Calcific Media Combined with Media from Oscillatory Flow-Conditioned Valve Endothelial Cells Leads to Valve Interstitial Cell Calcification

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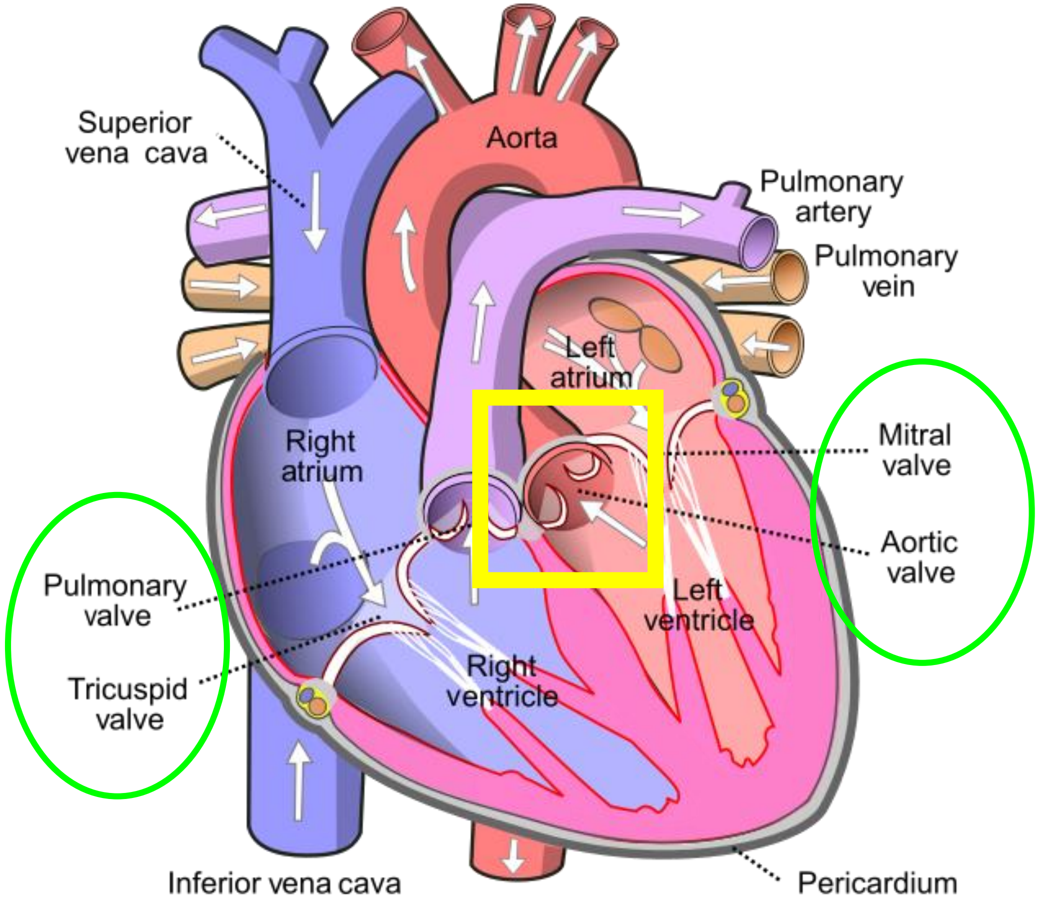
Cardiovascular Matrix Remodeling Laboratory

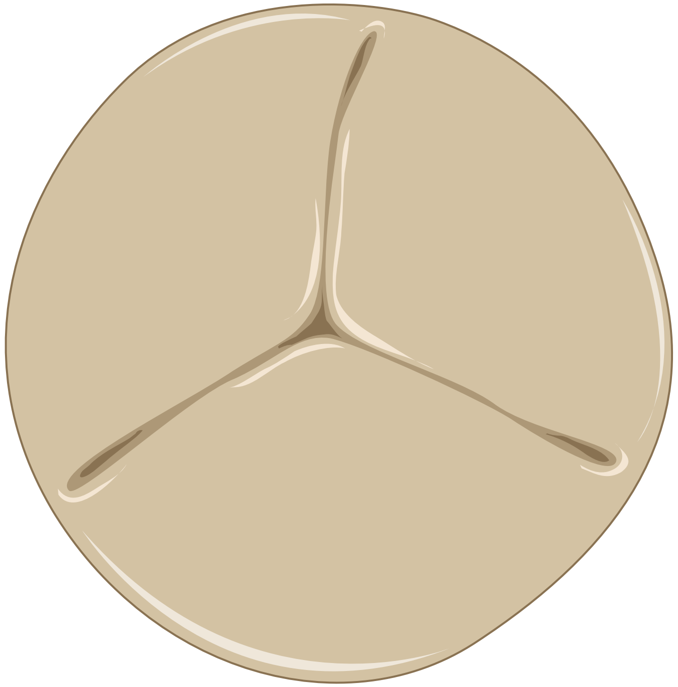
CV-PEUTICS
Cardiovascular Therapeutics Lab

Heart Valve Function

- 4 valves
  - Consist of leaflets
  - Facilitate unidirectional flow
- Aortic valve
  - Most commonly diseased
  - Situated between the left ventricle and the aorta



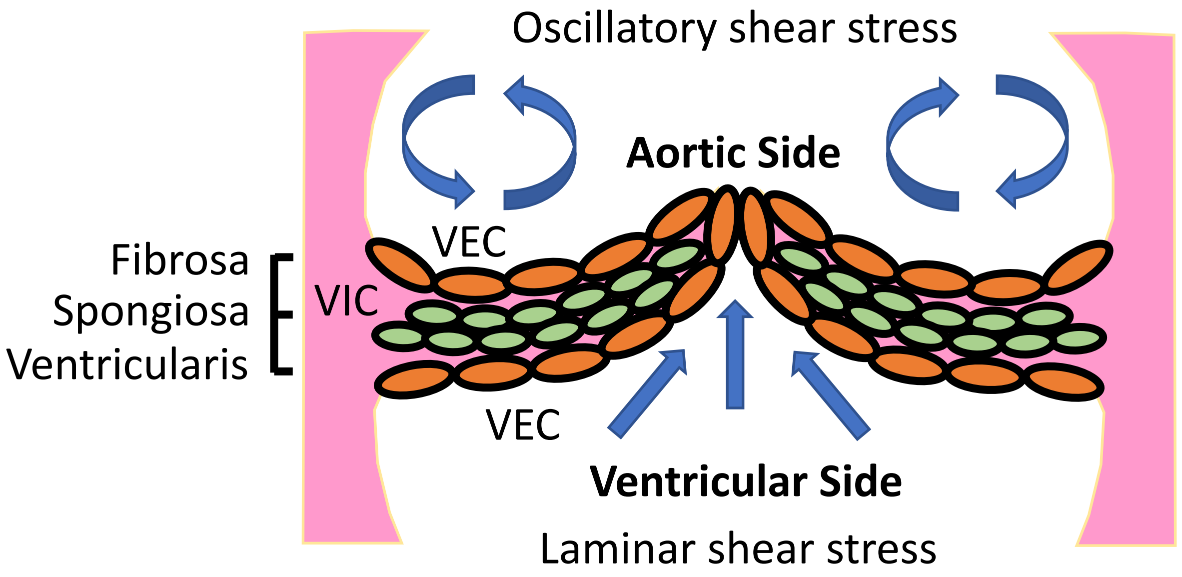
Calcific Aortic Valve Disease (CAVD)

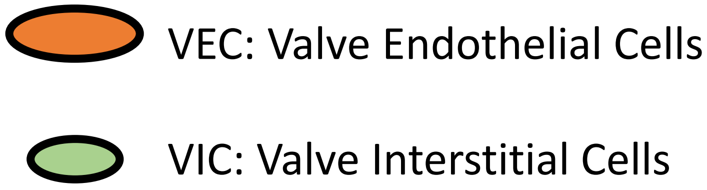


Closed health valve

Calcified valve - Stenosis

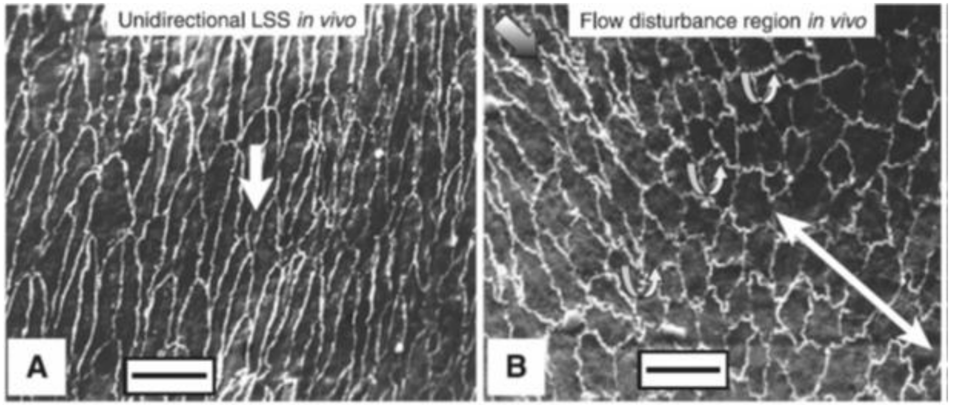
Valve Structure and Hemodynamics

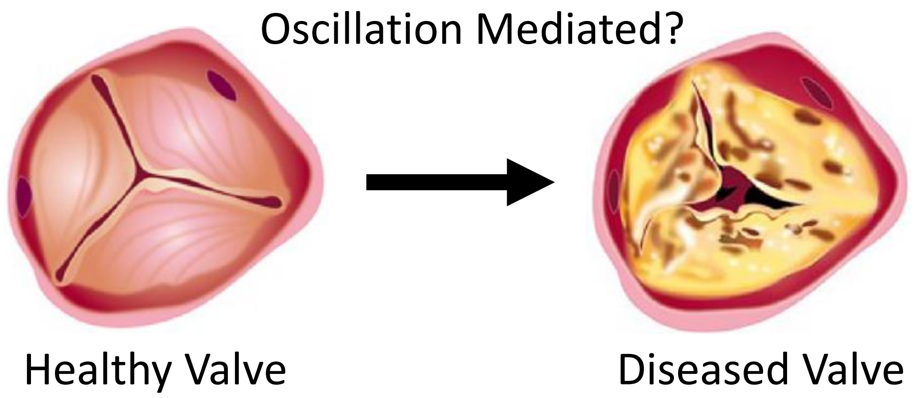




Problem Statement

- Past research shows alignment of cells change under flow patterns





- Problem Statement

Degree of Oscillation → Valve Endothelium injury? → VIC Phenotype change? → **Disease / remodeling?**

Chiu, J. et al.

Oscillatory Shear Stress

- Oscillatory Shear Index (OSI)
  - Measurement of flow disturbance
  - Quantifies ratio between forward shear and total shear
- $OSI = \frac{1}{2}\left(1 - \frac{\left|\int_0^T \tau_w dt\right|}{\int_0^T |\tau_w| dt}\right)$
  - T: duration of cycle
  - τ_w: wall shear stress
  - t: time
- $0 \leq OSI \leq 0.5$

Oscillatory Flow Profiles

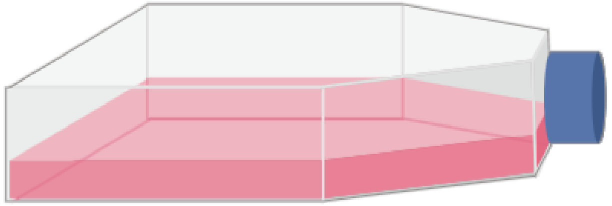
- 0 OSI, static (no oscillation)
- 0 OSI, steady flow (no oscillation)
- 0.25 OSI (moderate oscillation)

start

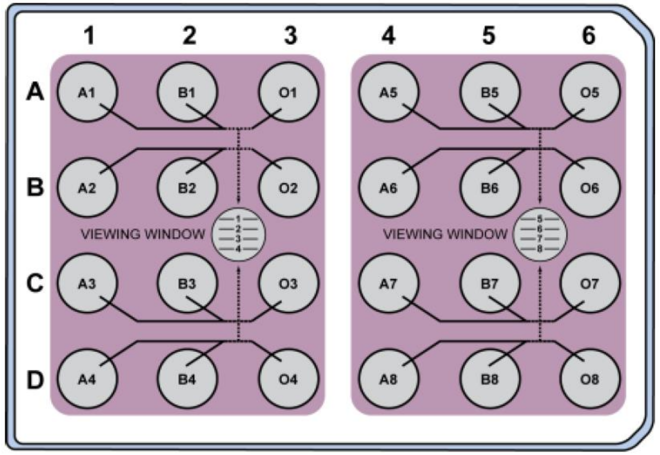
- 0.5 OSI (full oscillation)

start

Cell Culture and Expansion

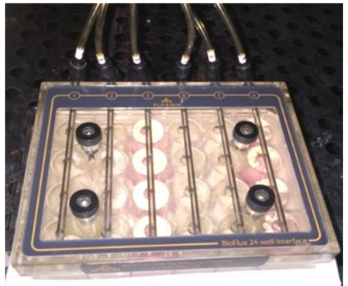
CATEGORY	Valvular Endothelial Cells (VEC)	Valve Interstitial Cells (VIC)
CULTURE MEDIA	Endothelial Cell Growth Medium 	Regular DMEM
SUPPLEMENTS	1% Penicillin/Streptomycin 	10% Fetal Calf Serum 1% Penicillin/Streptomycin
CULTURE VESSEL	T75 Flask, coated with endothelial matrix 	T75 Flask

Bioflux System

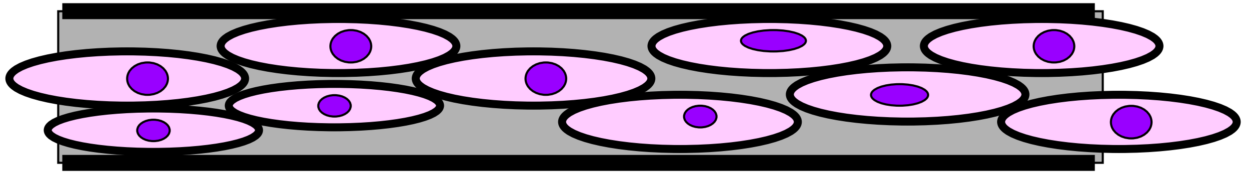


- Microfluidic channels
  - 8 channels per plate
- Seeding density (Bioflux protocol):
  - 200,000 cells/channel

Pneumatic Pump



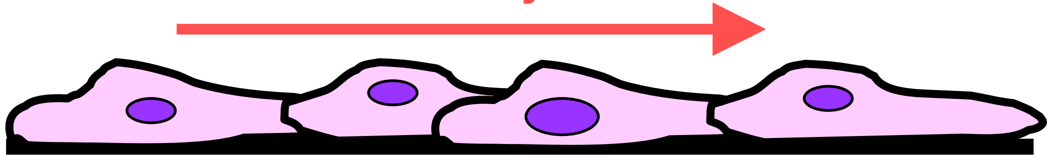
Top View



24-hours in static for cell attachment

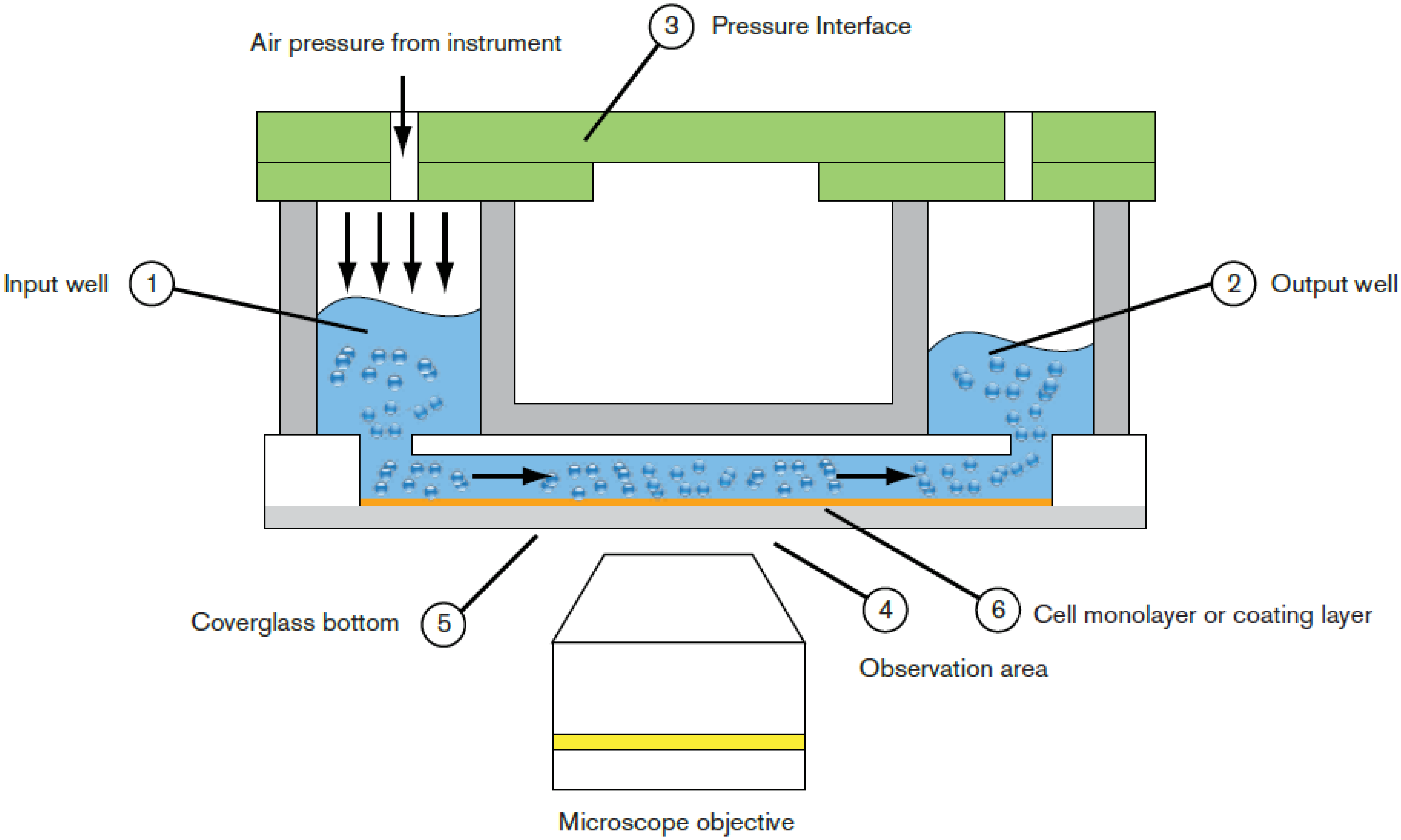
Fluid Shear at 1 dyne/cm^2 Yap et al.

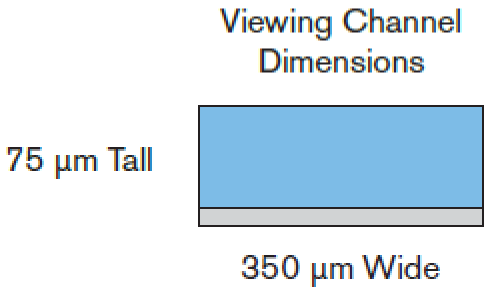
Side View



Bottom of plate/channel

Bioflux System





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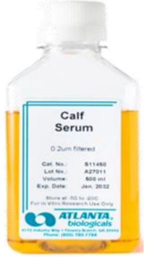
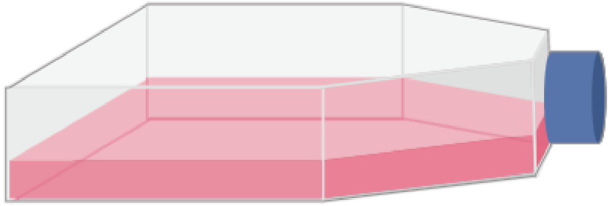
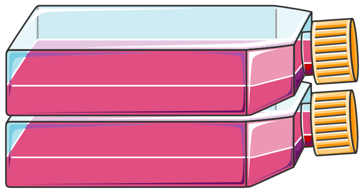
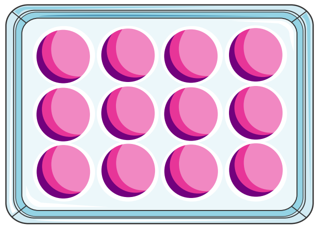
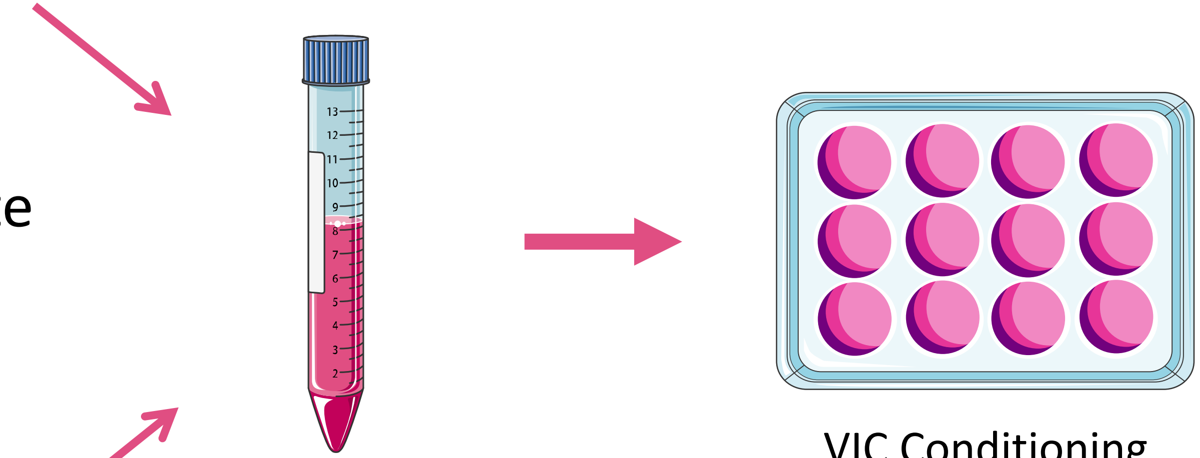
SUPPLEMENTS	1% Penicillin/Streptomycin	10% Fetal Calf Serum 1% Penicillin/Streptomycin  
CULTURE VESSEL	T75 Flask, coated with endothelial matrix	T75 Flask 

Paracrine Regulation

<table>
<tr><th>Cell Type</th><th>Flow Group</th><th>Media</th><th>Conditioning Time</th></tr>
<tr><td rowspan="4">
Rat VEC</td><td>Static (no flow)</td><td rowspan="4">Complete DMEM (flow condition)</td><td rowspan="4">48 hours</td></tr>
<tr><td>Steady Flow (0 OSI)</td></tr>
<tr><td>0.25 OSI</td></tr>
<tr><td>0.5 OSI</td></tr>
<tr><td>
Rat VIC</td><td>Static (culture with respective VEC flow group media)</td><td>50% Flow conditioned media from VEC + 50% Complete DMEM
50 % Flow conditioned media from VEC + 50% pro-calcifying media</td><td>7 days, 1 media change</td></tr>
</table>

Pro-Calcifying Media and VIC Conditioning

- Pro-Calcific Media
  - DMEM with 5% FBS, 1% P/S
  - 1.8 mM $CaCl_2$
  - 3.8 mM NaH_2PO_4
  - 0.4 units inorganic pyrophosphate
- VEC Conditioned Media
  - Static, 0 OSI
  - Steady, 0 OSI
  - 0.25 OSI
  - 0.50 OSI

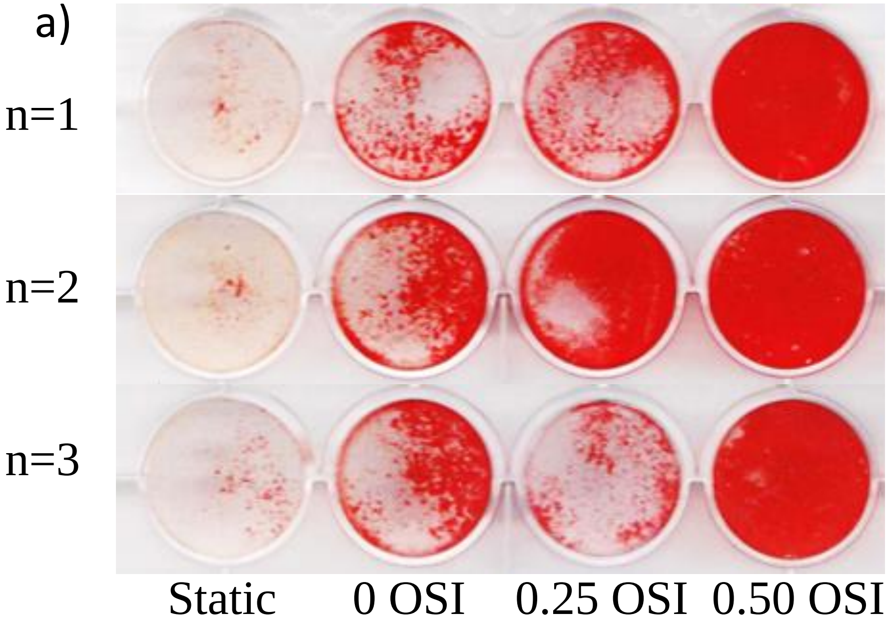


VIC Conditioning

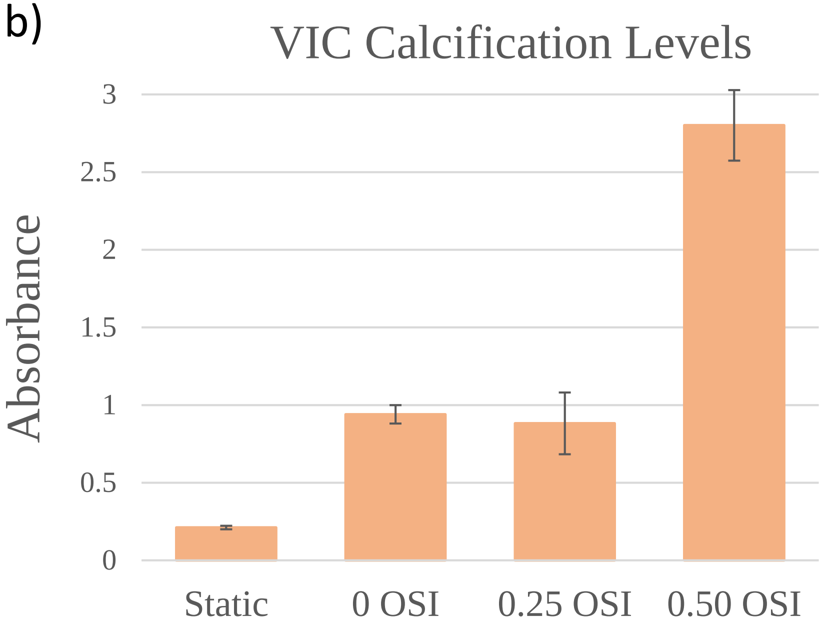
Rathan et al.
Goto et al.

Rat VIC Calcification - Alizarin Red Staining

a)

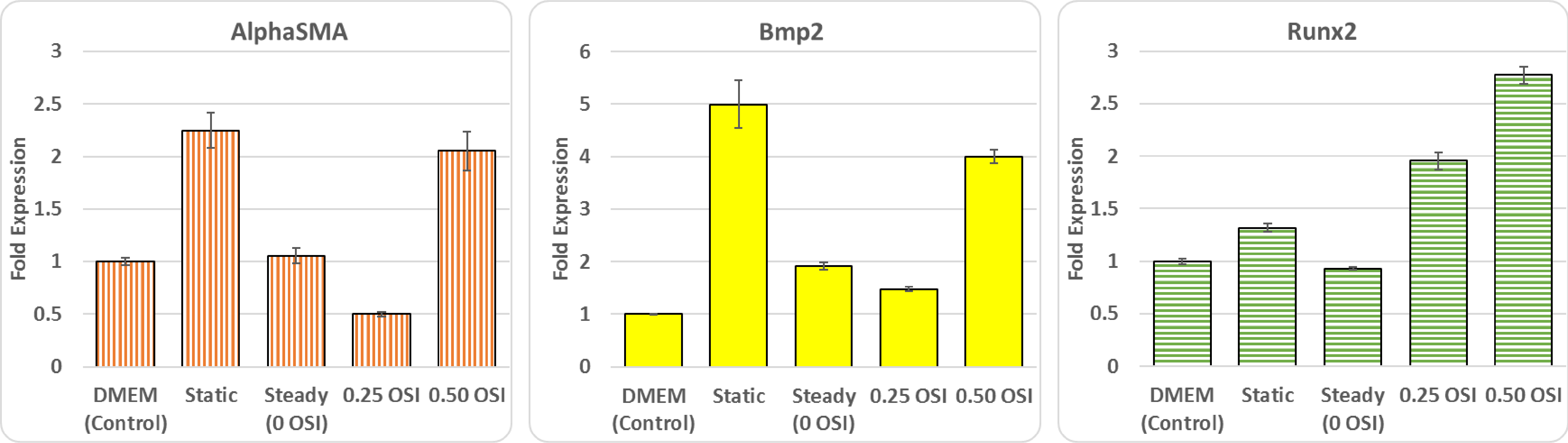


b)



- Significantly increased calcification in the 0.50 OSI group ($p<0.001$)
- Calcification between 0.25 OSI and Static or 0 OSI were not significant ($p>0.05$)

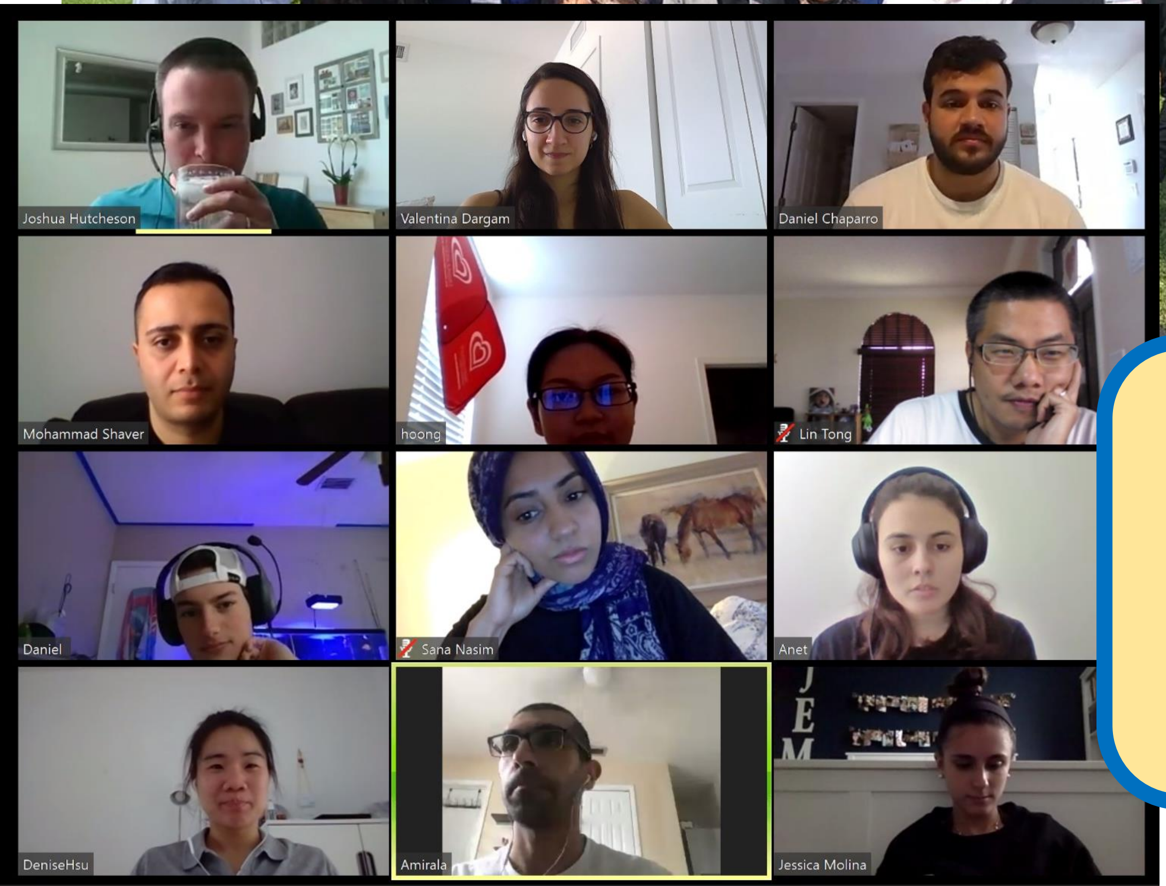
Gene Expression of Rat VICs

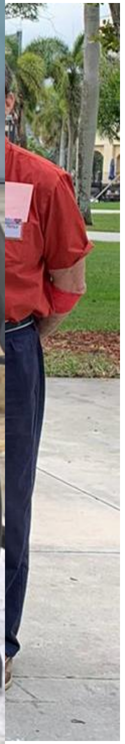


Conclusion & Future Works

- Shear stress of 1 dyne/cm^2 in a pro-calcific environment
    - VICs tend to calcify when exposed to high OSI
    - Low-to-moderate OSI maintains a quiescent VIC phenotype
- Further assessments of calcification in 3D tissues will be conducted

Thank you for your attention!

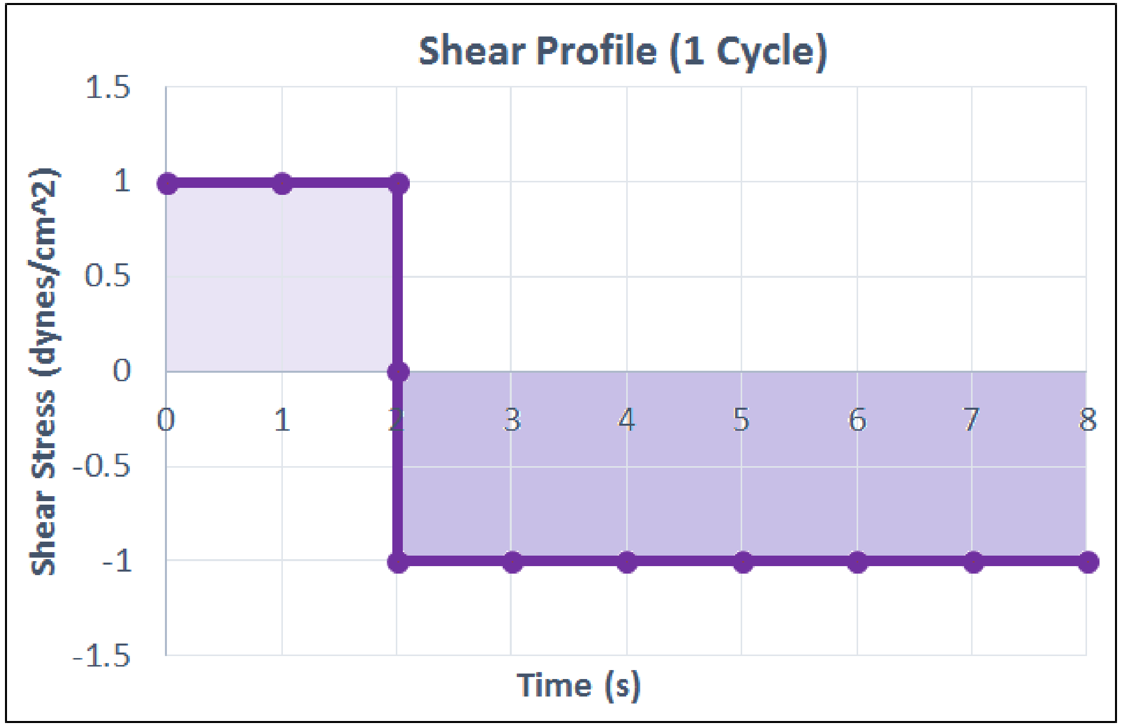




Now the bloopers....

0.25 OSI

- $OSI = \frac{1}{2}\left(1 - \frac{\left|\int_0^T \tau_w dt\right|}{\int_0^T |\tau_w| dt}\right)$
- $\frac{\left|\int_0^T \tau_w dt\right|}{\int_0^T |\tau_w| dt} = \frac{|Sum\ of\ \tau_w|}{Sum\ of\ |\tau_w|}$
- $= \frac{|1+1+(-1)+(-1)+(-1)+(-1)+(-1)+(-1)|}{|1|+|1|+|-1|+|-1|+|-1|+|-1|+|-1|+|-1|} = \frac{4}{8} = \frac{1}{2}$
- $OSI = \frac{1}{2}\left(1 - \frac{1}{2}\right) = \frac{1}{2} \times \frac{1}{2} = 0.25$



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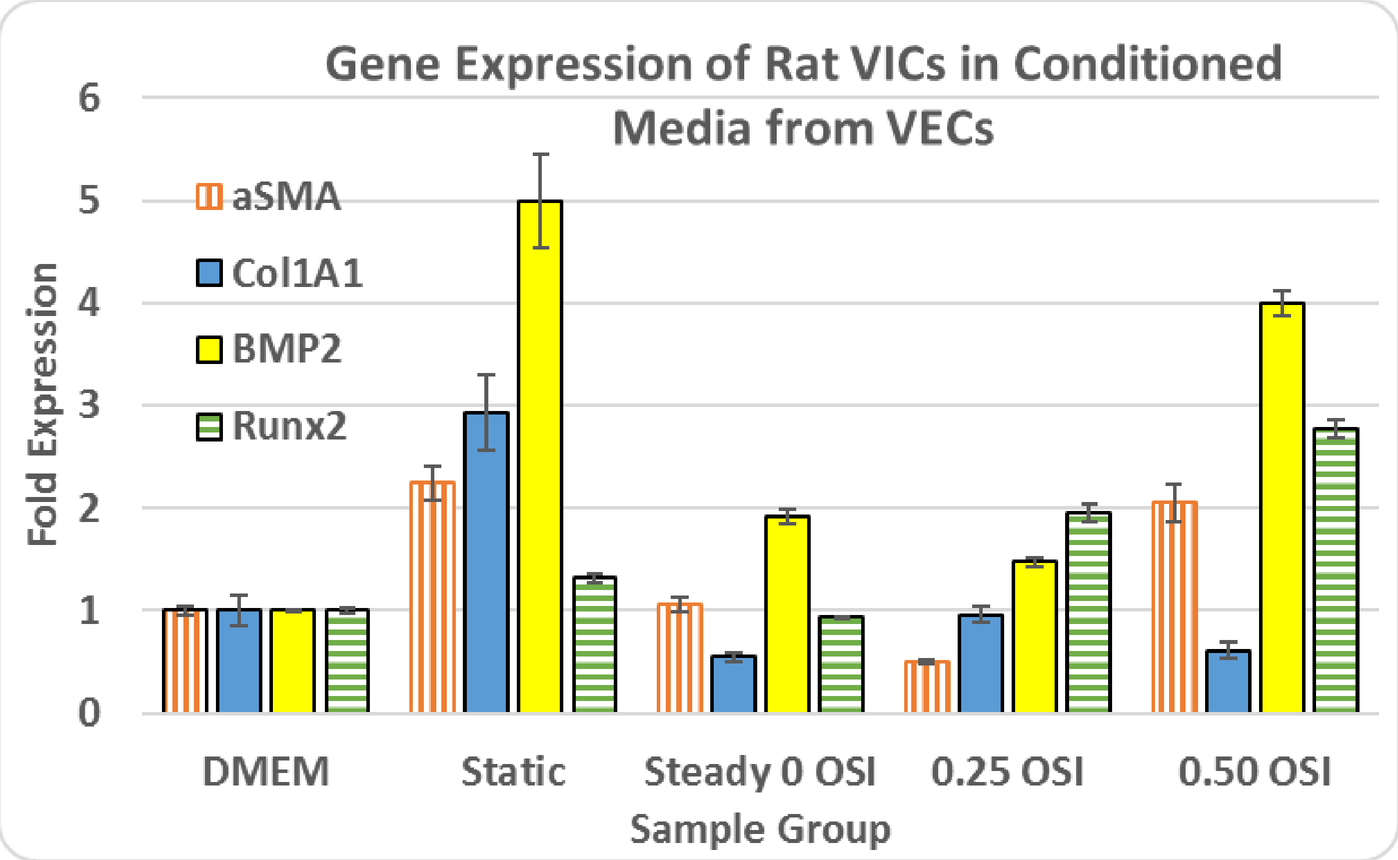

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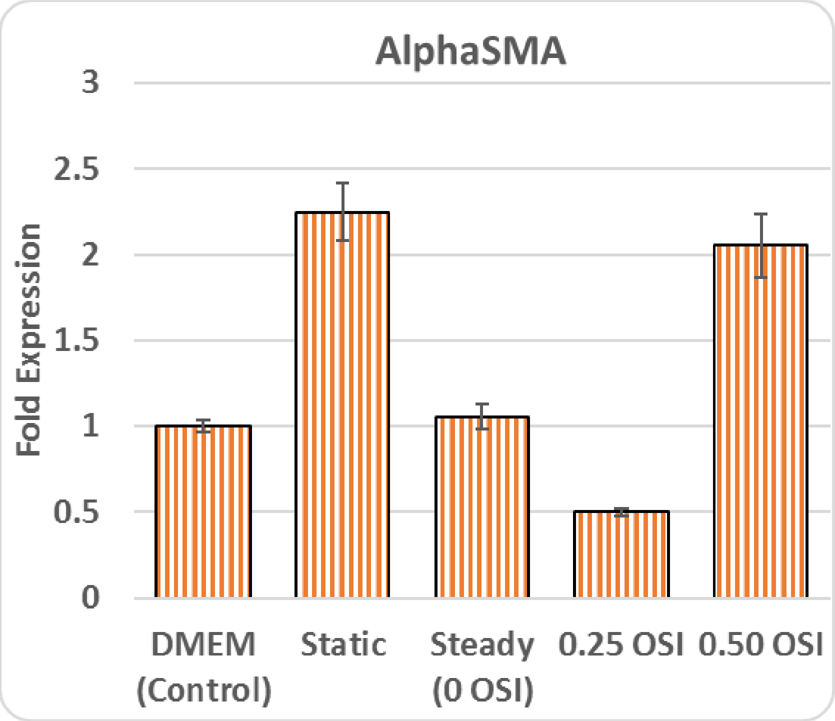

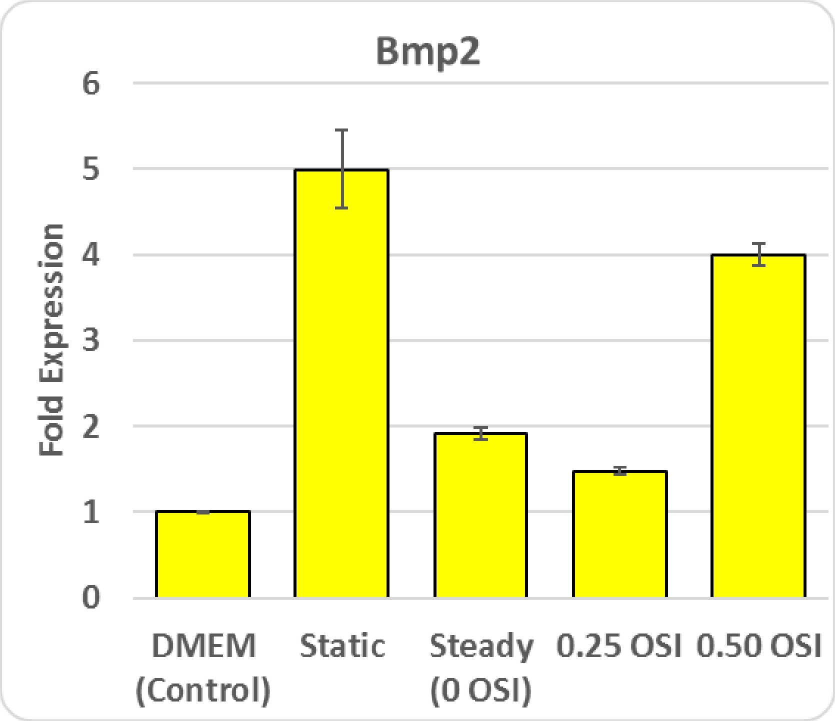

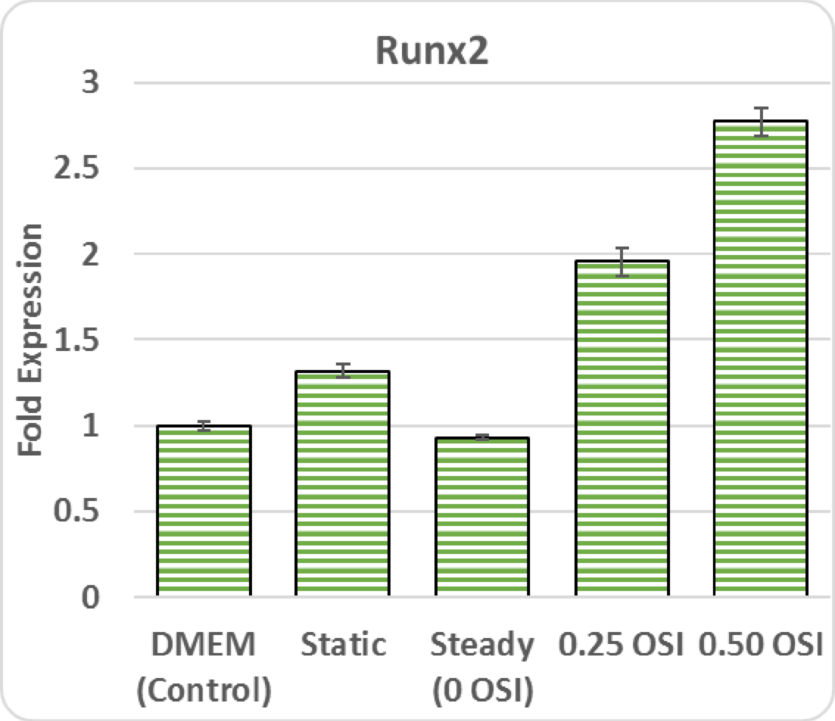

Fim