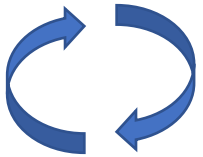
FIU Engineering & Computing
Biomedical Engineering

Valve Endothelial Cells 

Exposure to High Oscillatory Flow 

Leads to Valve Interstitial Cell Calcification 

Graduate Research Day

Denise Hsu

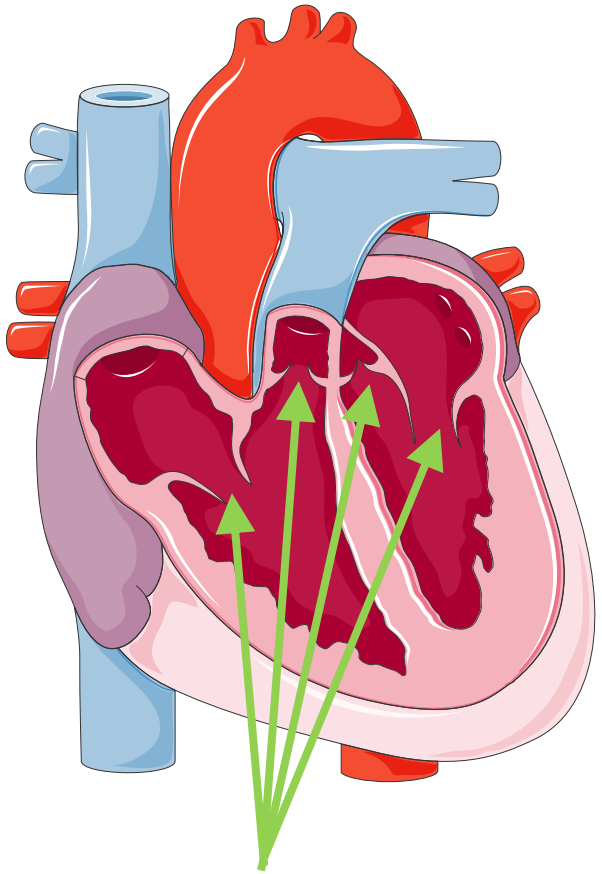
March 9th, 2022

Advisors: Dr. Joshua Hutcheson, Dr. Sharan Ramaswamy

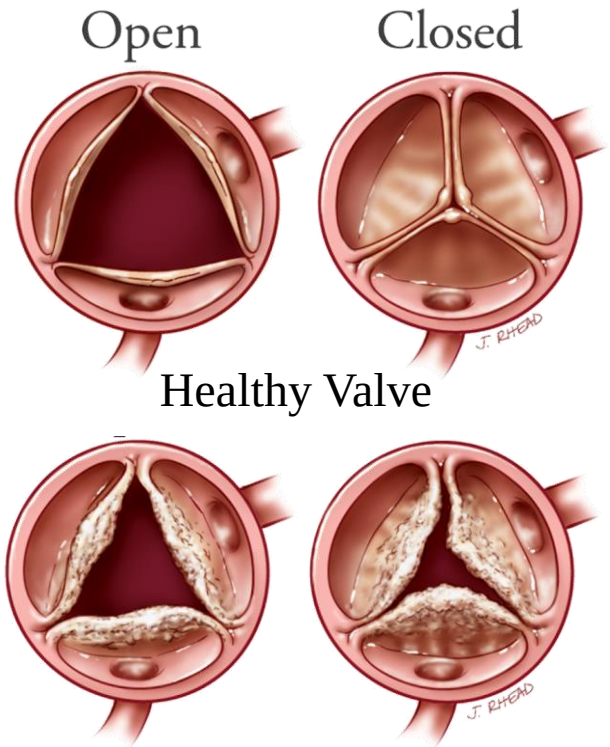
Cardiovascular Matrix Remodeling Laboratory

CV-PEUTICS
Cardiovascular Therapeutics Lab

Introduction



Valves



Healthy Valve

Valve Stenosis

CAVD

Calcific Aortic Valve Disease:

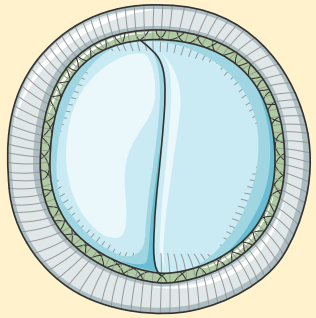
- One of the most prevalent chronic heart problems
- Global increase by 124% between 1990 and 2017.

- Stenosis
- Regurgitation
- Reduced cardiac output

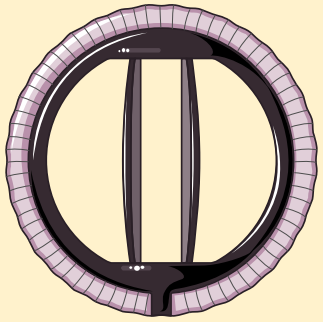
Yadgir et al.

Problem Statement

- No treatments for early and intermediate stages of disease
- Severe treatment options: **bioprosthetic** or **mechanical** valve replacements



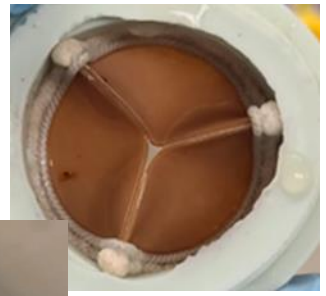
Bioprosthetic Valve



Mechanical Valve

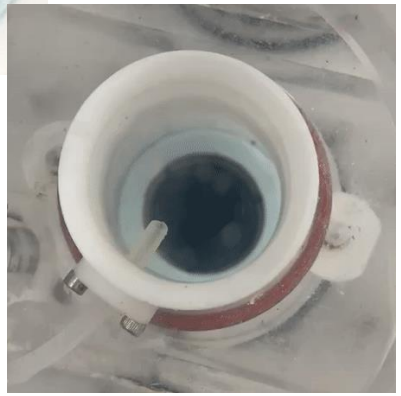
CAVD

Bioprosthetic Valve



- Re-calcification
- Multiple operations

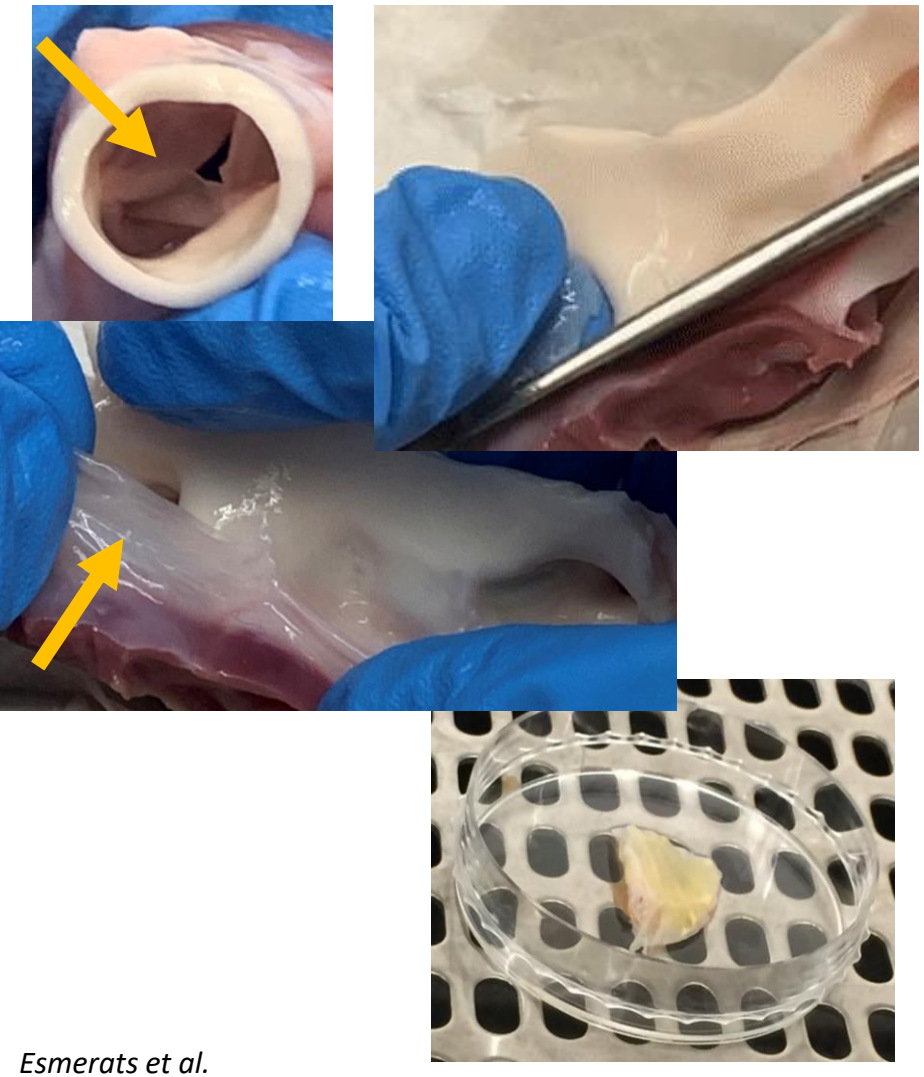
Mechanical Valve

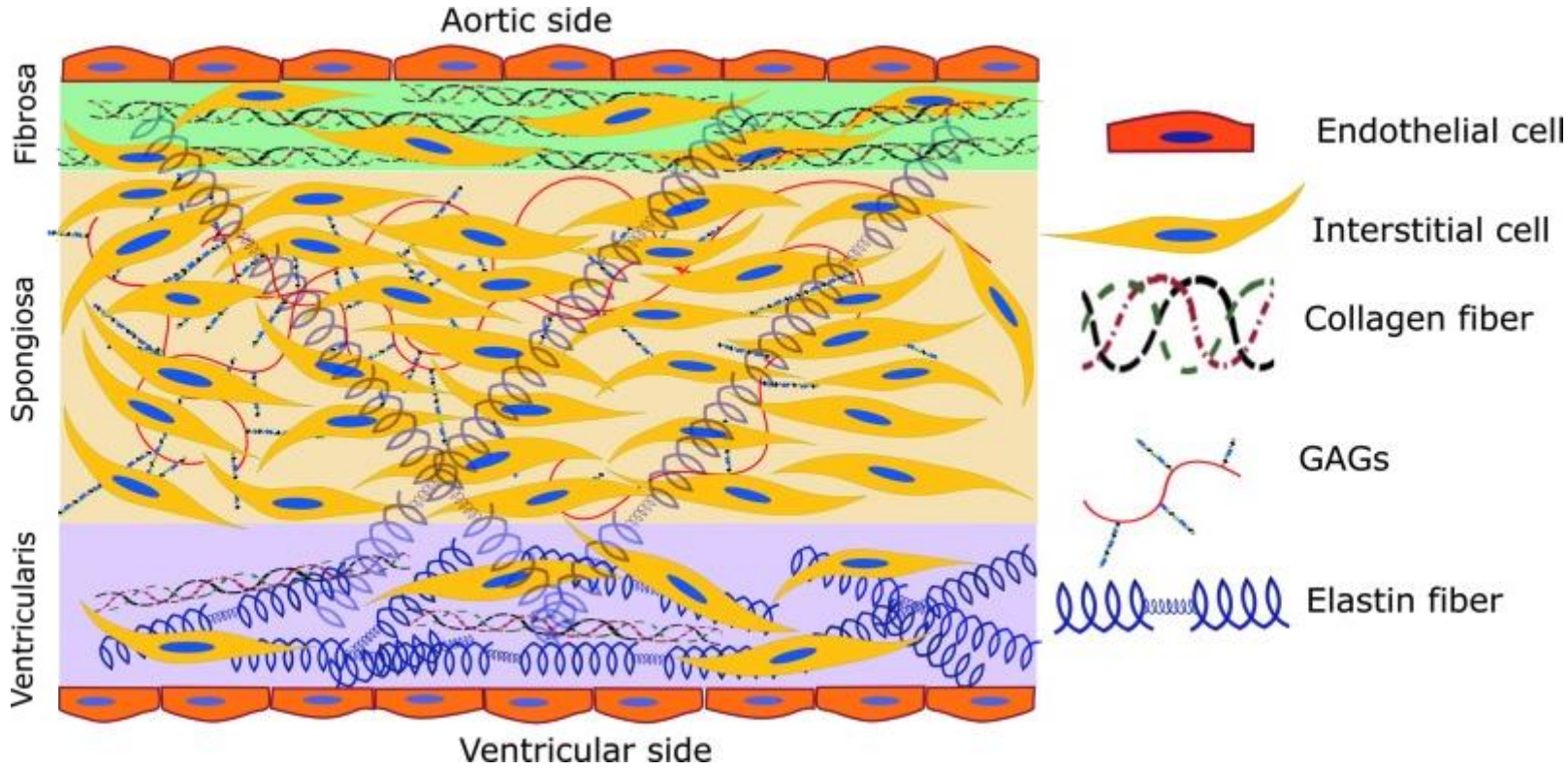


- Life-long anti-coagulant

- Highly invasive
- Limited to selective patient subset

Background – Valve Anatomy

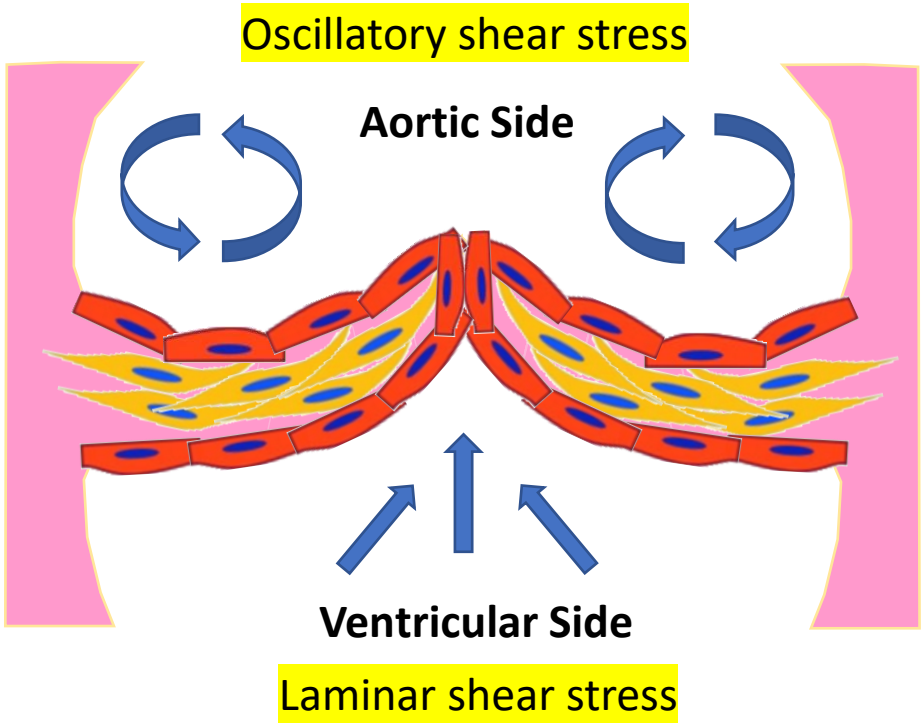


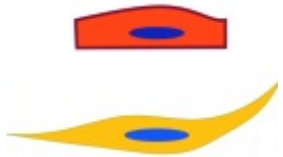


Esmerats et al.

Background – Valve Hemodynamics

Fibrosa
Spongiosa
Ventricularis





VEC: Valve Endothelial Cells

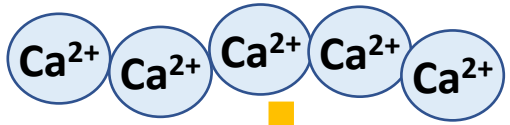
VIC: Valve Interstitial Cells

What we know…

- Low shear stresses
  (< 4 dynes/cm^2)

  ↓

  lesions and calcification
- Oscillatory flow with
  high calcium concentrations



  ↓

  inflammation on the valve
  fibrosa layer

What we want to know…

Relation between

precise flow oscillations

&

development of **CAVD**

OSI → CAVD

Malek et al., Chatzizisis et al., Gomel et al., Chiu et al.
Esmerats et al.

Oscillatory Shear Index

- Oscillatory Shear Index (OSI)
  - Measurement of flow disturbance
  - Ratio between forward shear and total shear
- $0 \leq OSI \leq 0.50$

$$OSI = \frac{1}{2}\left(1 - \frac{\left|\int_0^T \tau_w dt\right|}{\int_0^T |\tau_w| dt}\right)$$

OSI

T: duration of cycle

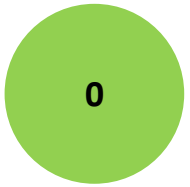
τ_w: wall shear stress

t: time

Oscillatory Shear Index OSI

Static
OSI=0

Steady
OSI=0



Moderate
OSI=0.25

High
OSI=0.50

He & Ku

Objective

- To correlate OSI with progression of CAVD

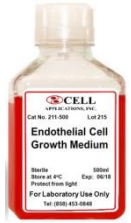
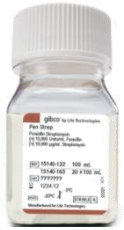
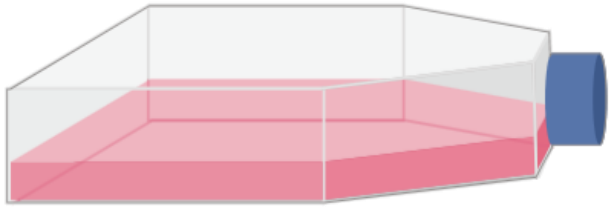
Hypothesis

We hypothesize that a combination of **high** OSI with **pro-calcific (PC)** environment promotes CAVD.

To test our hypothesis…

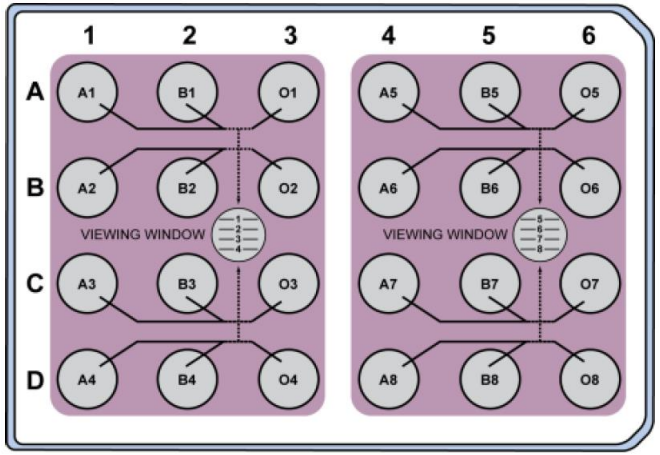
- Evaluate the extent to which paracrine signaling-mediated events from VECs cultured under dynamic conditions in low (OSI=0), moderate (OSI=0.25), and high (OSI=0.50) OSI environments lead to VIC calcification.

Cell Culture and Expansion

CATEGORY	Valvular Endothelial Cells (VEC) 	Valve Interstitial Cells (VIC)
CULTURE MEDIA	Endothelial Cell Growth Medium 	Growth medium
SUPPLEMENTS	1% Penicillin/Streptomycin 	10% Fetal Calf Serum 1% Penicillin/Streptomycin
CULTURE VESSEL	T75 Flask, coated with endothelial matrix 	T75 Flask

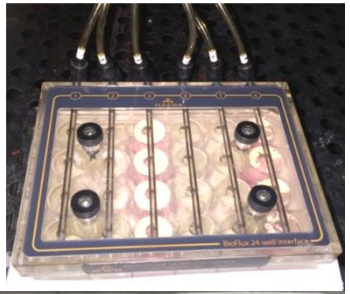
Bioflux System

- 24-well Plate
  - 8 microfluidic channels/plate
- Seeding density (Fluxion protocol):
  - 200,000 cells/channel



Pneumatic Pump

Fluxion Biosciences, Inc.

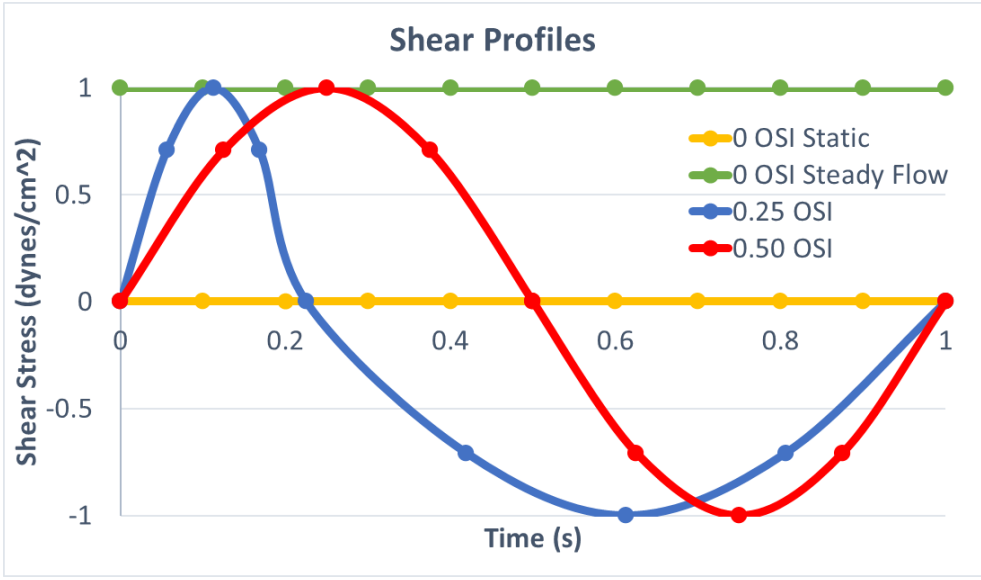


Fluid Shear at 1 dyne/cm^2

Yap et al.

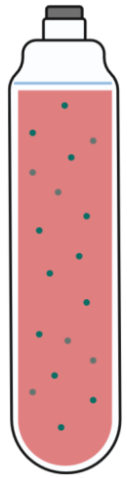
(48 hours)

Bottom of plate/channel

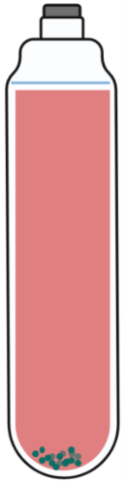


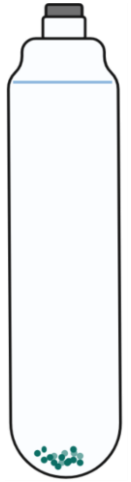
Ultracentrifuge

original



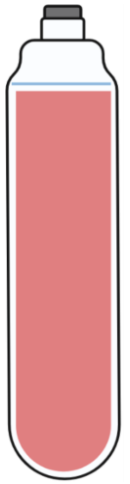
VEC-conditioned
media collected
from Bioflux
(added PC ingredients)





Exosomal Group
(resuspended in fresh PC media)

pellet

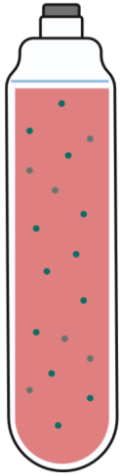


Non-Exosomal Group
(added PC ingredients)

supernatant

Pro-Calcific (PC) Ingredients

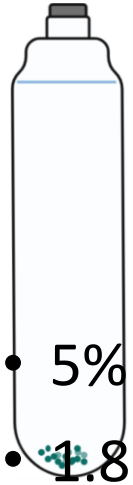
original



Org-PC

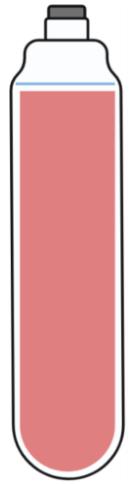
EX-PC

CY-PC



pellet

- 5% FBS, 1% P/S
- 1.8 mM $CaCl_2$
- 3.8 mM NaH_2PO_4
- 0.4 units inorganic pyrophosphate



supernatant

Rathan et al.
Goto et al.

Cell Culture and Expansion

CATEGORY	Valvular Endothelial Cells (VEC)	Valve Interstitial Cells (VIC)
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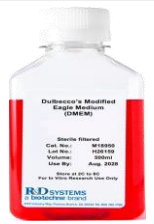
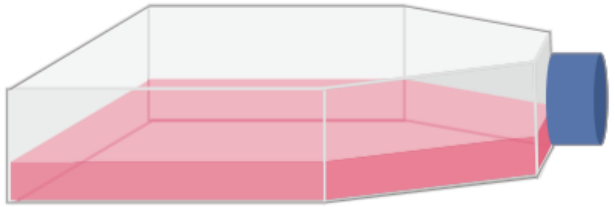
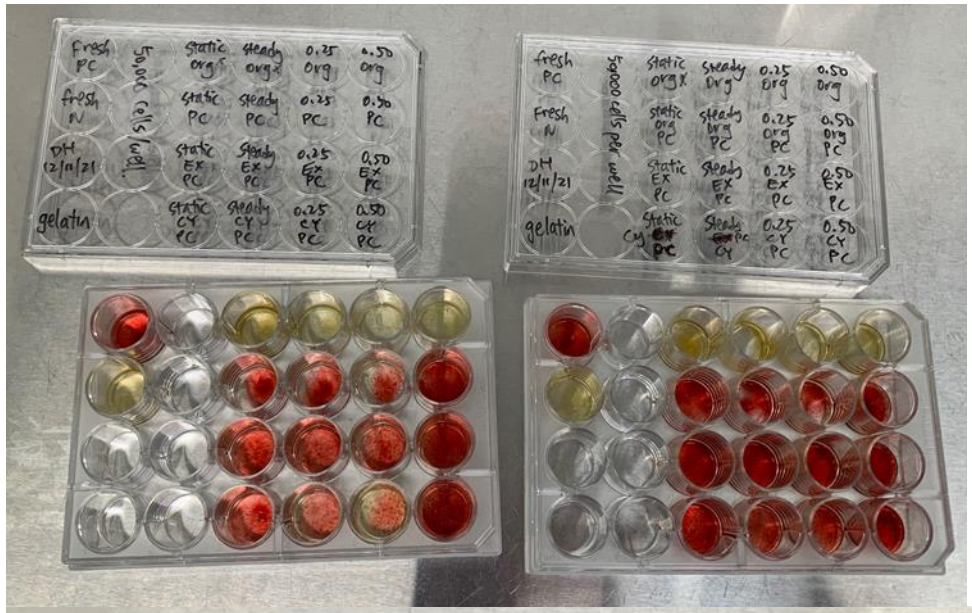
CULTURE VESSEL	T75 Flask, coated with endothelial matrix	T75 Flask

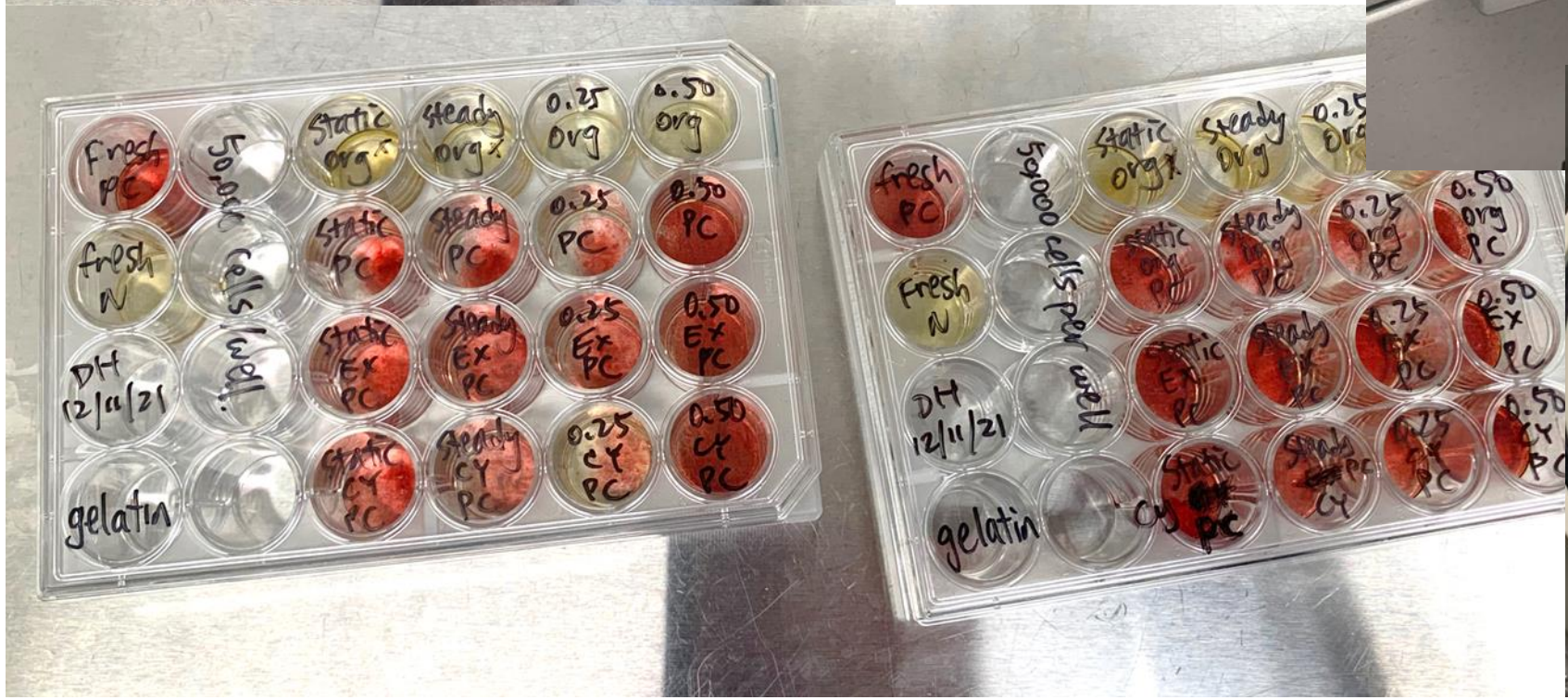




Paracrine Regulation

Cell Type	Flow Environment	Conditioning Media	Conditioning Time	Vessel
 VEC	Static (no flow)	Fresh Media	48 hours	 Bioflux
	Steady Flow (0 OSI)			
	0.25 OSI			
	0.5 OSI			


Fresh PC
50,000 cells/well
Static org
Steady org
0.25 Org
0.50 org
Fresh N
Static PC
Steady PC
0.25 PC
0.50 PC
DH 12/11/21
Static EX PC
Steady EX PC
0.25 EX PC
0.50 EX PC
gelatin
Static CY PC
Steady CY PC
0.25 CY PC
0.50 CY PC


Fresh PC
50,000 cells/well
Static org
Steady org
0.25 Org
0.50 org
Fresh N
Static PC
Steady PC
0.25 PC
0.50 PC
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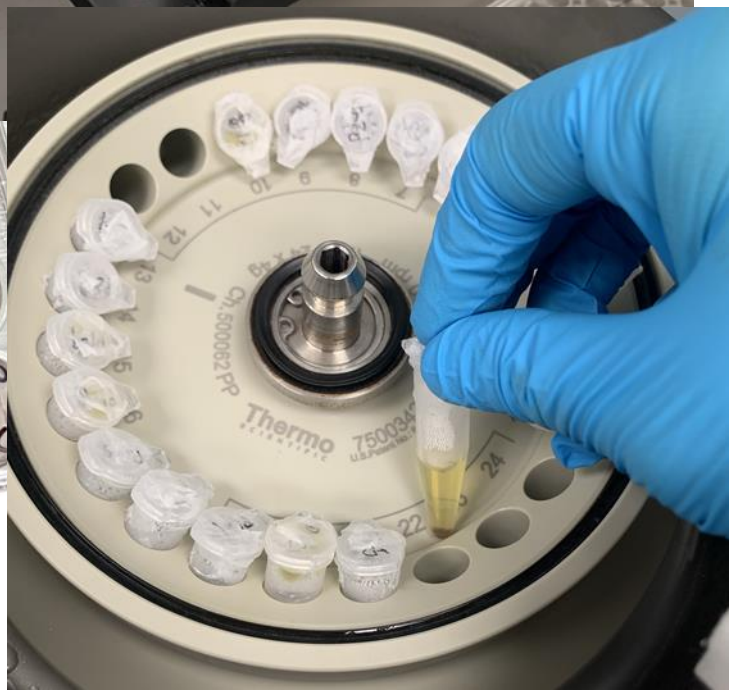
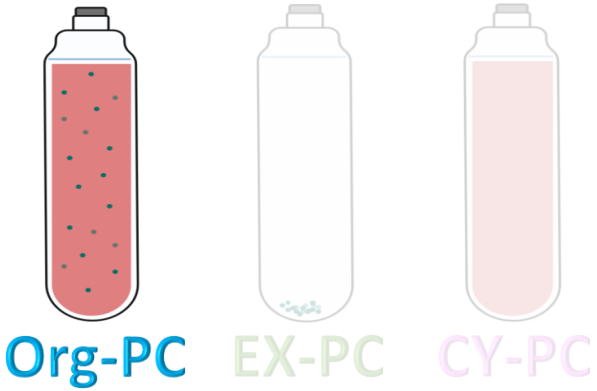
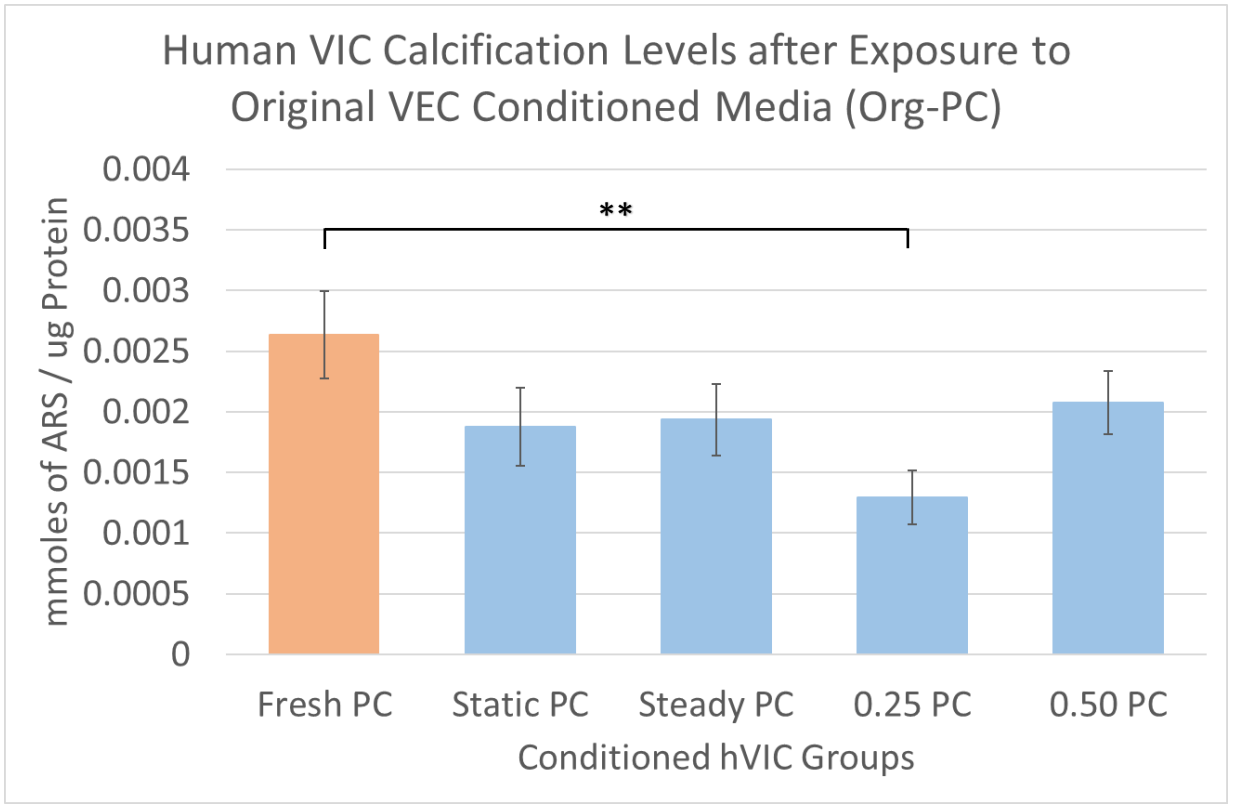
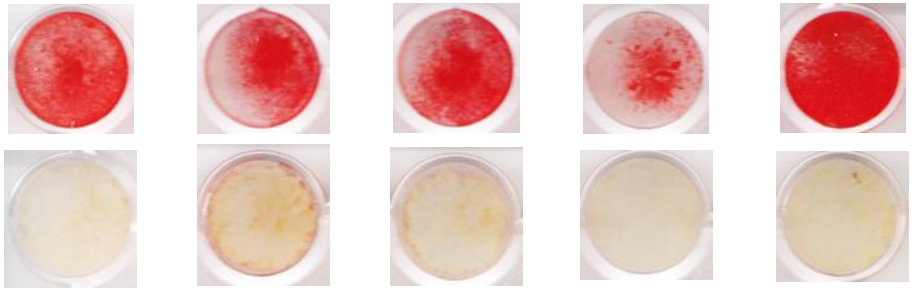
Static EX PC
Steady EX PC
0.25 EX PC
0.50 EX PC
gelatin
Static CY PC
Steady CY PC
0.25 CY PC
0.50 CY PC


Thermo

Results – Original PC



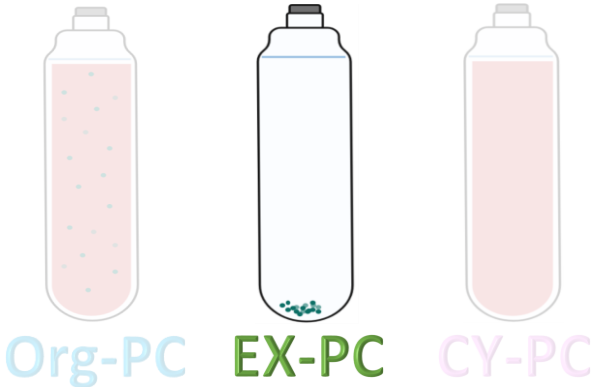


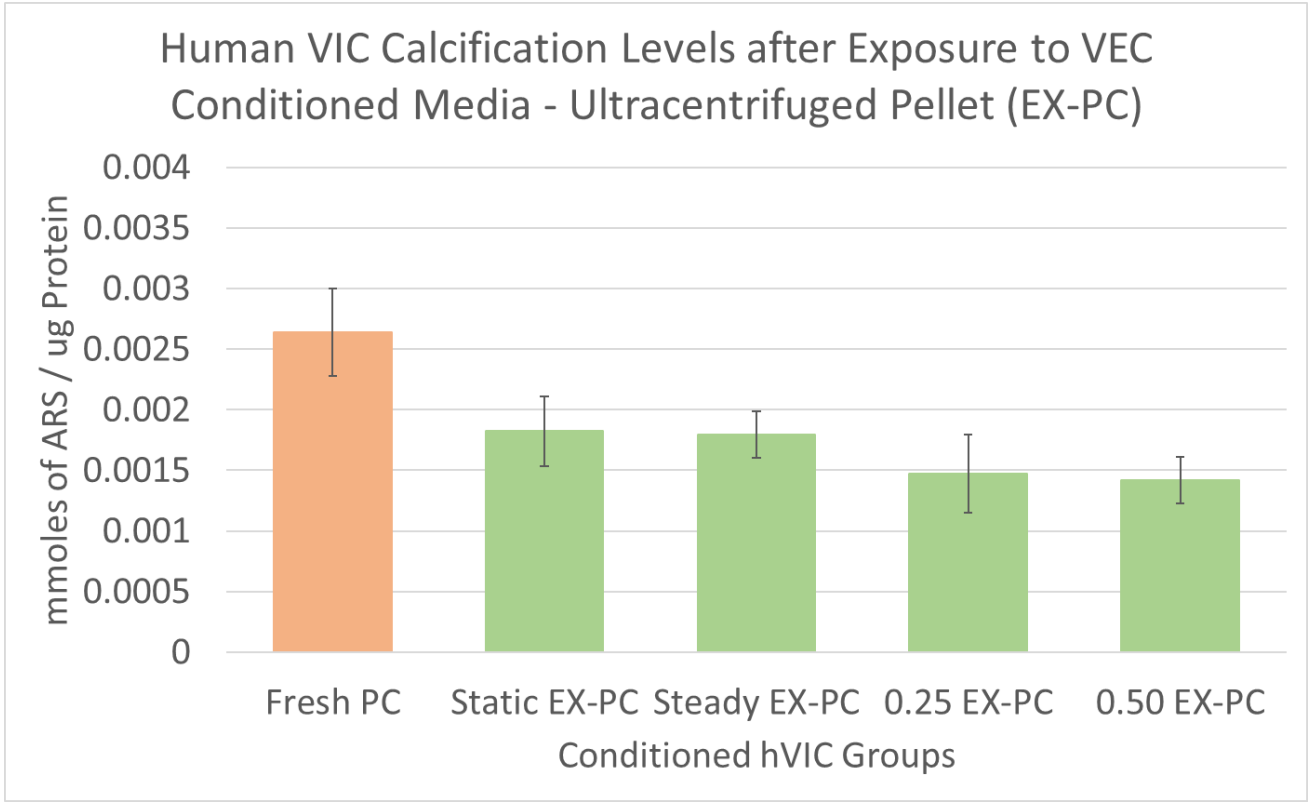


(Negative controls)

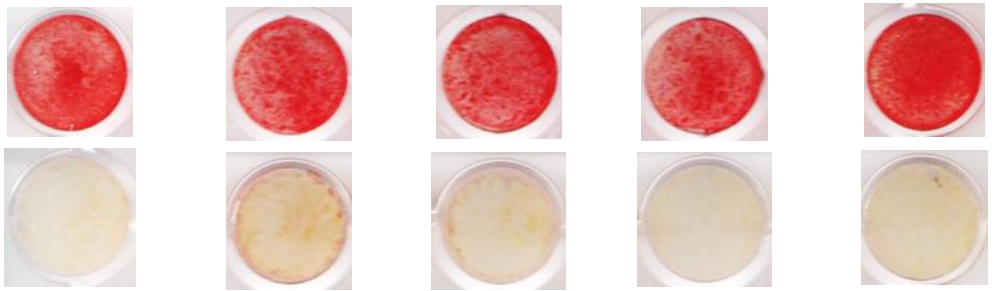
** p<0.05
*** p<0.005

Results – EX-PC

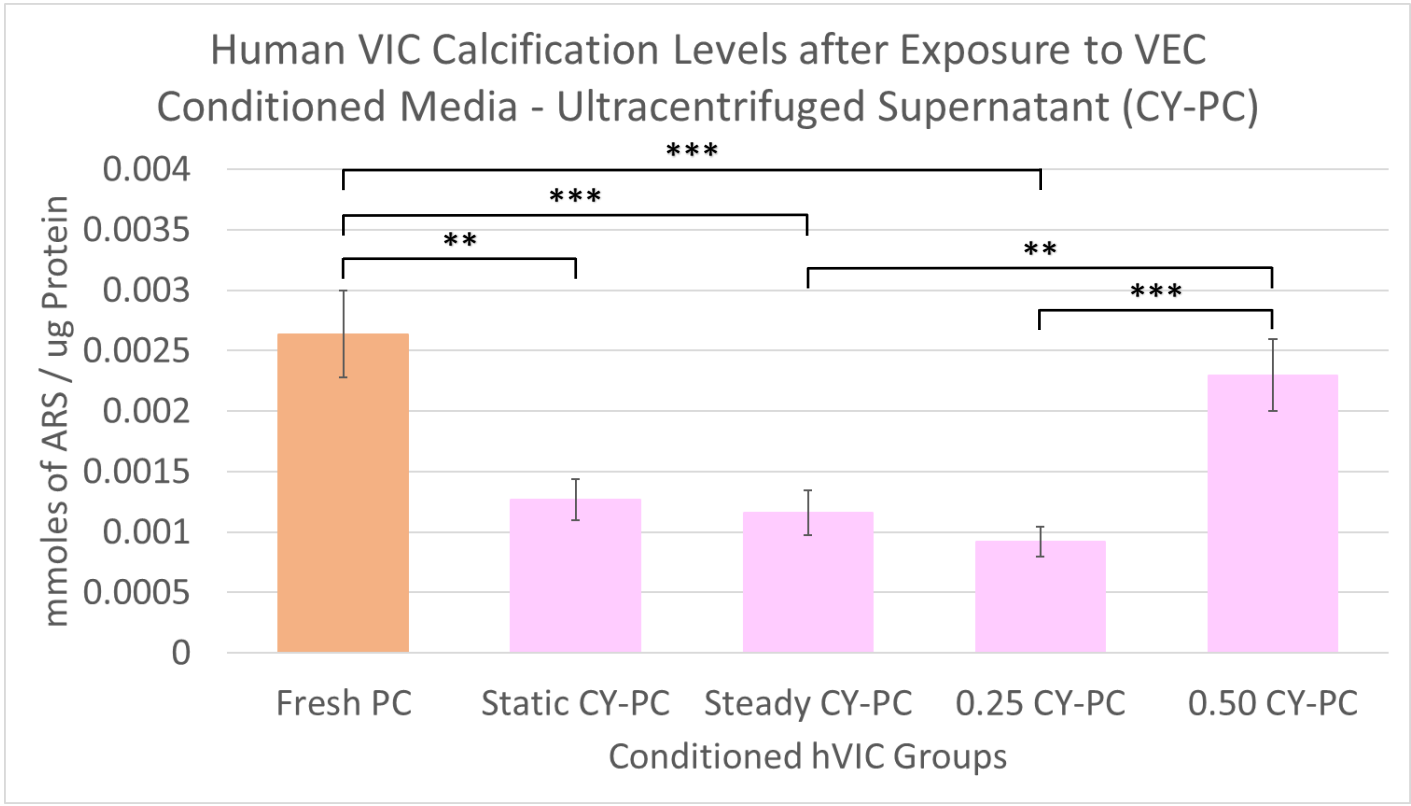


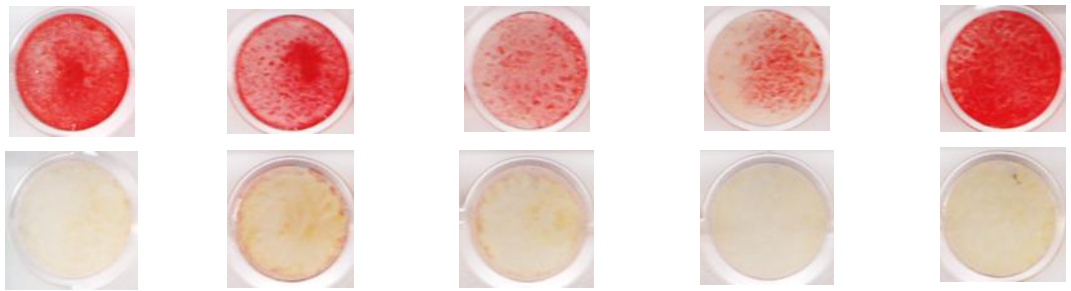


(Negative controls)



Results – CY-PC





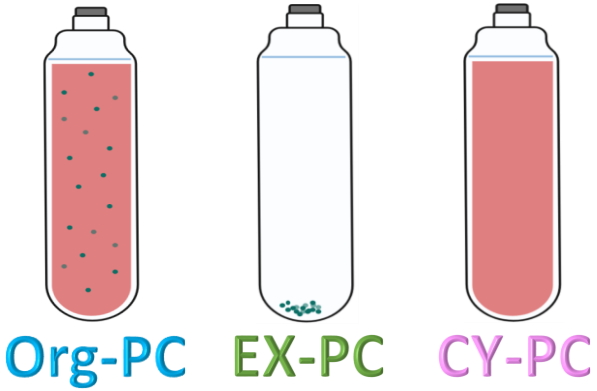
(Negative controls)

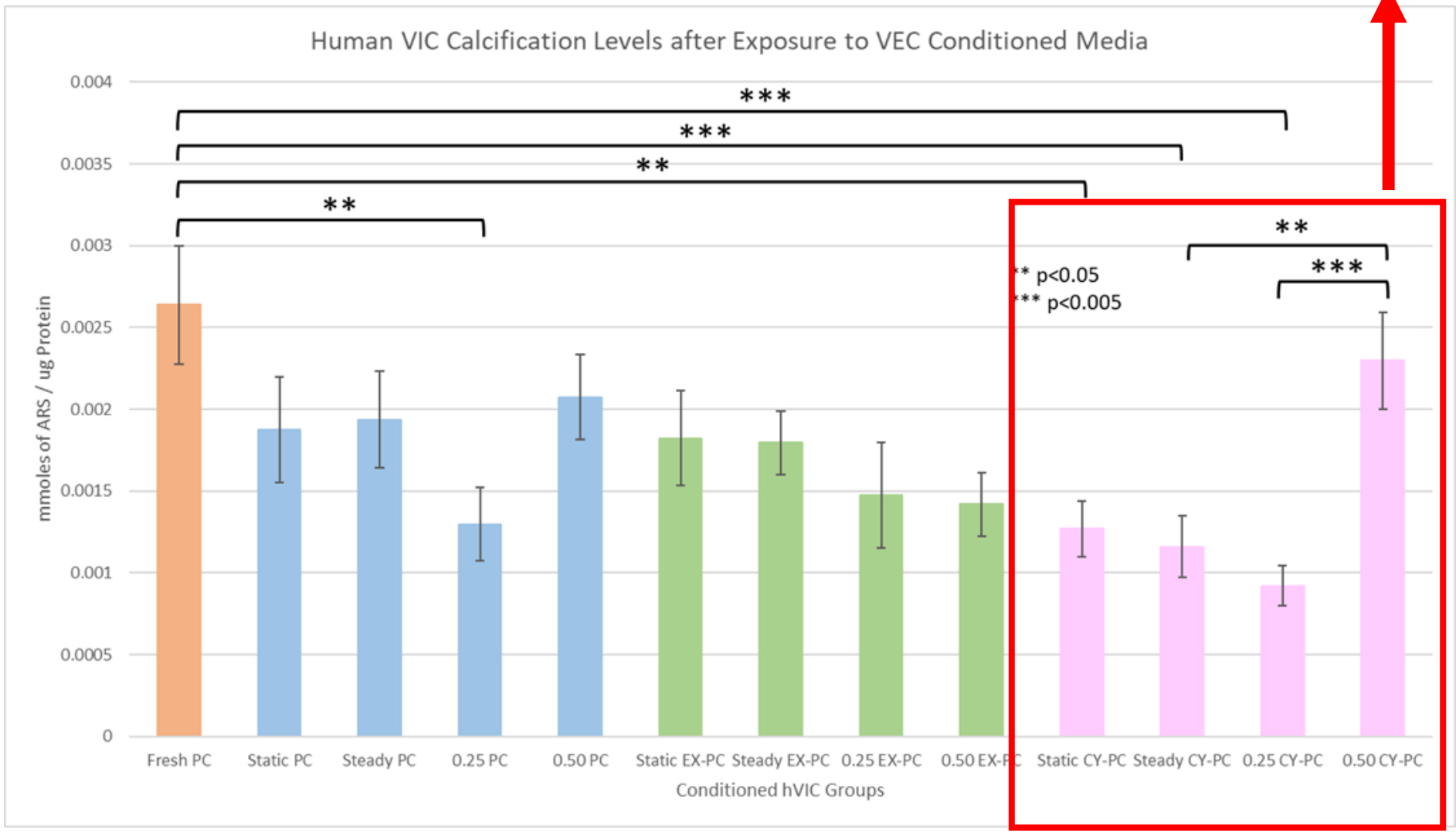
** p<0.05
*** p<0.005

Conclusion/Discussion

High OSI + PC = CAVD ??

non-exosomal cytokine pathways





On-going Work

- Conditioned media ELISA cytokine panel

- TGFb
- VEGF
- TNFa
- IL-1b
- IL-6
- IL-8
- MCP-1
- GM-CSF

Thank You!

Cardiovascular Matrix Remodeling Laboratory

FIU Engineering & Computing
Biomedical Engineering

CV-PEUTICS
Cardiovascular Therapeutics Lab

Questions/Comments?