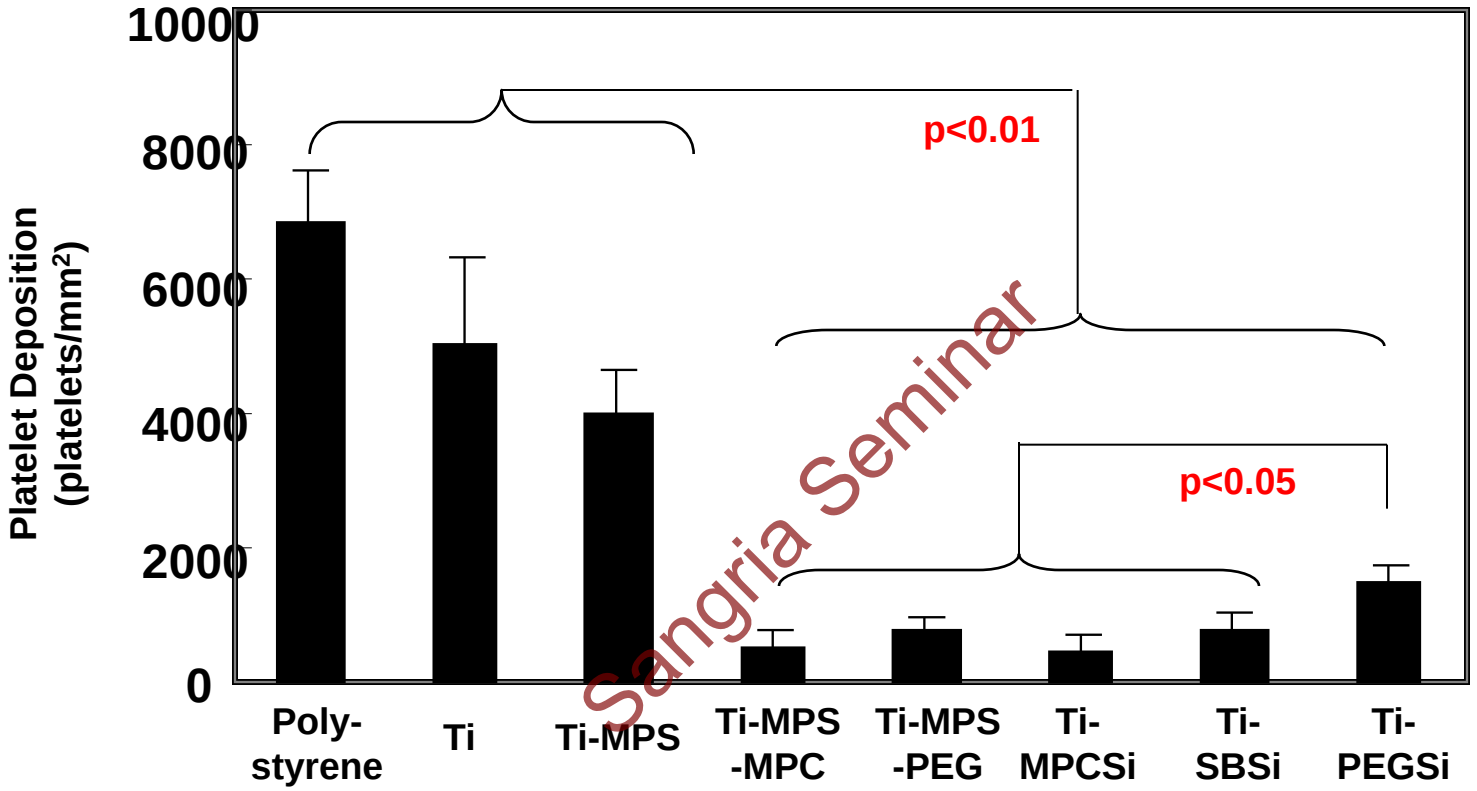
**SEM micrographs of unmodified and modified TiAl6V4 samples after contact with fresh ovine blood (heparin 3.0 U/mL) for 2 hr at 37˚C.**

# Quantification of Surface Adherent Platelets

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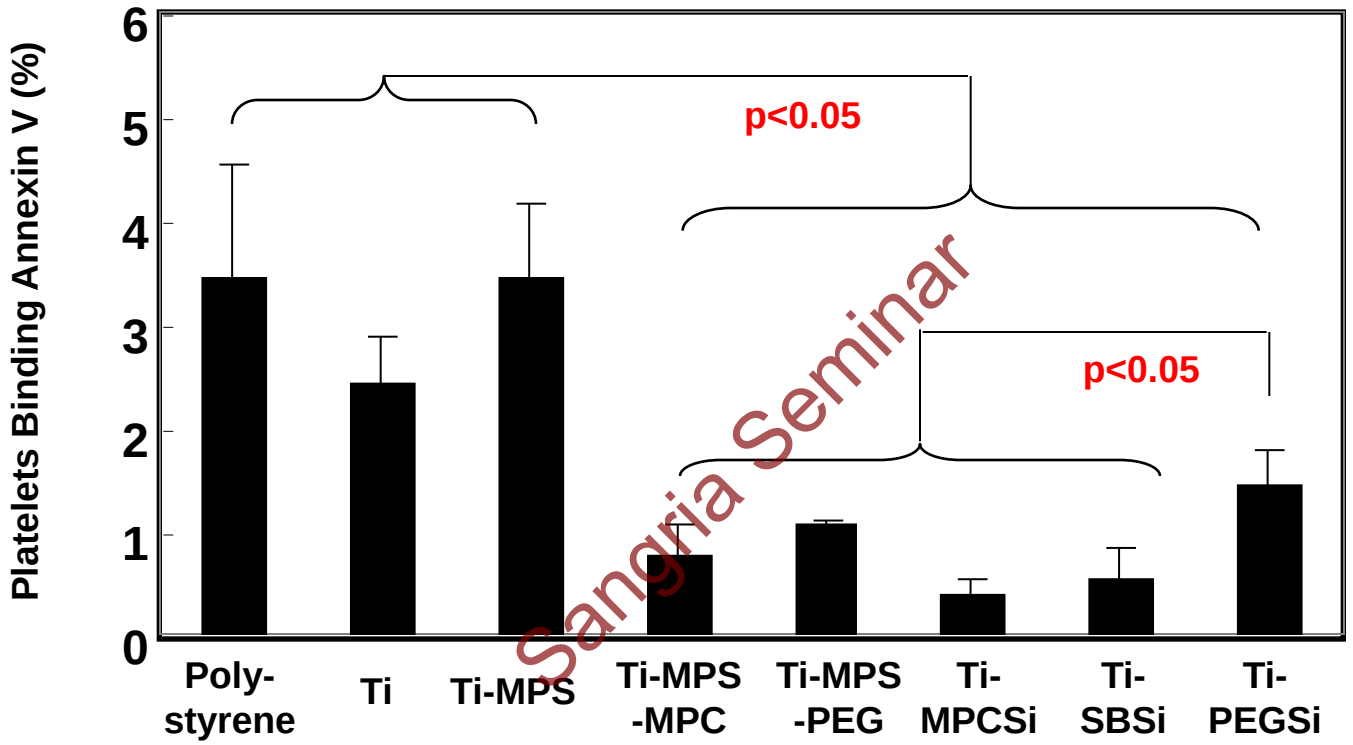
**Platelet deposition onto surfaces after contact with ovine blood as determined by a lactate dehydrogenase (LDH) assay.**

# Quantification of Platelet Activation by Flow Cytometry

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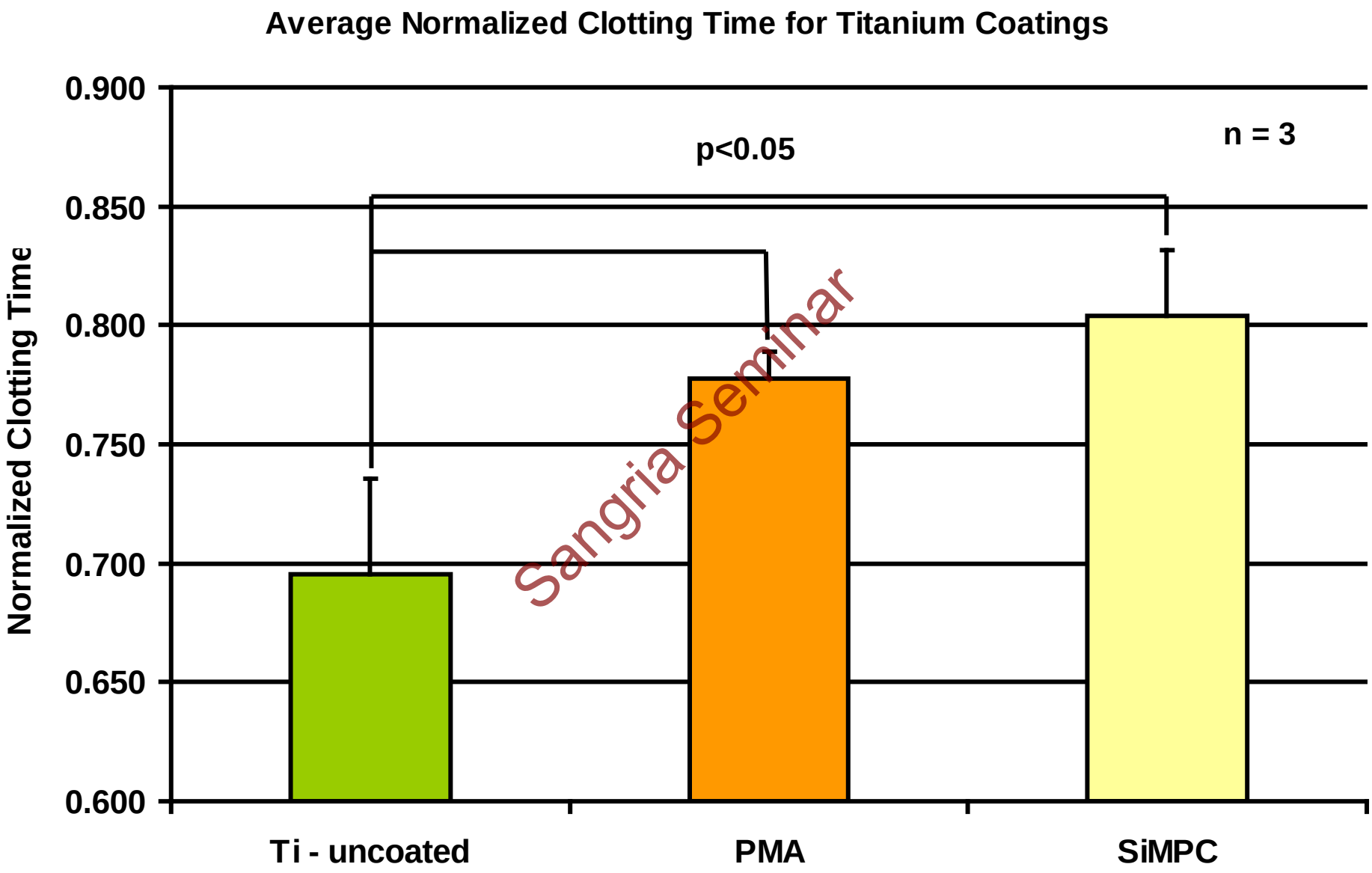
Quantification of activated platelets in the bulk phase of ovine blood after surface contact under continuous rocking. Platelet activation was quantified by flow cytometric measurement of Annexin V binding (No test surface result was subtracted).


Average Normalized Clotting Time for Titanium Coatings
Normalized Clotting Time
0.900
0.850
0.800
0.750
0.700
0.650
0.600
p<0.05
n = 3
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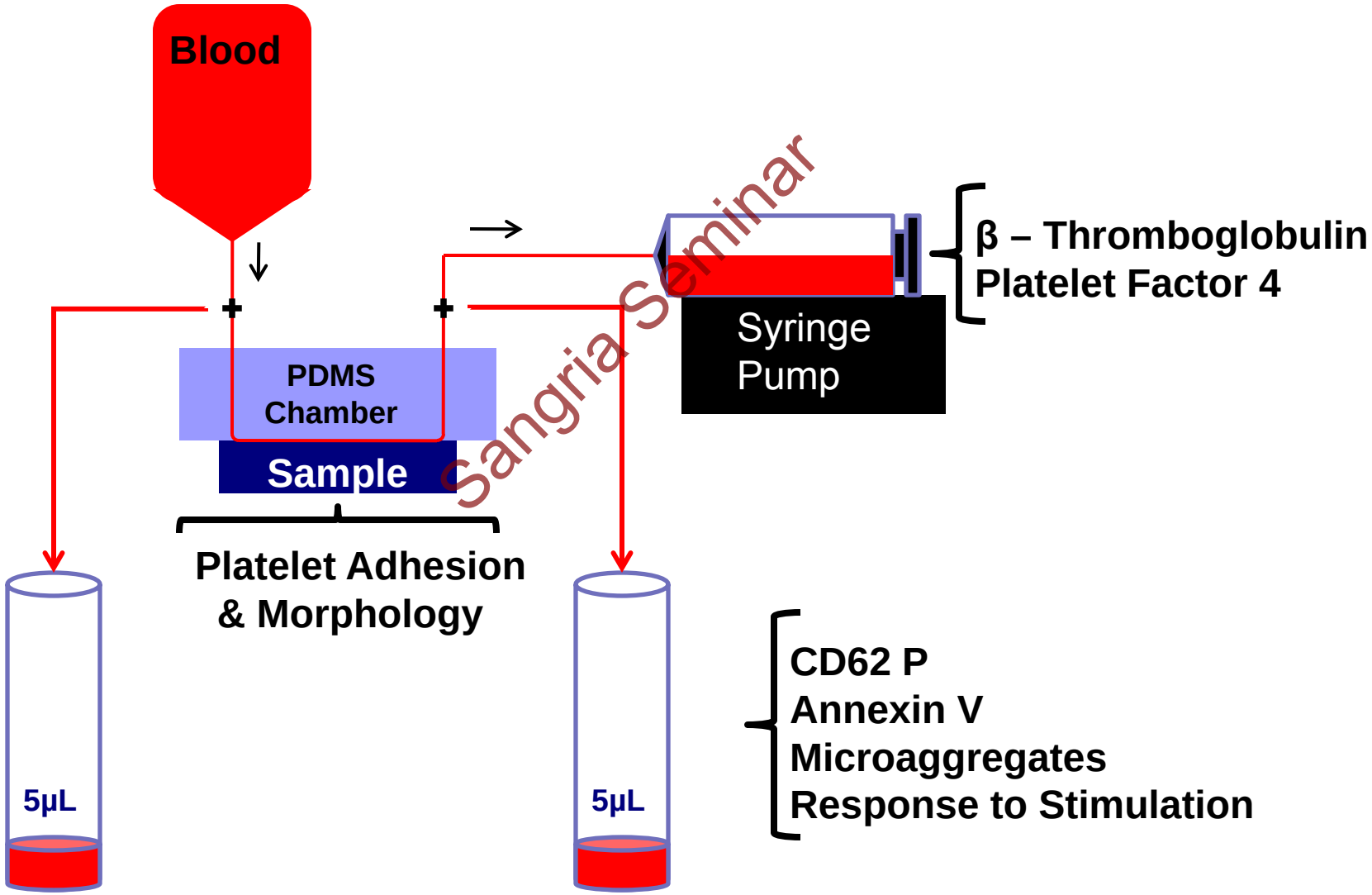
Ti - uncoated
PMA
SiMPC

# Research Design and Methods

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# Research Design and Methods

## Assessment of platelet adhesion

- Dynamic visualization
  - Fluorescent labeling
    - Dyes such as mepacrine, acridine red, DiOC6
    - Conjugated antibodies
- End-point analysis
  - SEM
  - LDH concentration

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# Research Design and Methods

## Assessment of bulk phase platelet activation

- Flow Cytometry (Cellular assessment)
  - CD62P, CD63 Expression on Platelets
  - Annexin V Binding to Platelets
  - Circulating Microaggregates
  - Platelet Response to Stimulation (Platelet Dysfunction)
- ELISA (Global assessment)
  - β – Thromboglobulin
  - Platelet Factor 4

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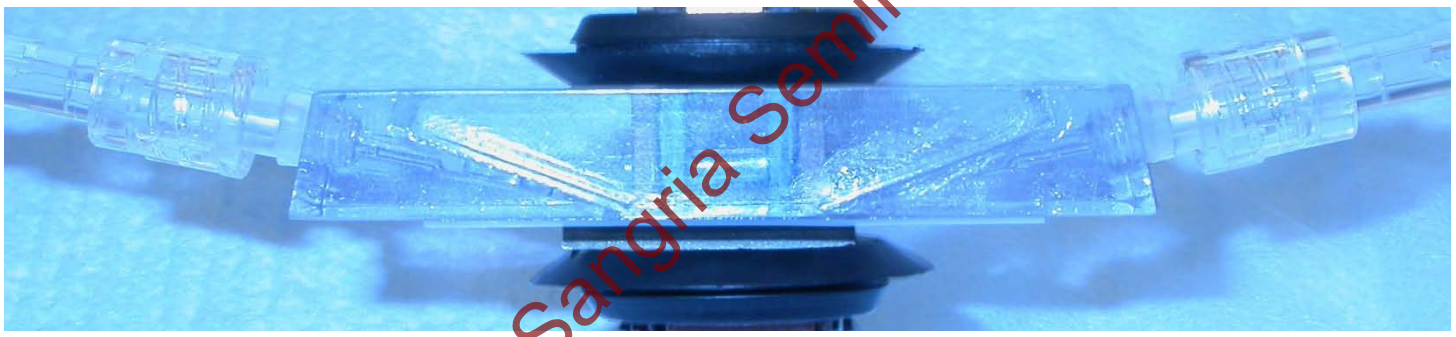
# Parallel Plate Chamber Design

- Polycarbonate Prototype
  - Expensive to make
  - Fragile
  - Many seems along flow path



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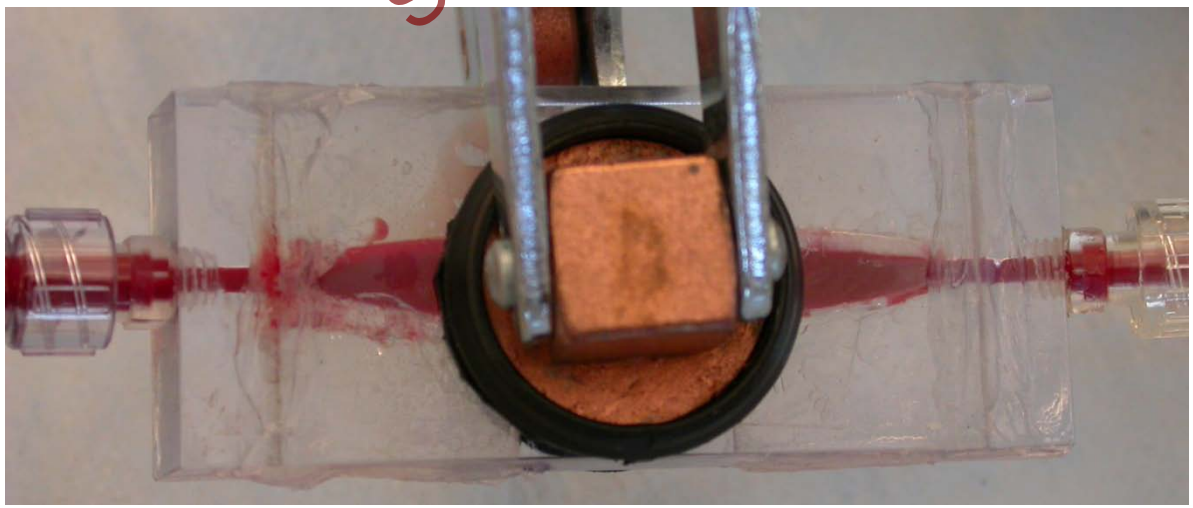
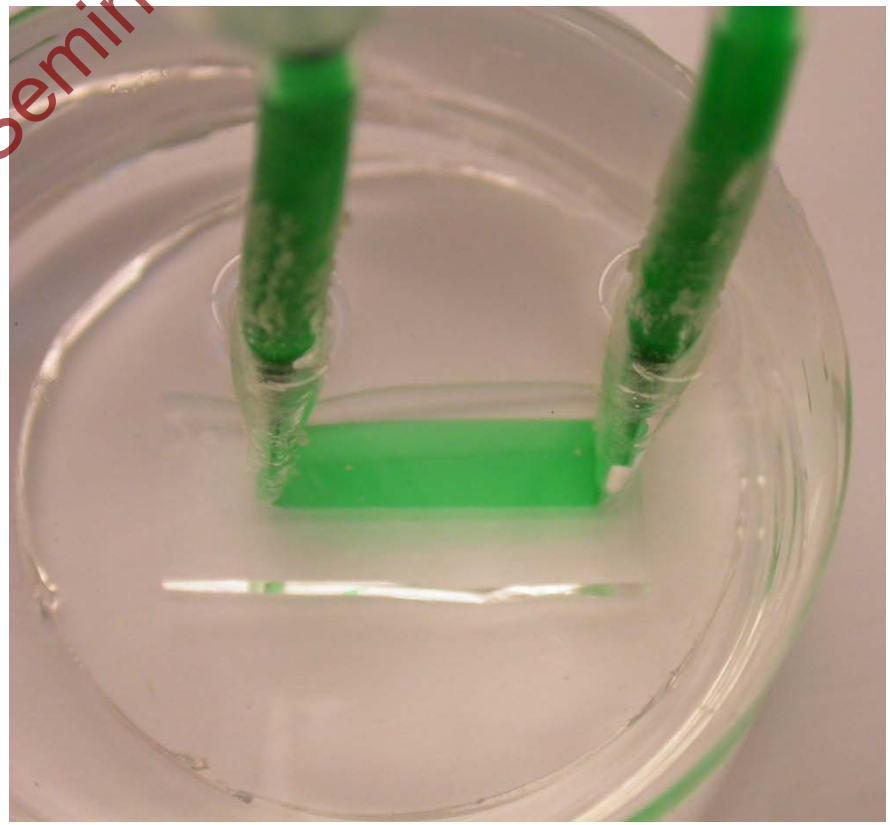


# Parallel Plate Chamber Design

- PDMS Prototype
  - Easy, reproducible design
  - Only allows for end-point determinations



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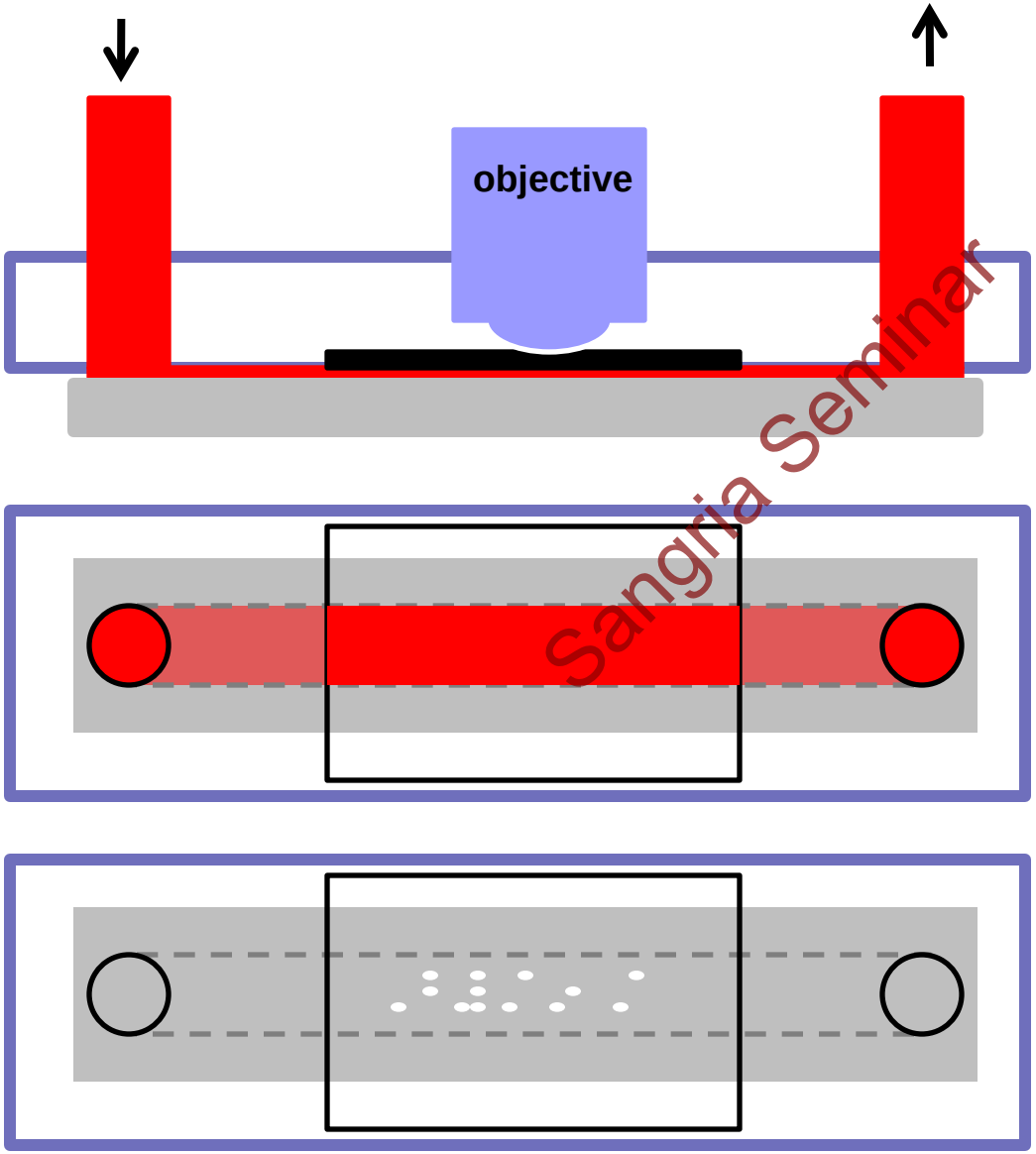
# Proposed Chamber Design




PDMS chamber with glass slide for visualization of fluorescent platelets

Chamber with blood contacting the material (which blocks visualization of platelets)

Short bolus of platelet-poor plasma to allow photograph of platelets.

# Specific Aim 3: Model Validation with Steps and Crevices

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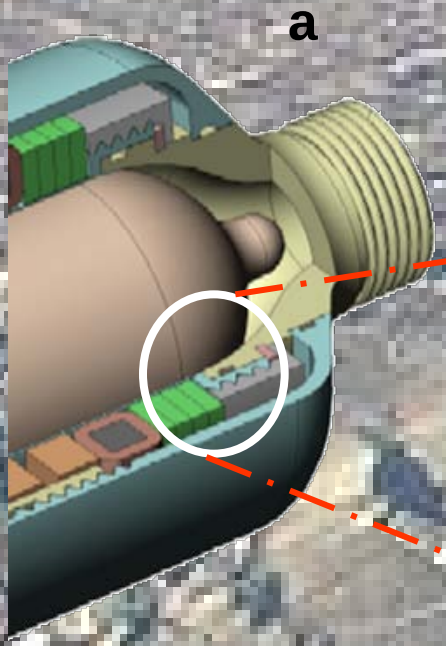
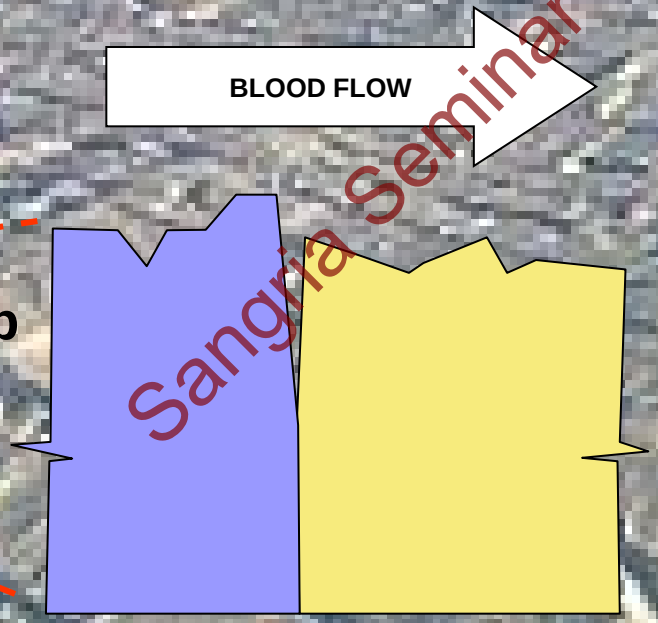
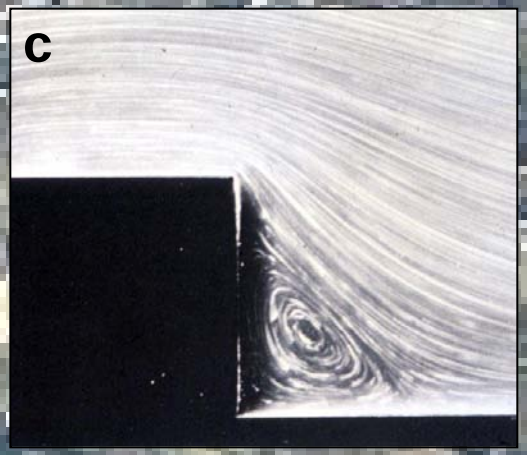
# Steps, Crevices, and Crevasses

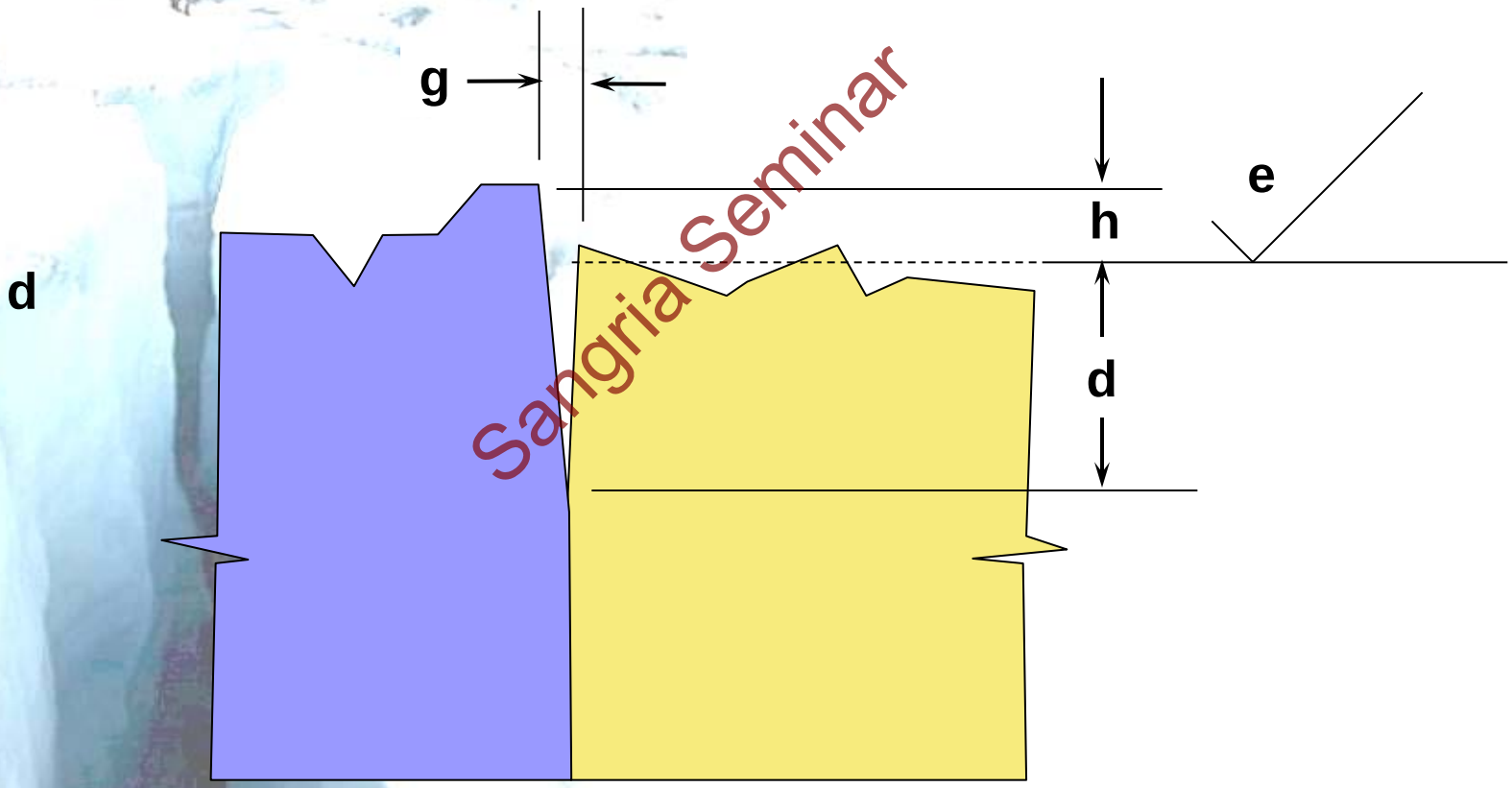
# NOMENCLATURE
# Backward Step - Crevice



# NOMENCLATURE
# Backward Step - Crevice

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**Blood Flow**

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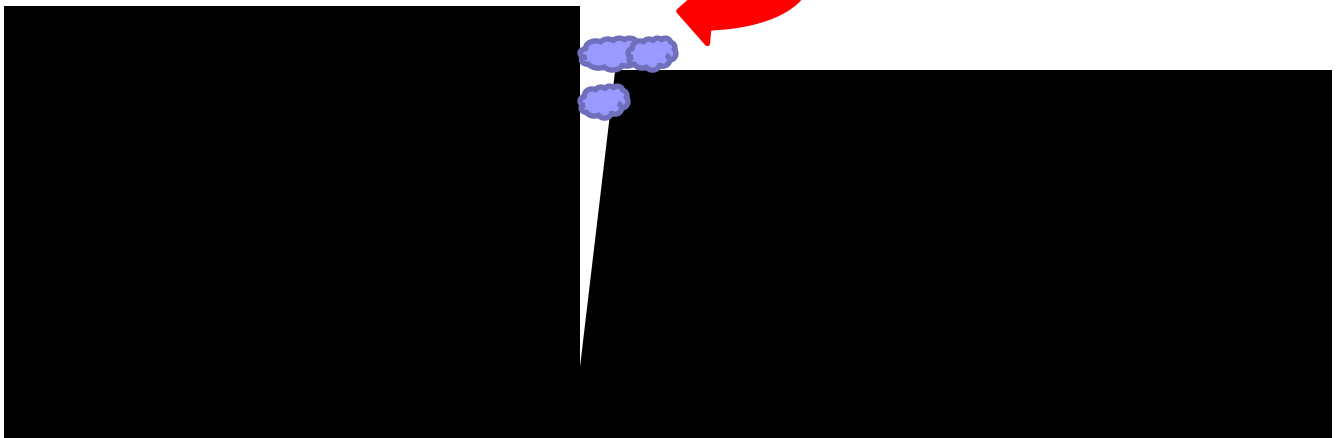
**Recirculation and Platelet Deposition**





**Blood Flow**

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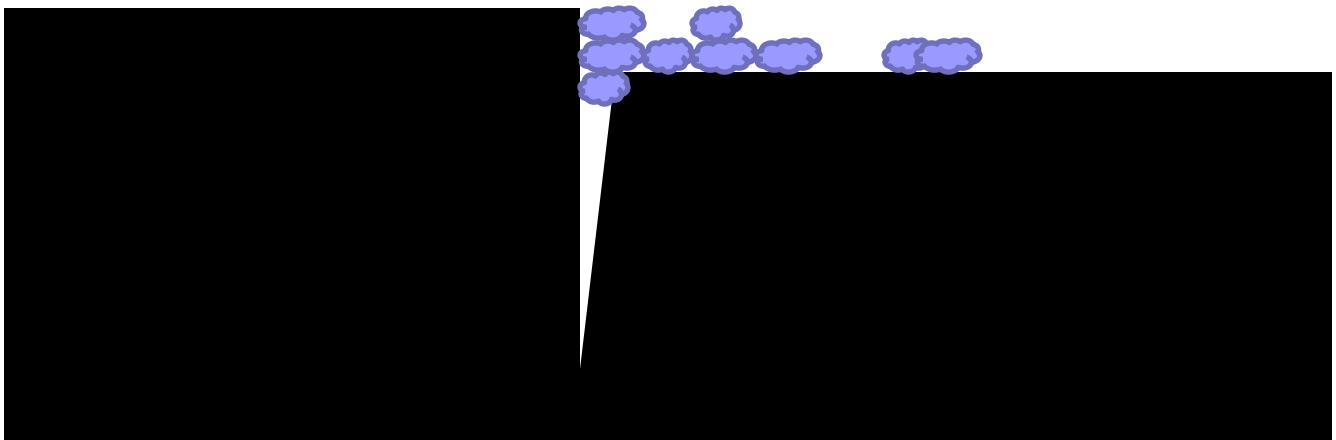
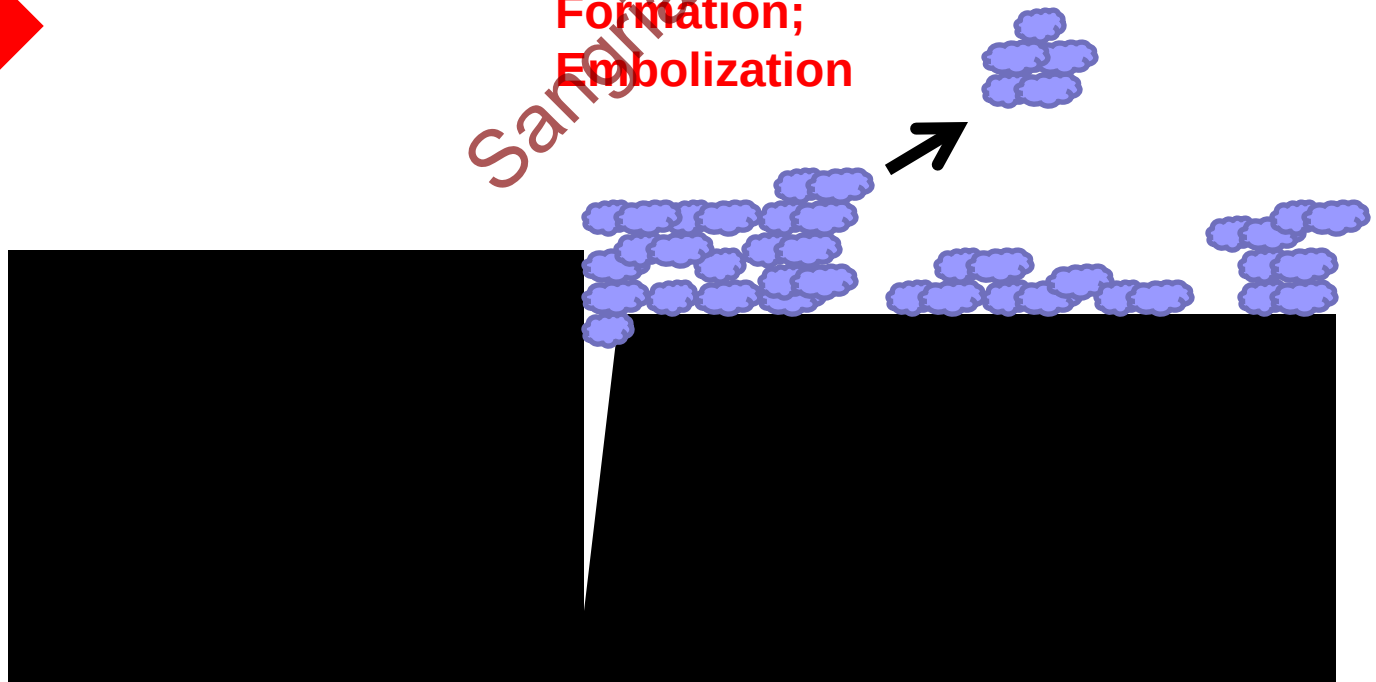
**Platelet**
**Recruitment**
**Downstream**



**Blood Flow**

**Thrombus Formation; Embolization**



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$t_1 = t$

**Blood Flow**

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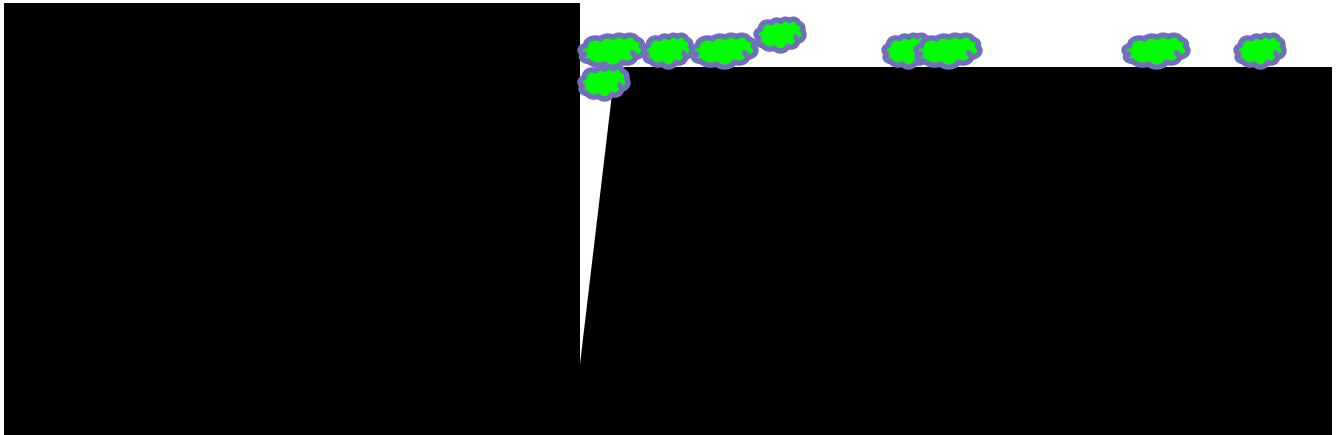

$$t_2 = t_1 + t$$

**Blood Flow**

**Thrombus Formation; Embolization**

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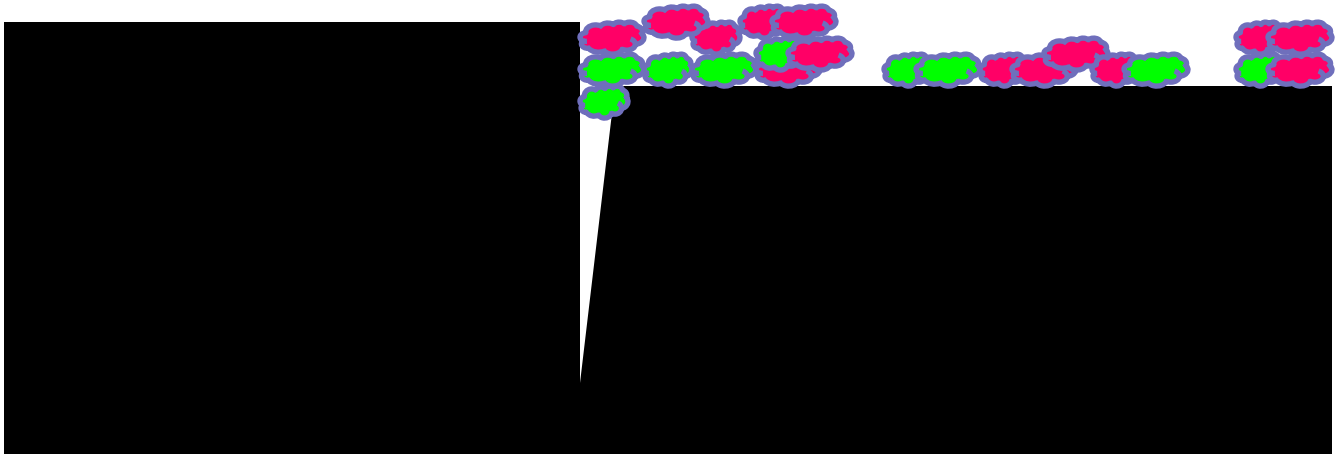

$$t_3 = t_1 + t_2 + t$$

**Blood Flow**

**Thrombus Formation; Embolization**

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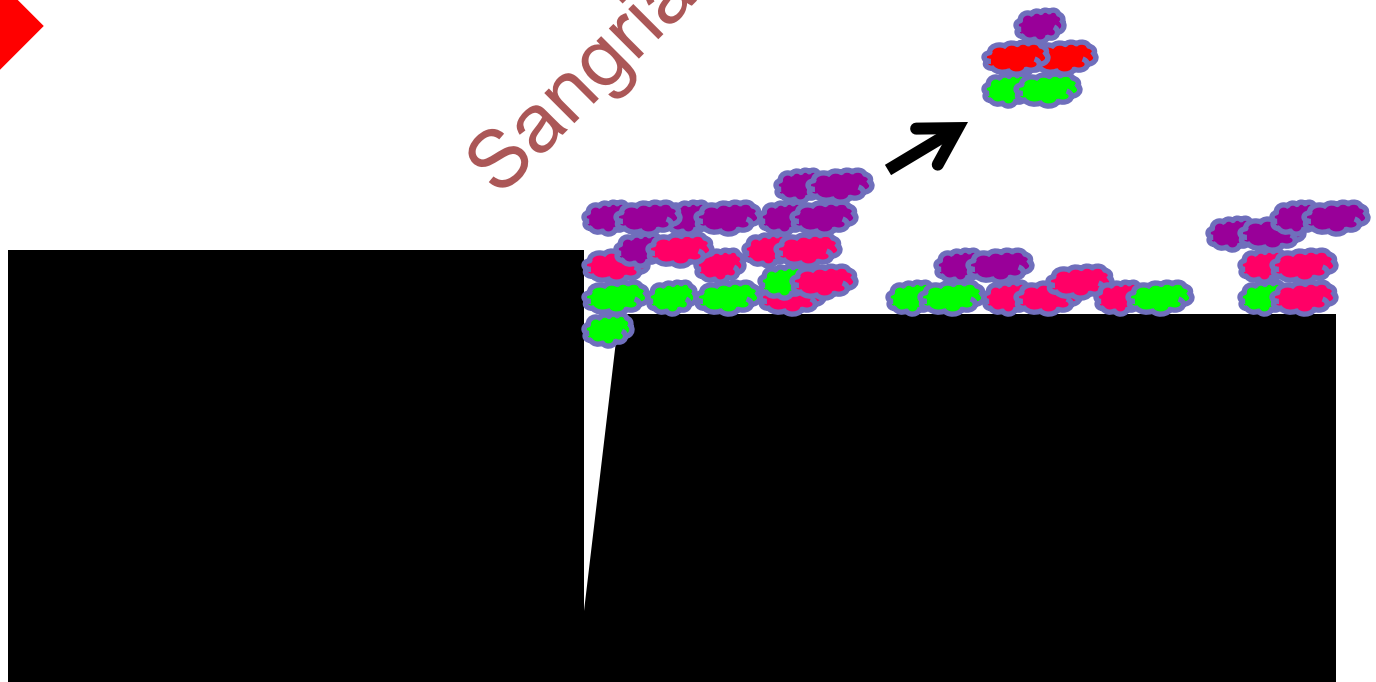
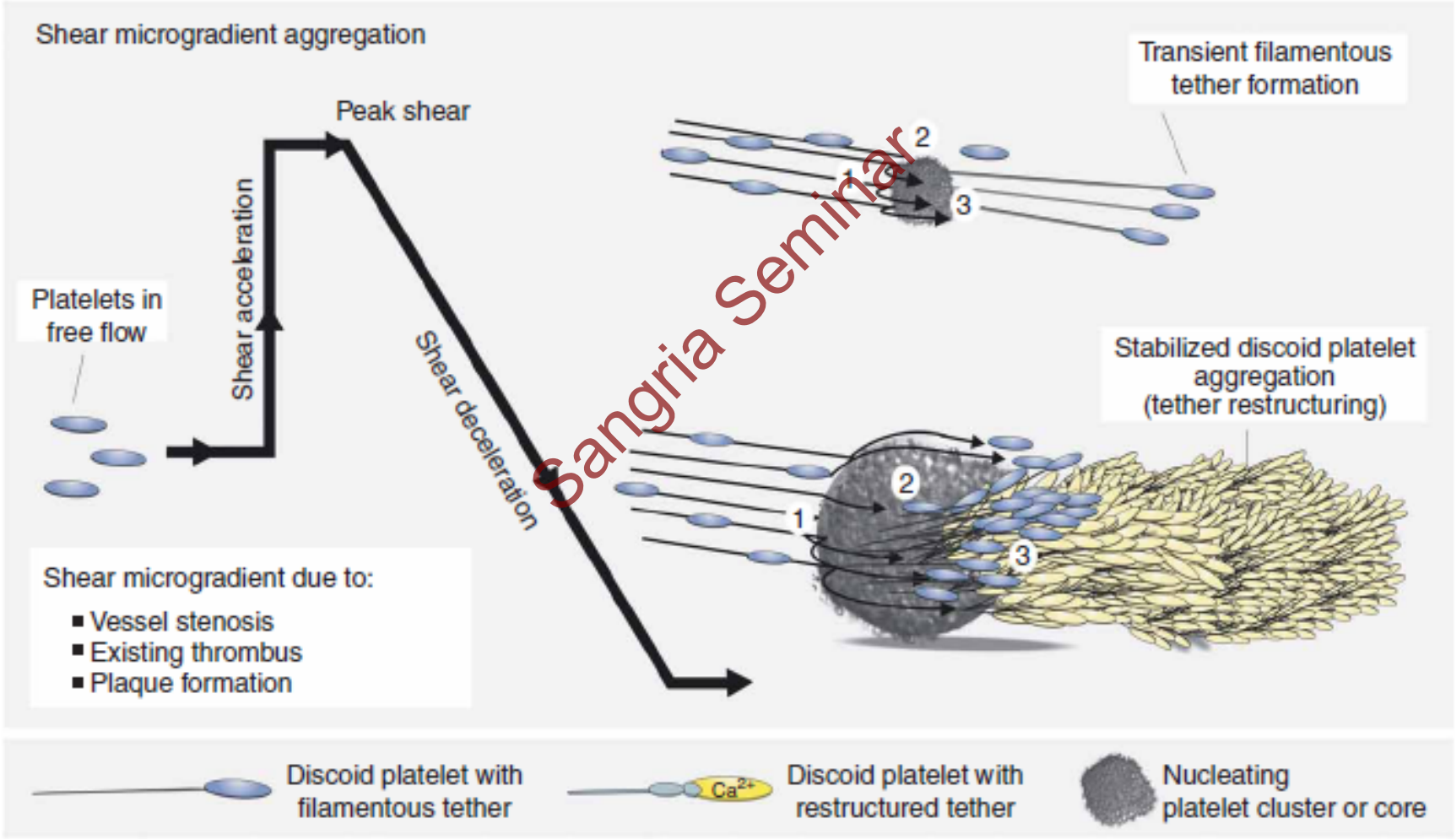


# Platelet Recruitment

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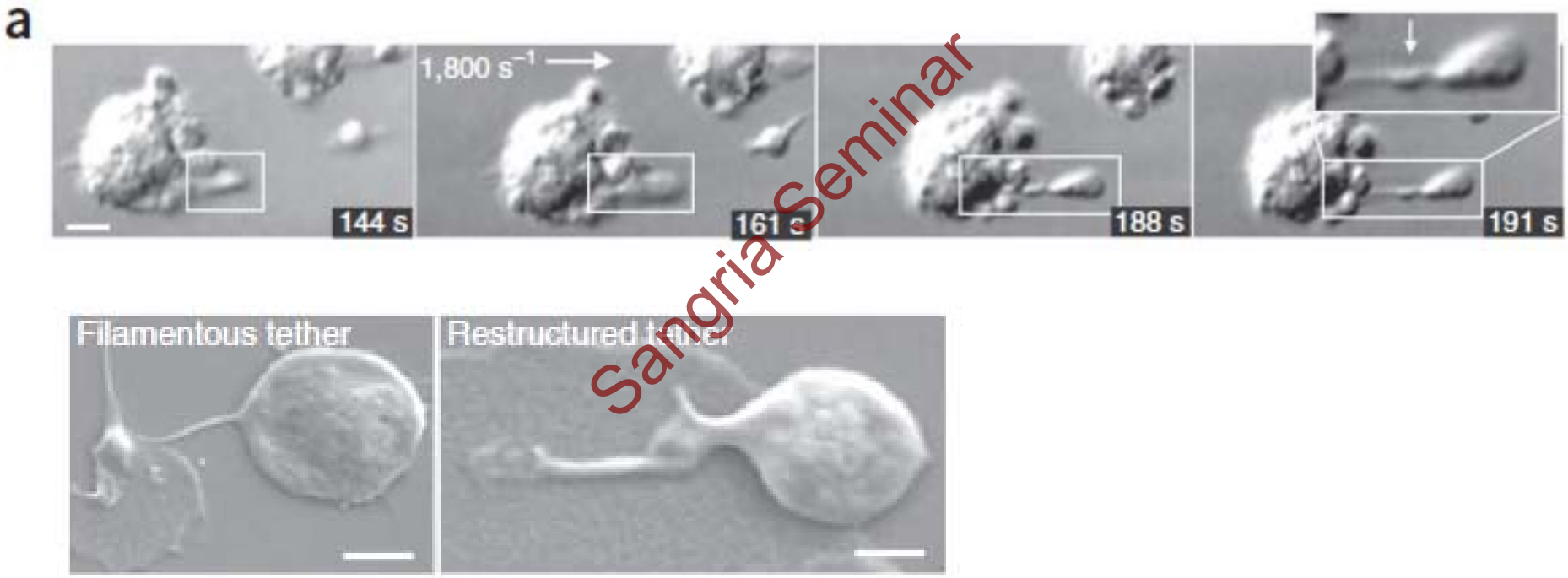
Nesbitt, Nature Medicine, 2009

# Platelet Recruitment

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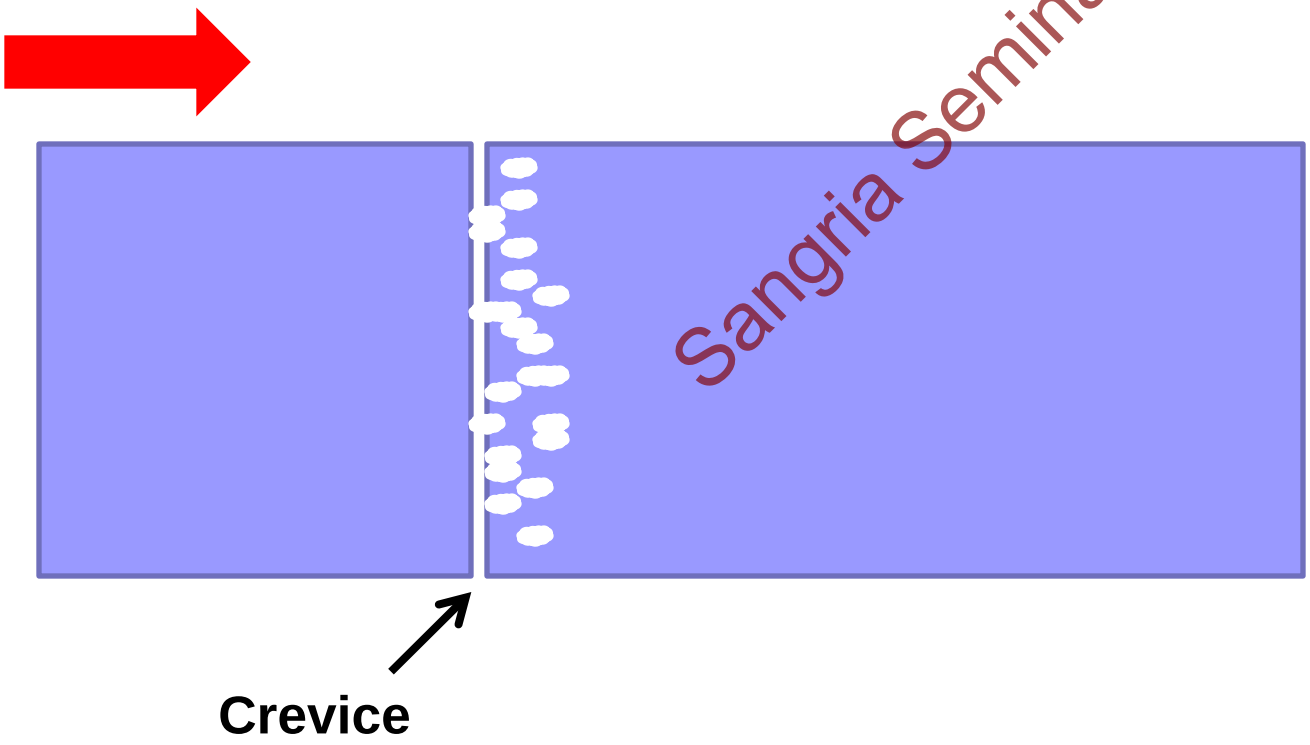
Nesbitt, Nature Medicine, 2009

Top View

$t_1 = t$

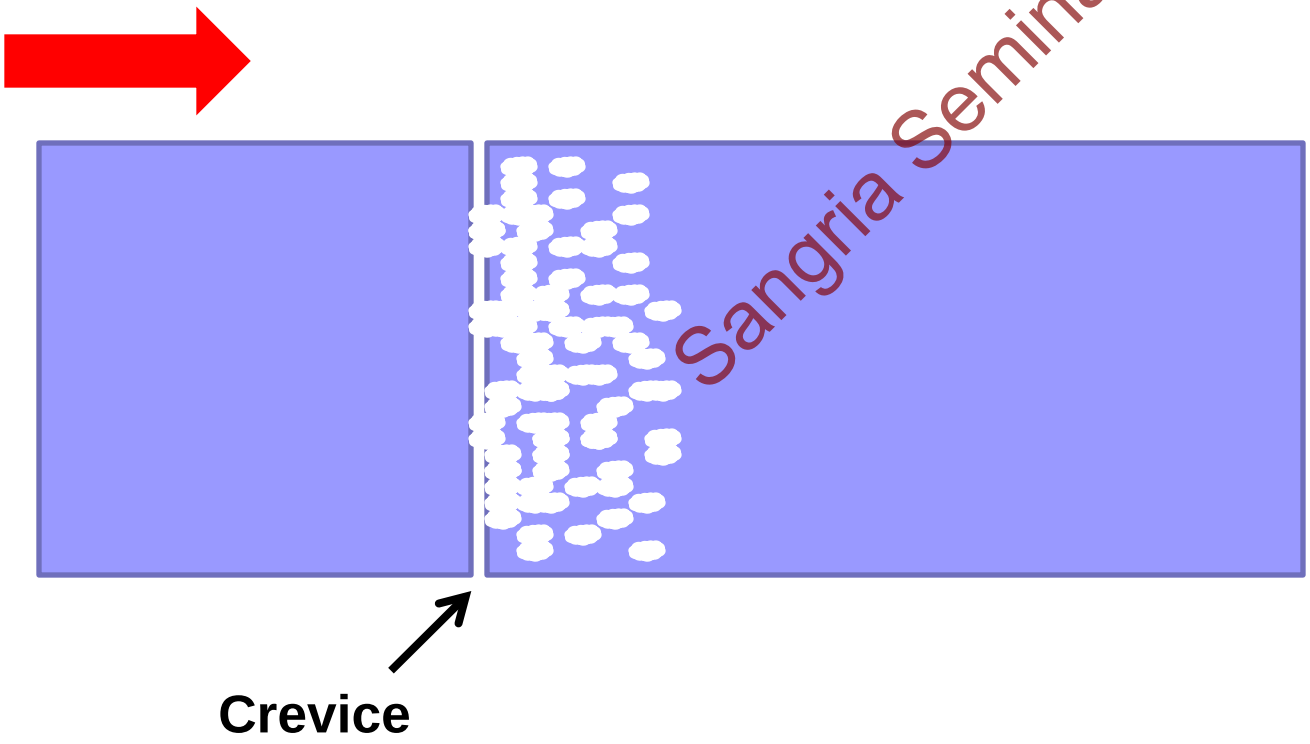
Blood Flow



Top View

$t_2 = t_1 + t$

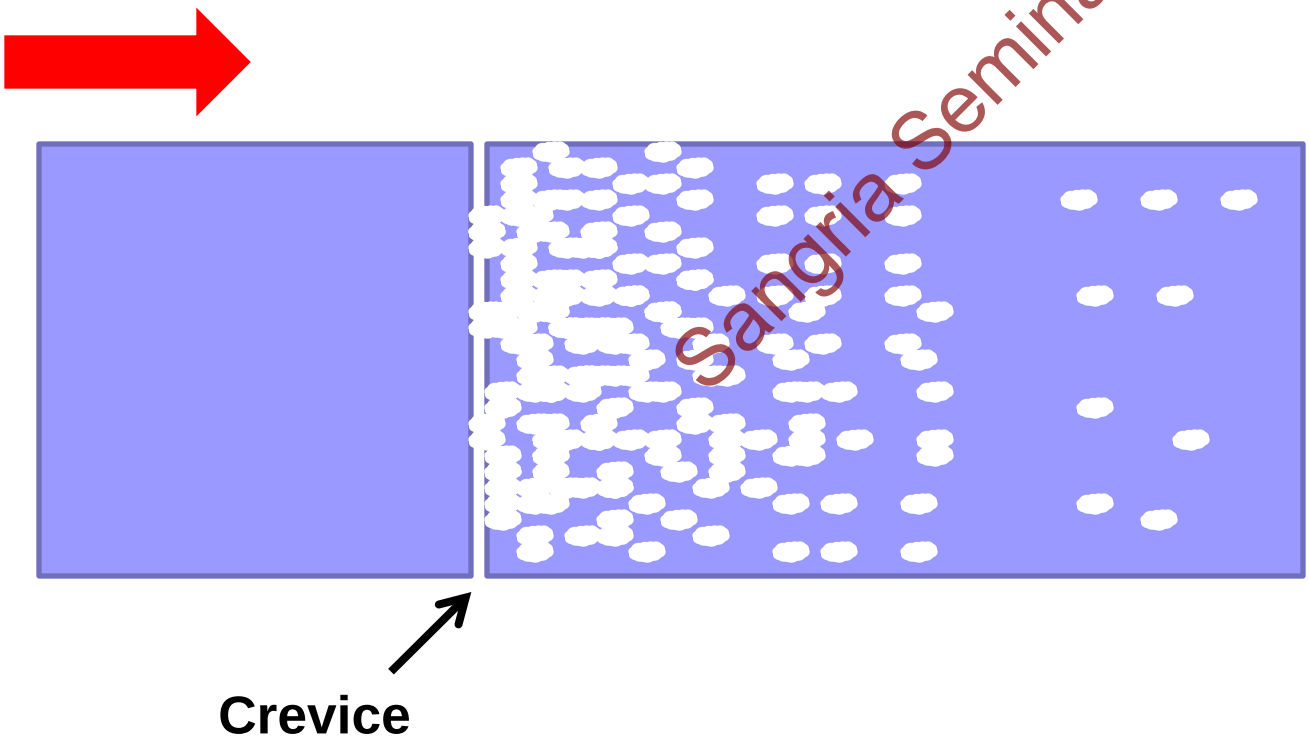
Blood Flow



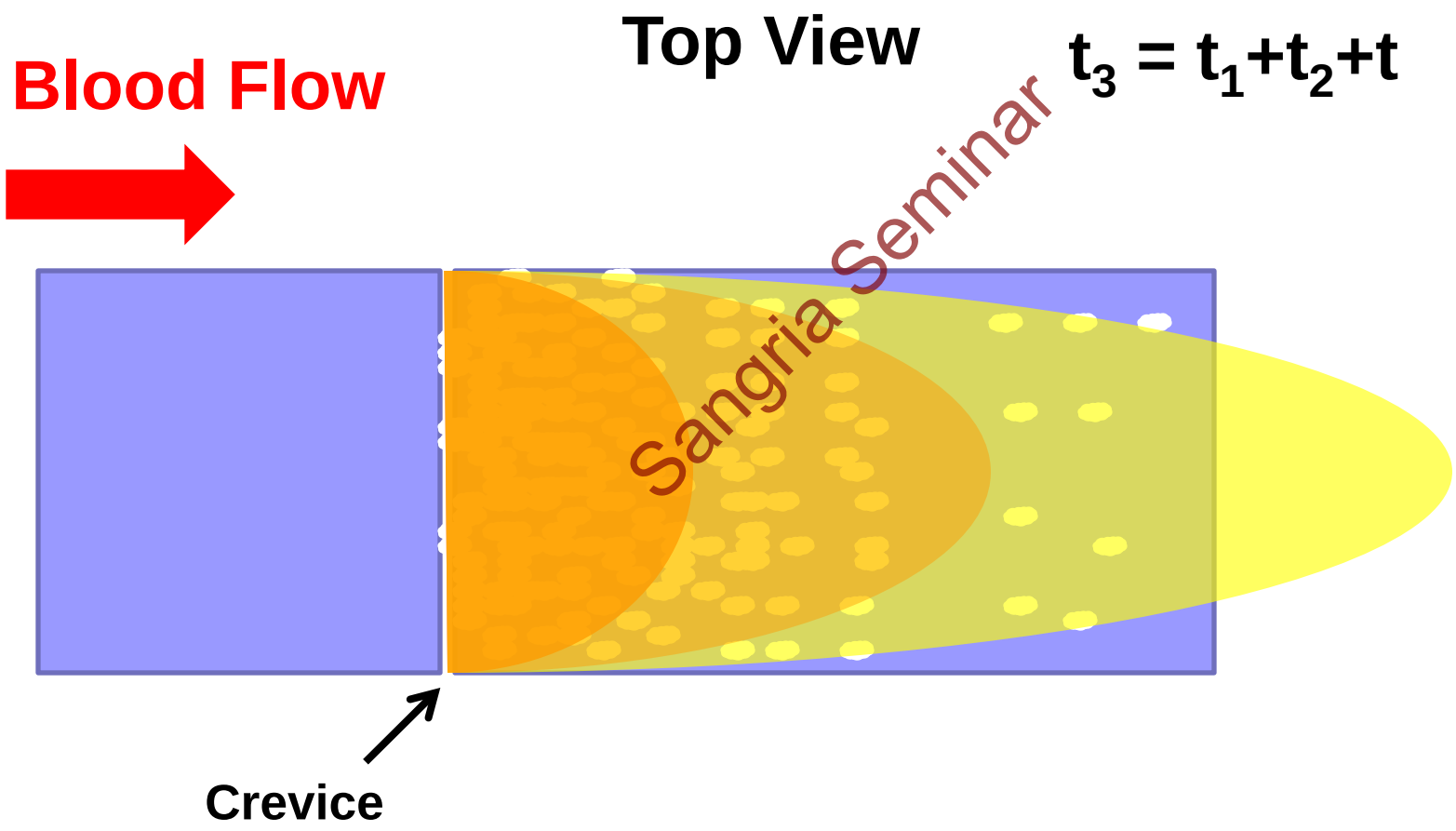
Top View

$t_3 = t_1 + t_2 + t$

Blood Flow



# Model Validation Example



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# Research Design and Methods

**Evaluation of platelet deposition and morphology**

- Incubate blood with mepacrine pre – perfusion for epifluorescent imaging after the test
  - Allows quick and simple imaging and sample recovery (as compared to SEM)
  - Imaging software may be used to determine number of adhered platelets and area of spread platelet coverage
- Scanning Electron Micrographs (SEM) will also be performed on some samples to validate the mepacrine data

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# Flow Cytometry Overview

•Fluorescently labeled antibodies or proteins are incubated with very small volumes of blood (~5 µL) in test tubes

- **•Wide varieties of antibodies enables the quantification of many different cellular markers**

•Blood components are lined up in the injector tip and passed in front of a laser

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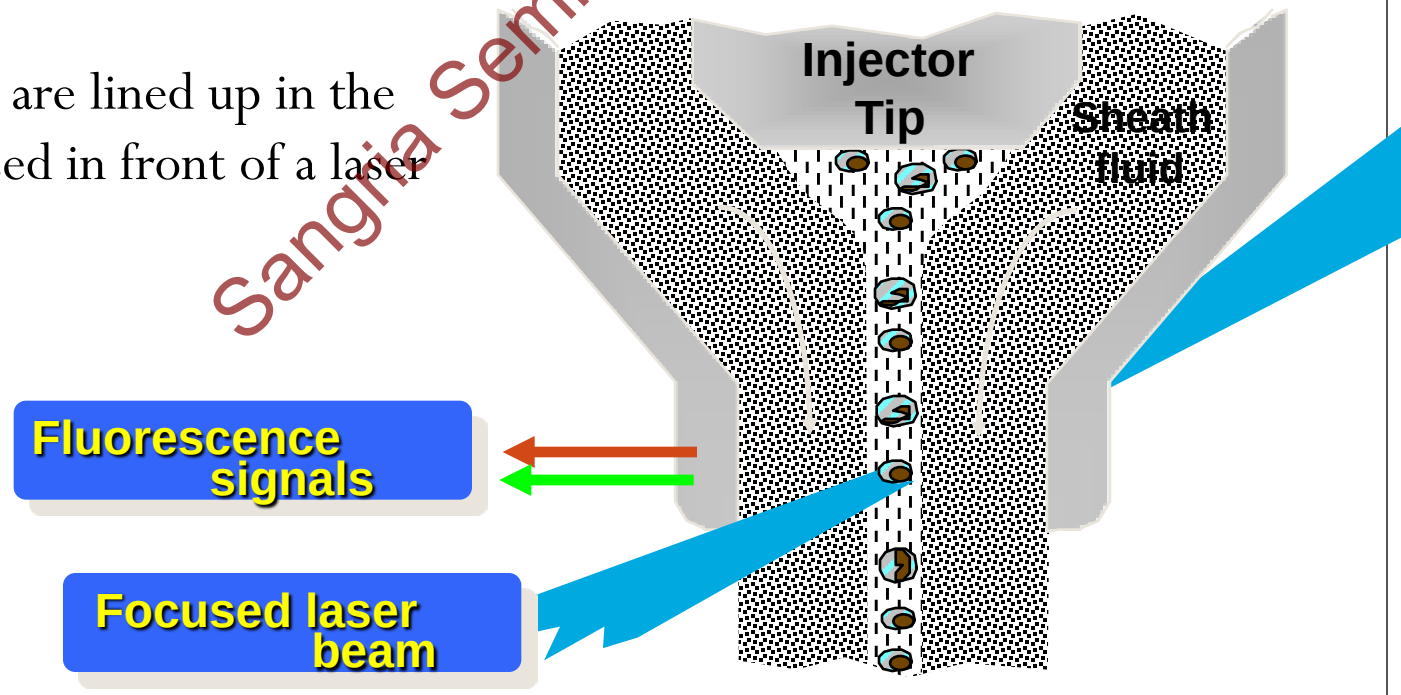
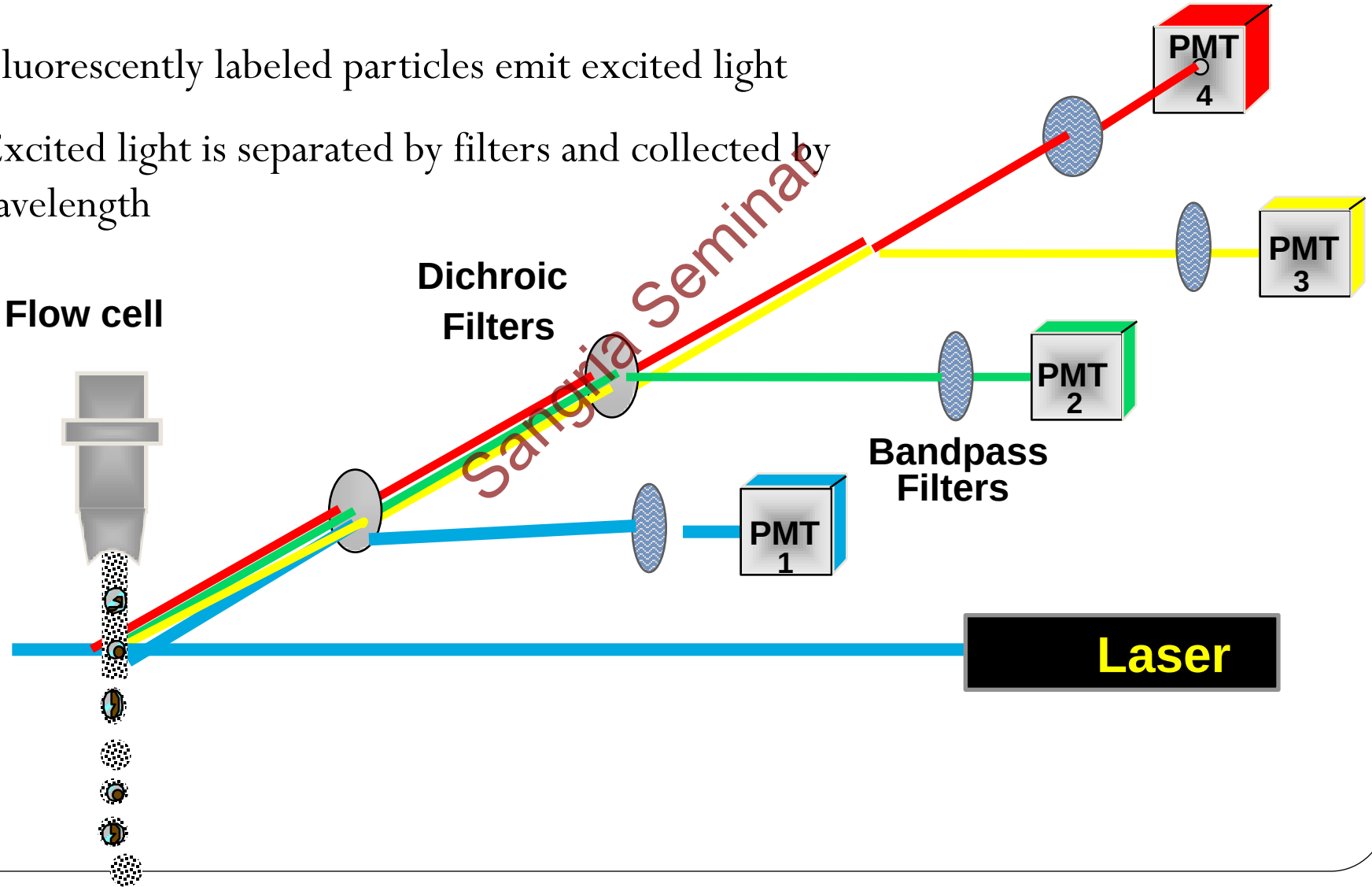


# Flow Cytometry Overview

- Fluorescently labeled particles emit excited light
- Excited light is separated by filters and collected by wavelength

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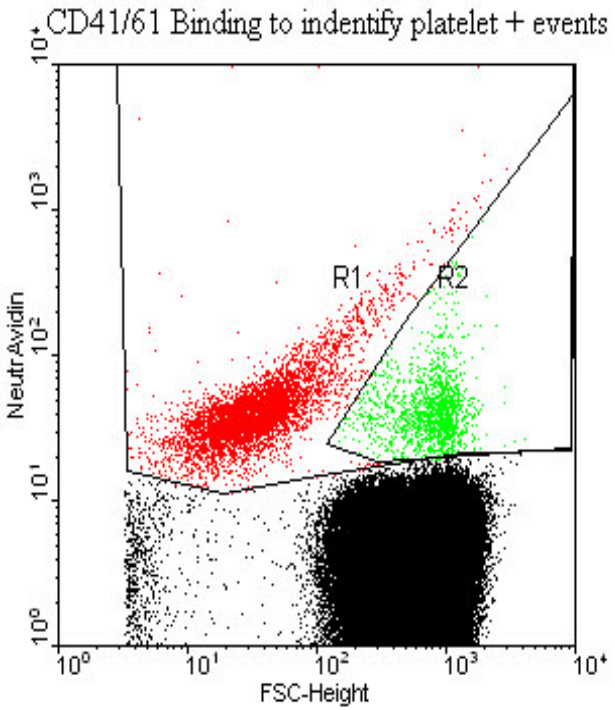
# Research Design and Methods

Flow cytometric determination of platelet activation (continued)

- Gating is placed on platelet positive events to determine the percent of platelets that express CD62P, CD63 or binding of Annexin V

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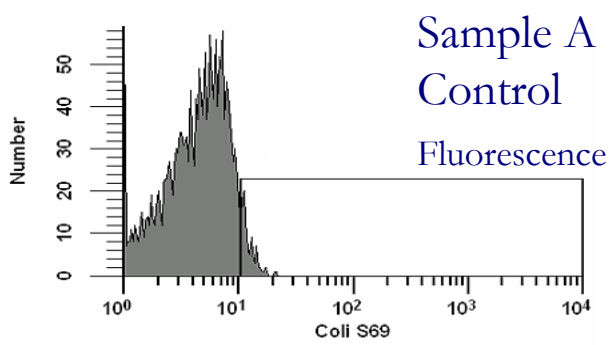
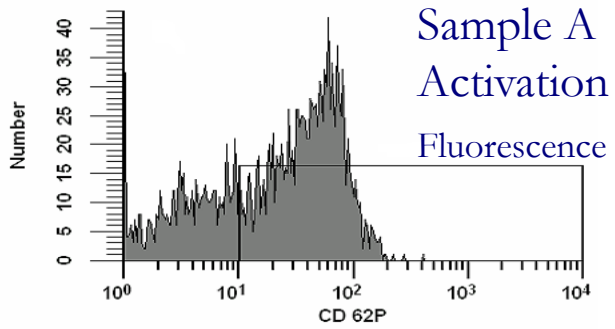
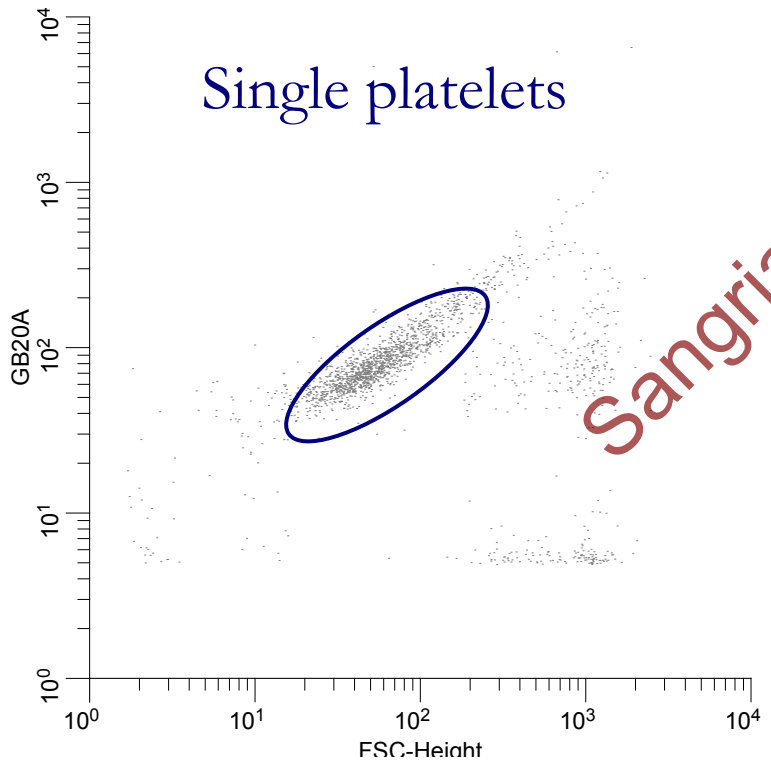


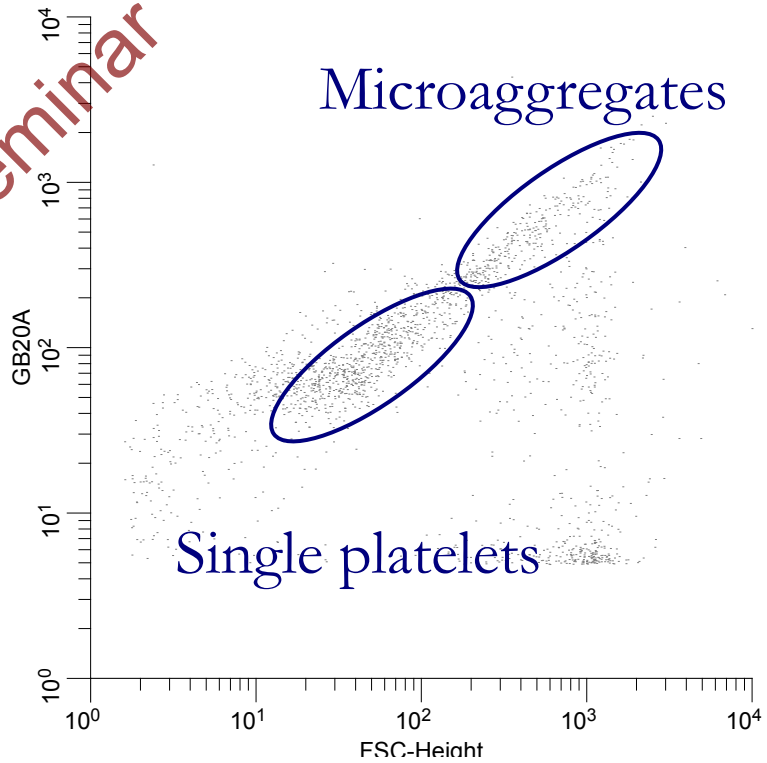



# Research Design and Methods

- Flow cytometric determination of platelet – platelet microaggregates
  - Use particle size and fluorescence intensity to choose microaggregate region




Control Sample




Activated Sample

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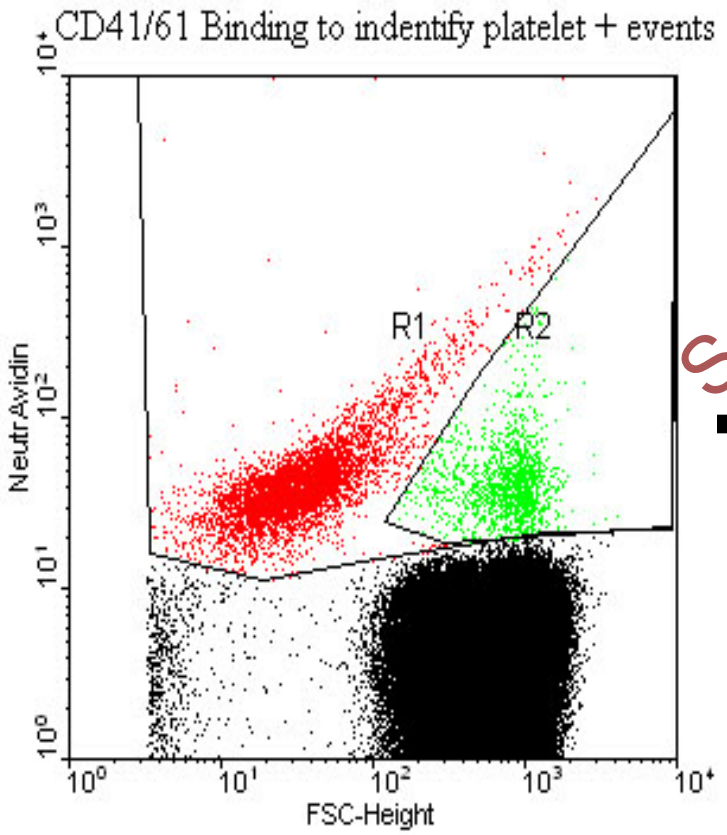
# Analyzing Flow Cytometry Results

- The filtered and collected information is presented by dot plots and histographs
- We can selectively analyze groups of particles from the dot plots to determine the amount of fluorescence (i.e. antibody labeling)

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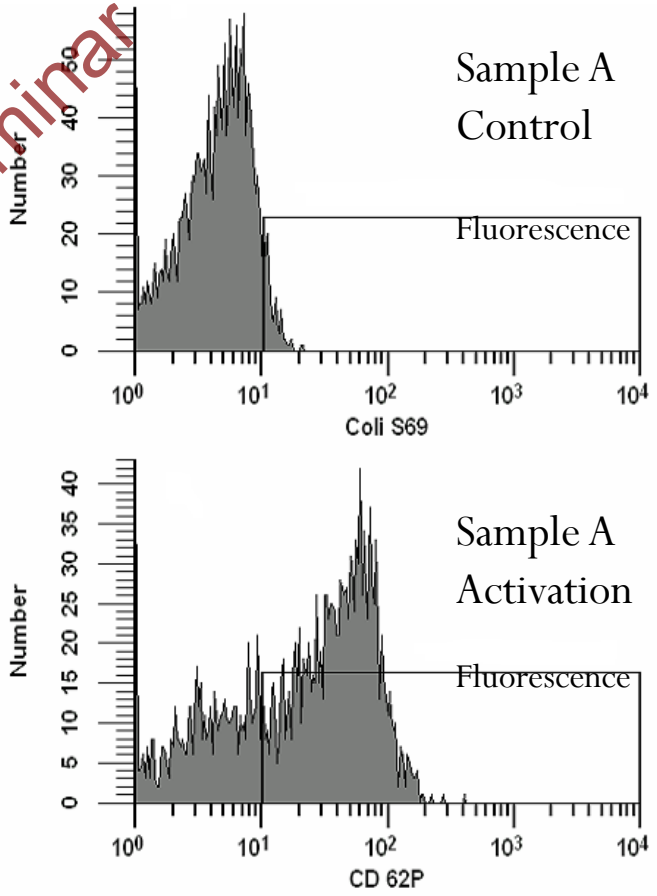

# Research Design and Methods

- **Chung et al. suggest defining (and maximizing) an Active Test Region (ATR) where laminar fluid flow and uniform shear on the blood may be confidently assumed**
  - **Wall and entrance length effects will decrease the size of the ATR**
  - **Flow visualization could be used to verify laminar flow and identify the ATR; analysis of platelet deposition could then be limited t[illegible] nar flow conditio[illegible]**

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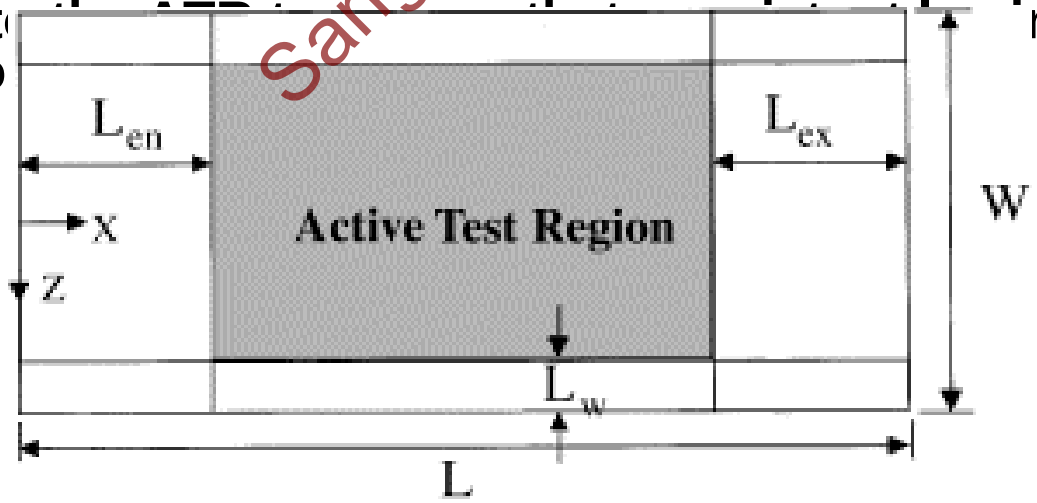


Chung BJ, Robertson AM, Peters DG. The numerical design of a parallel plate flow chamber for investigation of endothelial cell response to shear stress. *Computers & Structures.* 2003; 81:535 – 546

# Methods/Experiment Design

- **Governing equations**
  - **Nauman et al. reduces the design of a parallel plate chamber to three parameters – plate shear stress, Reynolds number and entrance length:**

$$\tau_w = \frac{6\mu Q}{wh^2}, \qquad \mathrm{Re} = \frac{v_{avg}\rho h}{\mu} = \frac{\tau_w \rho h^2}{6\mu^2}, \qquad L_{ent} = 0.04h\,\mathrm{Re} = 0.04\frac{\tau_w \rho h^3}{6\mu^2}.$$

  - **Limiting the height of the flow chamber will decrease the Re and the entrance length effects, while making the height too small may cause excessive shear stress and shear rates**
  - **This simplification of the governing equations may be acceptable for these proposed studies; however, Chung et al. found that this equation for entrance length will underestimate the true entrance length found in a flow chamber and suggest that a numerical approach should be used instead**

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Nauman E, Risic KJ, Keaveny TM, Satcher RL. Quantitative assessment of steady and pulsatile flow fields in a parallel plate flow chamber. *Ann Biomed Eng.* 1999; 27:194 – 199

# Methods/Experiment Design

- **Blood flow dynamics and validation**
  - **Chung et al. suggest defining (and maximizing) an Active Test Region (ATR) where laminar fluid flow and uniform shear on the blood may be confidently assumed**
    - **Wall and entrance length effects will decrease the size of the ATR; optimization of the design should focus on reducing these effects**
    - **Computational Fluid Dynamics (CFD) could be used to identify (and maximize) the ATR; analysis of platelet deposition and protein absorption could then be limited to the ATR to ensure that consistent laminar flow conditions are evaluated by the chambers**

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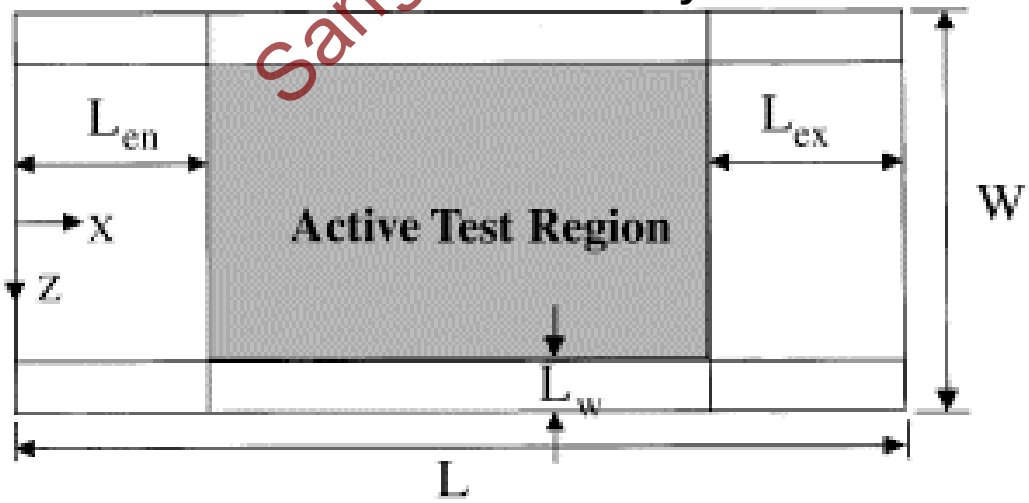


Chung BJ, Robertson AM, Peters DG. The numerical design of a parallel plate flow chamber for investigation of endothelial cell response to shear stress. *Computers & Structures.* 2003; 81:535 – 546

# Methods/Experiment Design

- **Blood flow dynamics and validation (continued)**
  - **The flow chamber should be constructed out of a clear polymer so that flow visualization may be performed to validate the assumed flow conditions and ATR region**
  - **The flow chamber used by Schaub et al. may be employed but with modifications**
    - **Corners created at the entrance and exit of the flow chamber may cause recirculation and stagnation of flow, which will lead to increased platelet activation**
    - **Fiber optic access will not be needed for this proposal and would be eliminated from this design**
    - **CFD and flow visualization could be used to optimize this design**

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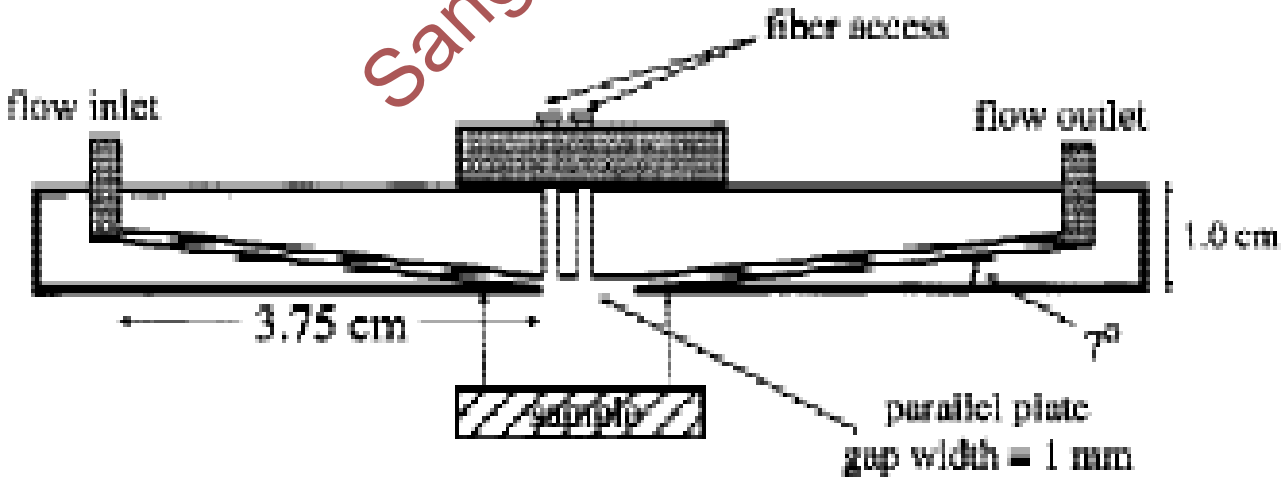


Schaub RD, Kameneva MV, Borovetz HS, Wagner WR. Assessing acute platelet adhesion on opaque metallic and polymeric biomaterials with fiber optic microscopy. *J. Biomed Mater Res.* 2000; 49(4):460 – 468