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Mammosphere Bioreactor For Life-Stage Specific Toxicology:

considerations for toxicant bioavailability in organ-on-chip bioreactors

People: Lisa J. McCawley, Dmitry A. Markov, Phllip Fryman, Gloria Bazilevich, Alex Auner, Emily Schafer, Kazi Tasneem

Vanderbilt-**P**ittsburg **R**esource for **O**rganotypic **M**odels for **P**redictive **T**oxicology

We are working toward advancing alternative methods of testing chemical toxicity using organotypic culture models (OCMs) – 3D cultures of heterotypic cells with appropriate extracellular matrices to better approximate the in vivo cellular microenvironment

Our three center-wide objectives:

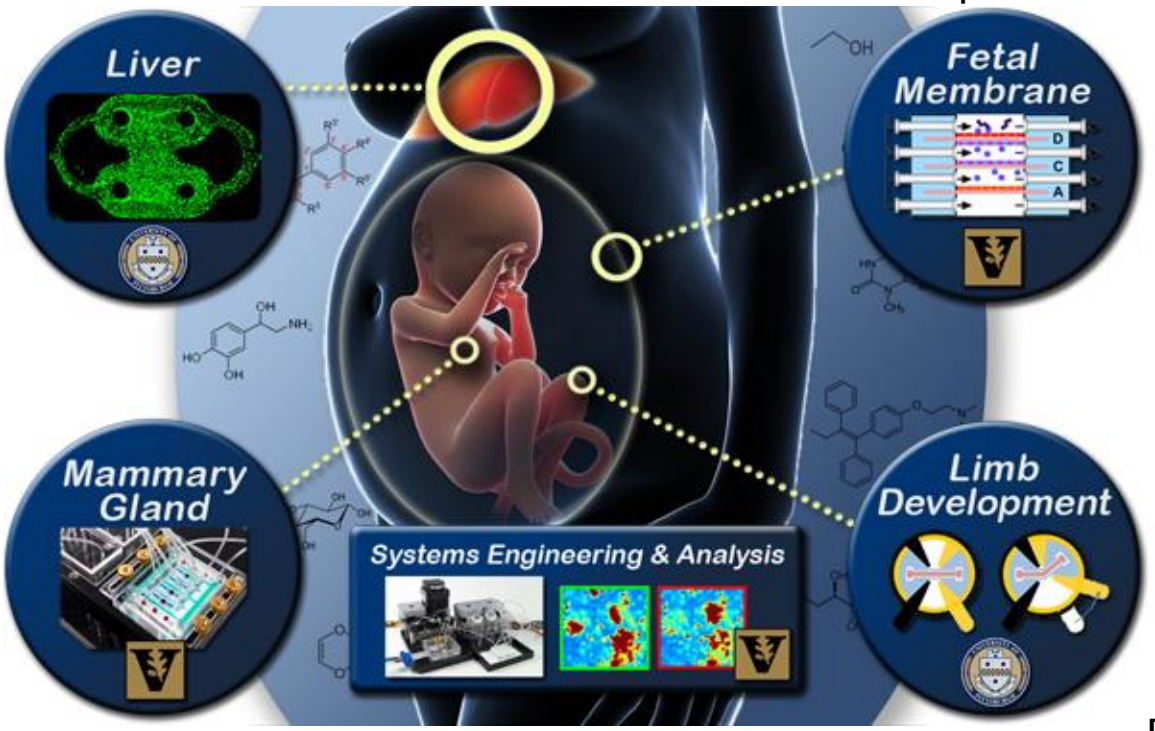
1. to use OCMs to identify the scale(s) of organismal complexity that most severely confound predictions of in vivo toxicity from in vitro data sets;
2. to use OCMs to elucidate the details of selected Adverse Outcome Pathways (AOPs) from molecular initiating events to tissue-level responses; and
3. to engineer and develop OCM platforms and control systems that will be amenable and affordable for mid- to high-throughput chemical screening.

Vanderbilt-Pittsburg Resource for Organotypic Models for Predictive Toxicology

http://www.vanderbilt.edu/vprompt/

P4. Organotypic Liver Model for Predictive Human Toxicology and Metabolism

P3. Validating a Fetal Membrane-on-a-Chip Model for Characterizing Reproductive Toxicant Exposure Risks



P1. Mammosphere Bioreactor For Life-Stage Specific Toxicology

P5. Systems Engineering & Analysis for OCM

P2. OCM to Analyze Developmental Limb Malformations Resulting from Toxicant/Teratogen Exposure

SPECIAL THANKS

Vanderbilt:

- Alex Auner
- Phillip Fryman
- Gloria Bazilevich
- Emily Schafer
- John Wikswo
- Shane Hutson
- **Lisa McCawley**

Past members:

- Tyler Moon
- Rahul Telange
- Jenny Liu

Funding:
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This research is funded by
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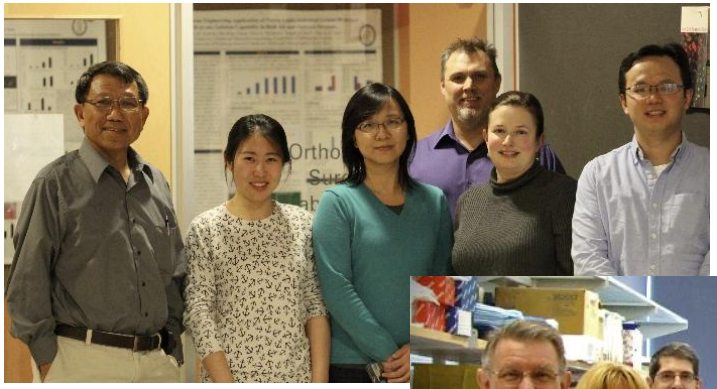
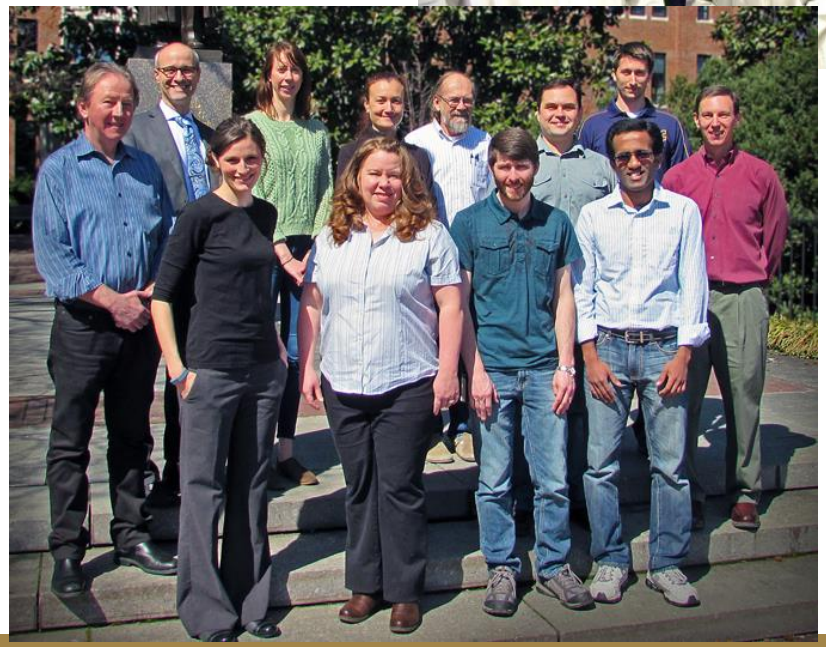
Grant # 83573601

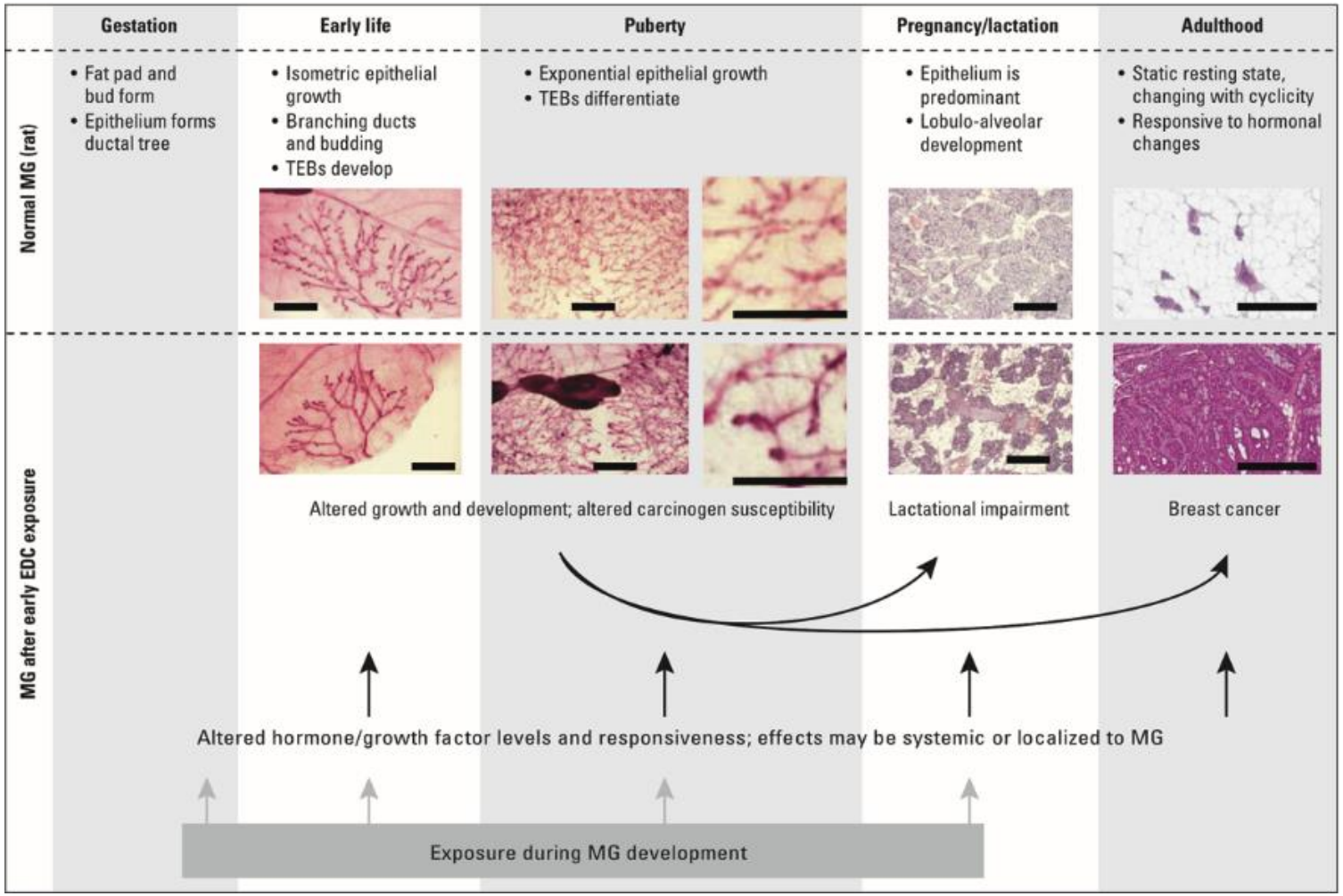


Pitt Crew

VU Crew



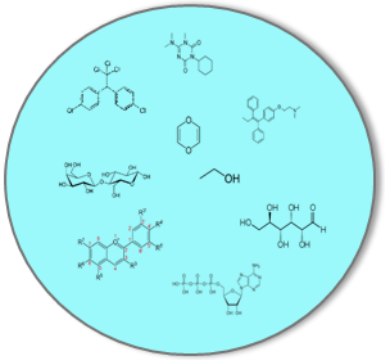
Mammary Gland development and effects of environment on subsequent events

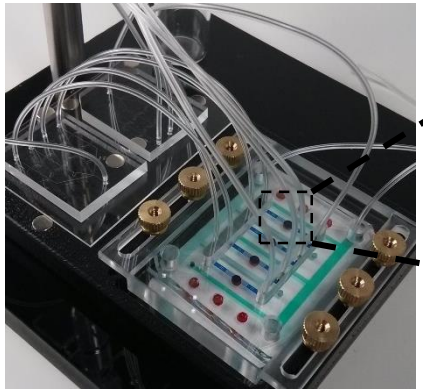


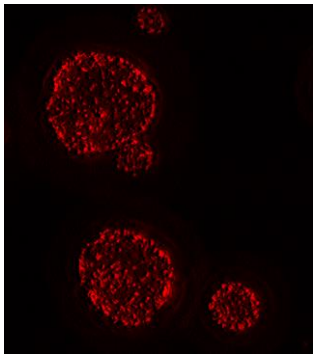
Rudel et al, Environ Health Perspect 119:1053-1061 (2011).

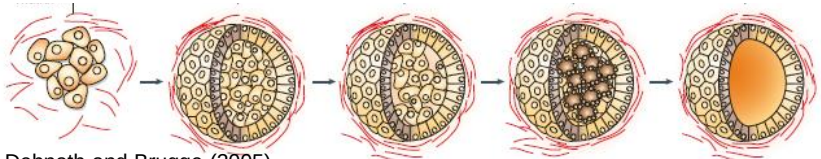
On-chip analysis using known toxicants of mammary development and development of chronic and “acute”, stage specific delivery of compounds

Toxicant?









Debnath and Brugge (2005)

Mammosphere Formation

Toxicant exposure:

Continuous

Acute/Stage Specific

Dynamic Changes

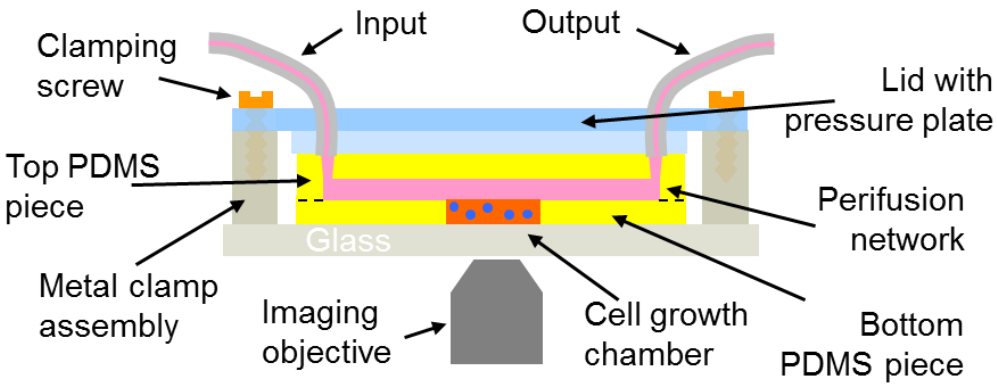
-Organoid Size
-Organoid Morphogenesis
-Cell Proliferation
-Apoptosis

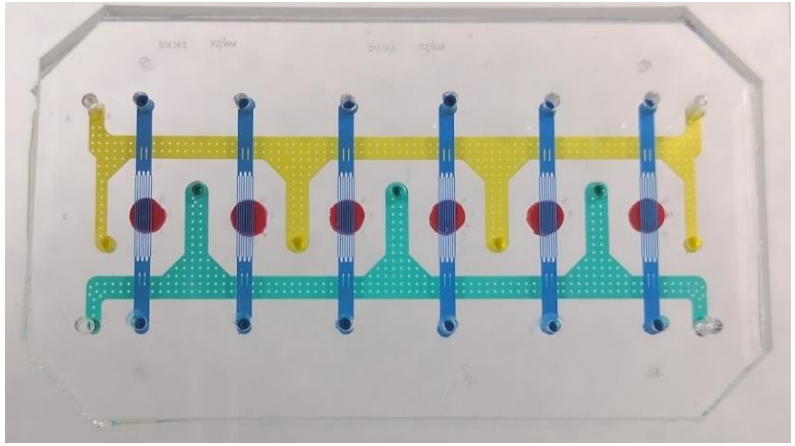
End Point

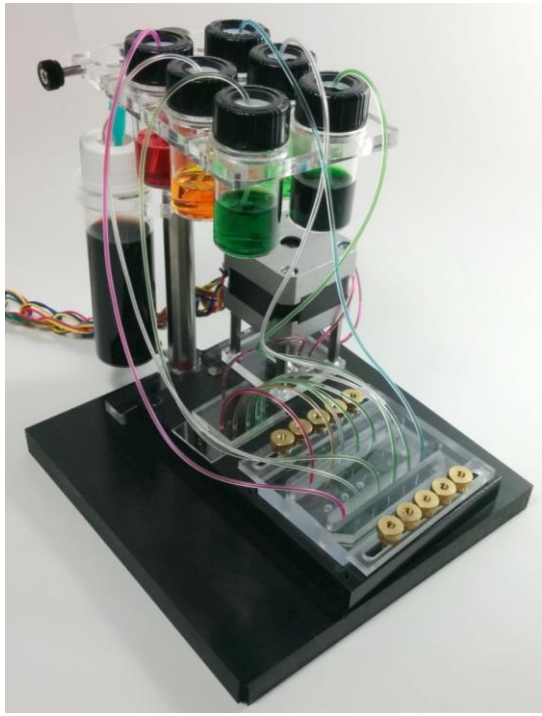
-Cell Differentiation
-Polarity Establishment

- Is there concordance of this system with already identified toxicant effects?
- What are the fewest number of measures needed for medium through-put screen analysis?

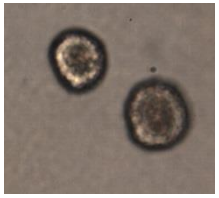
Thick Tissue Bioreactor for long-term mammosphere culture

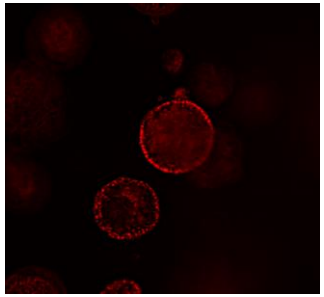






Day 10 +/- Toxicant

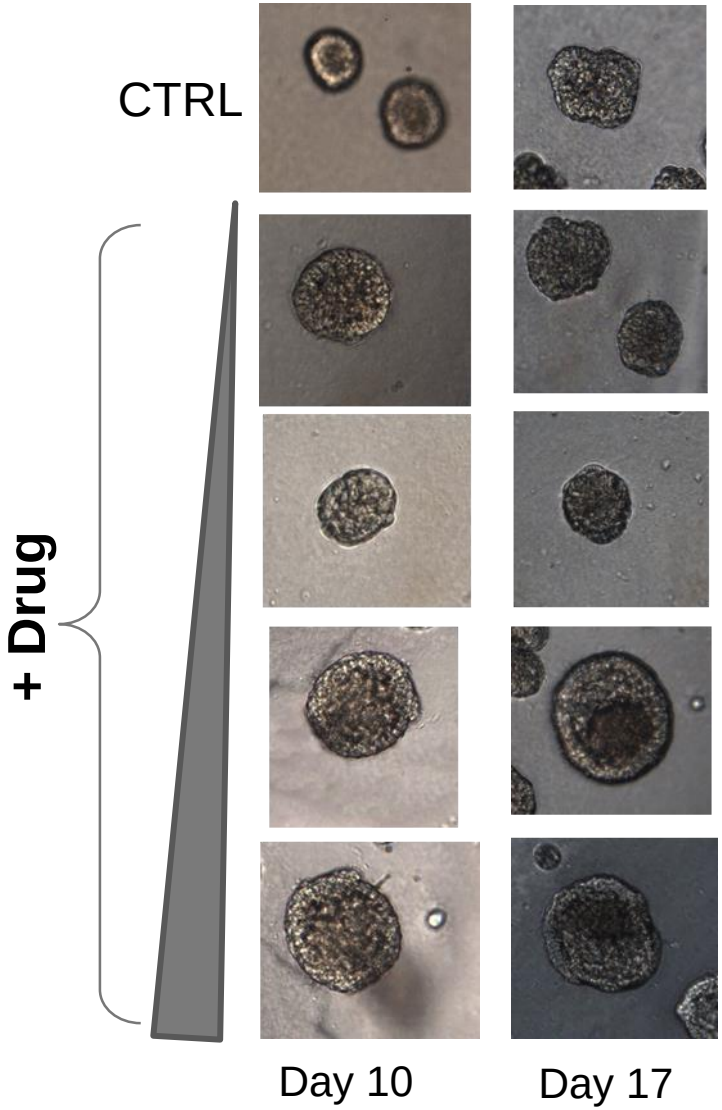


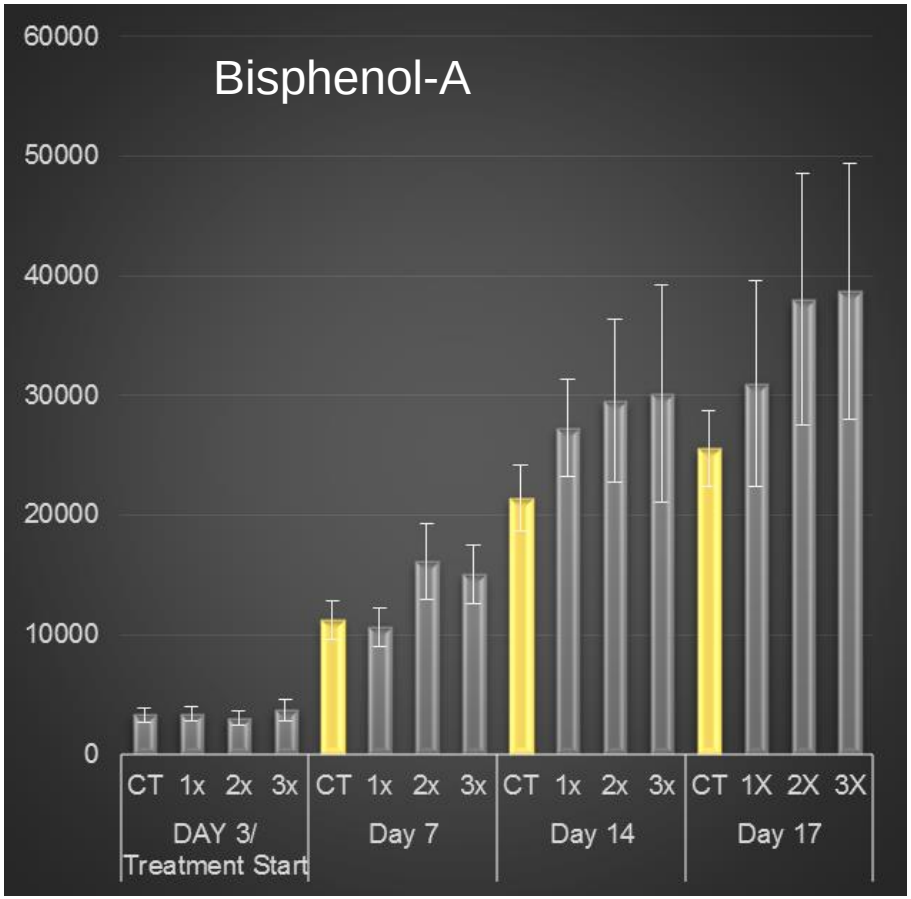


- 96-well footprint
- Magnetically attaches to the stand between imaging session
- Holds bioreactor cartridge with 6 culture chambers that are on 9-mm grid
- RPPM and RPV are magnetically attached to the IOM
- Compatible with conventional microscopy and ImageXpress Micro XLS High Content Analysis system

Markov et al, *Lab on a Chip*, 2012
Markov et al., *Biomedical Microdevices*, 2014

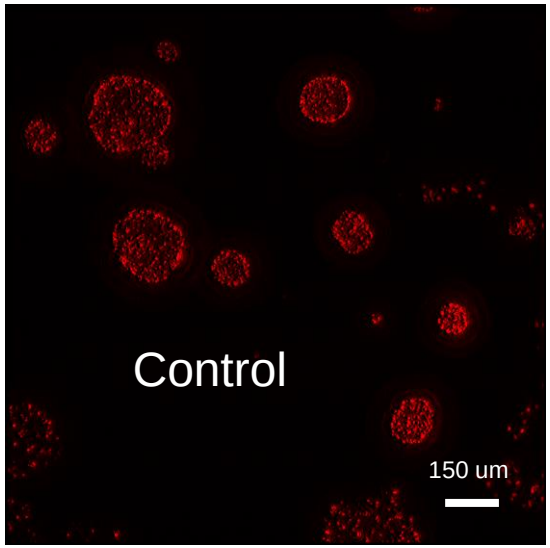
Mammosphere Growth Overtime With Increasing Log Dose Of a Toxicant

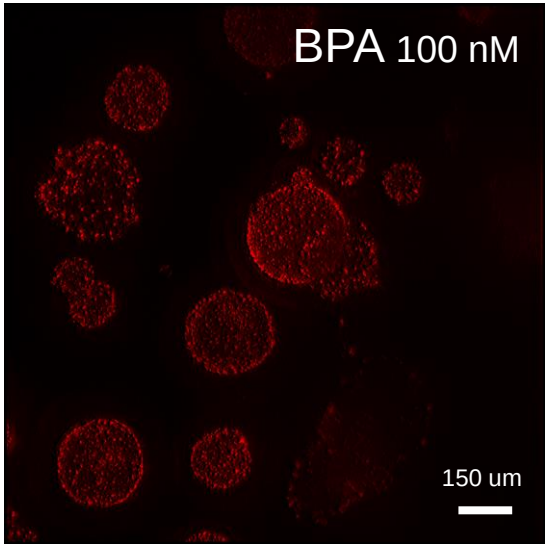


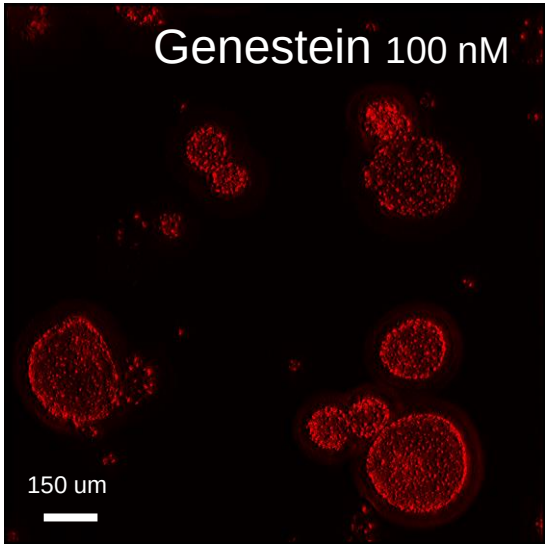


Concentrations: 1, 10, and 100 nM

Difference in mammosphere sizes due to toxicant exposure at 21 days

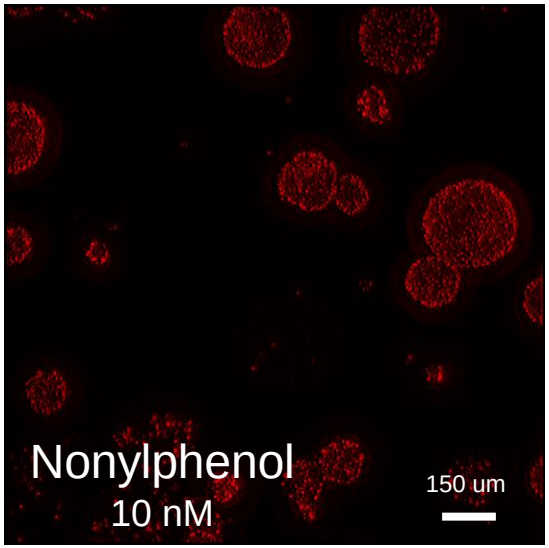






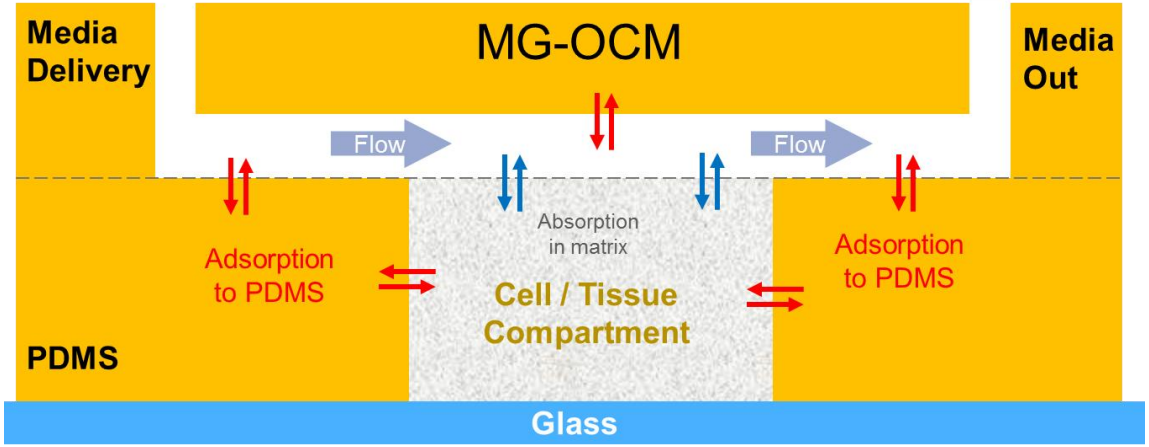
MCF10A at 21 days
2D projection of the 3D reconstructed stack

- Bisphenol A has effect early in mammosphere development.
- Genestein has and effect midway in mammosphere development
- Nonylphenol has effect later in mammosphere development

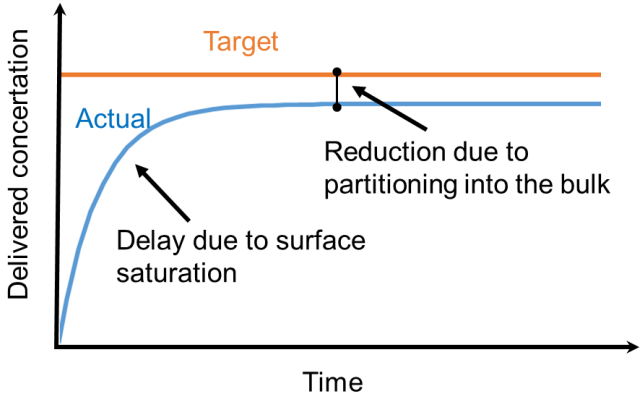


Potential PDMS – Toxicant interactions that could affect our measured outcomes

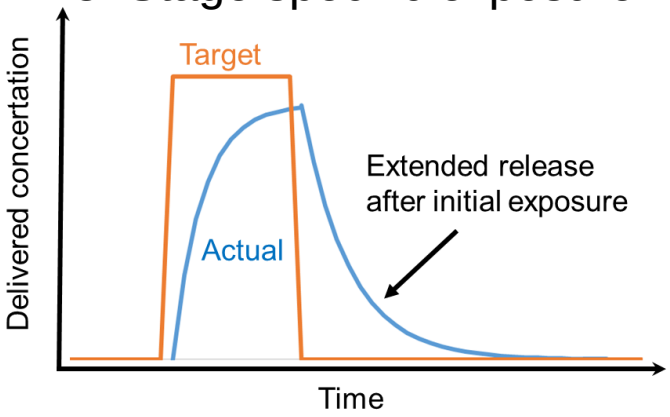
1.



2. Chronic exposure



3. Stage specific exposure



1. Is there PDMS surface – toxicant interaction?
2. What is PDMS surface saturation dynamics?
3. Is there any carry forward during stage specific exposure?

UV-Vis and ATR approaches

UV-Vis spectroscopy – relies on reduction of compound concertation in bulk solution as it adsorbs to the surface.

Pros:

- Cheap
- Simple and easy to use
- Multiple conditions could be screened in parallel
- Fast

Cons:

- Works best with compounds with high extinction coefficients
- Not well suitable for complex mixtures or compounds with poor solubility in water
- Requires multiple controls

Used for preliminary bulk screening as well as with selected compounds for "on" and "off" rates

Attenuated Total reflection (ATR) – FTIR spectroscopy that uses evanescent waves to probe solid or liquid surface *directly*

Pros:

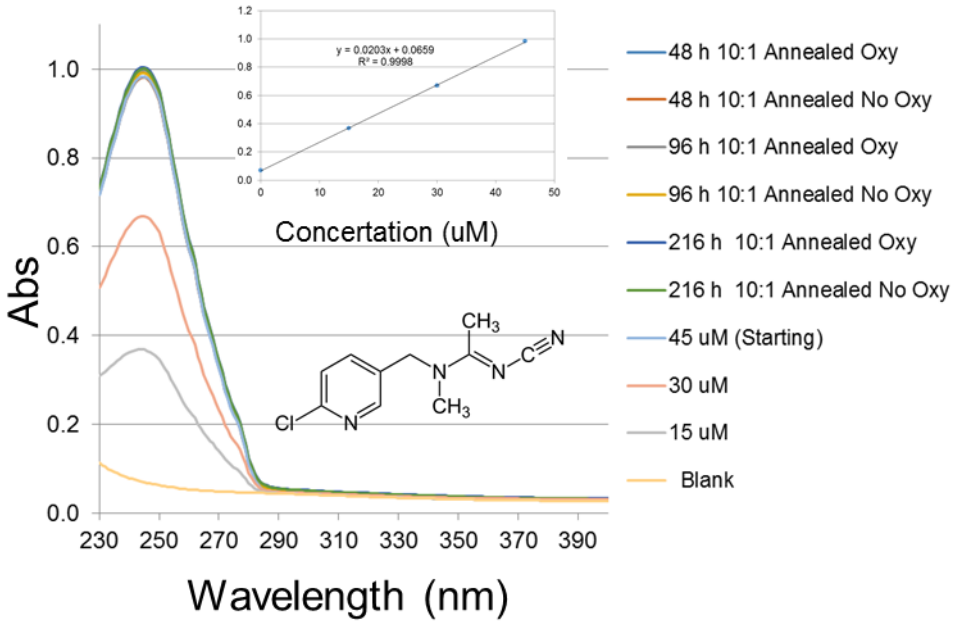
- No sample preparation required
- Direct sample measurements
- Measurements confined to the surface (limited penetration depth)

Cons:

- Slow
- Requires acquisition of reference spectra
- Spectra could be very complicated to decipher

Useful for compounds with low aqueous solubility.

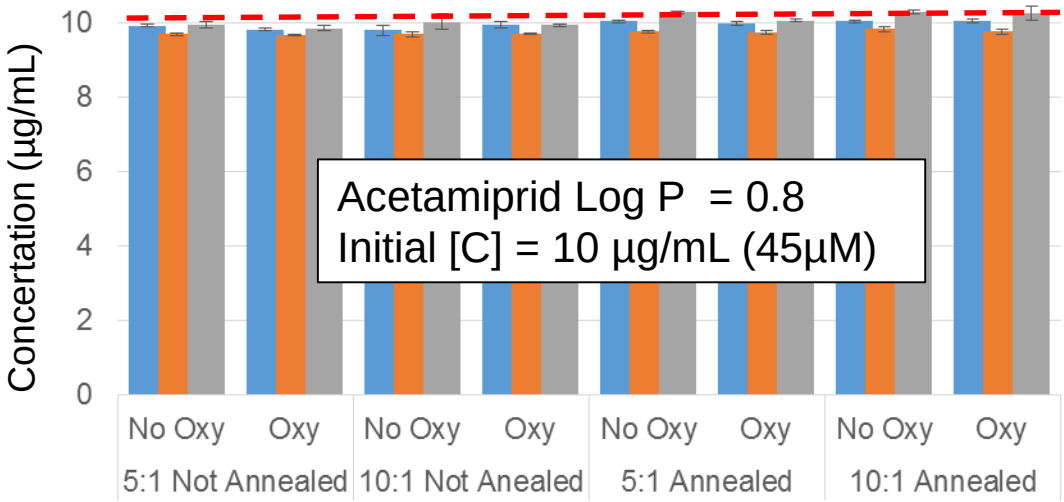
Monitoring decrease in Acetamiprid concentrations for multiple PDMS treatments



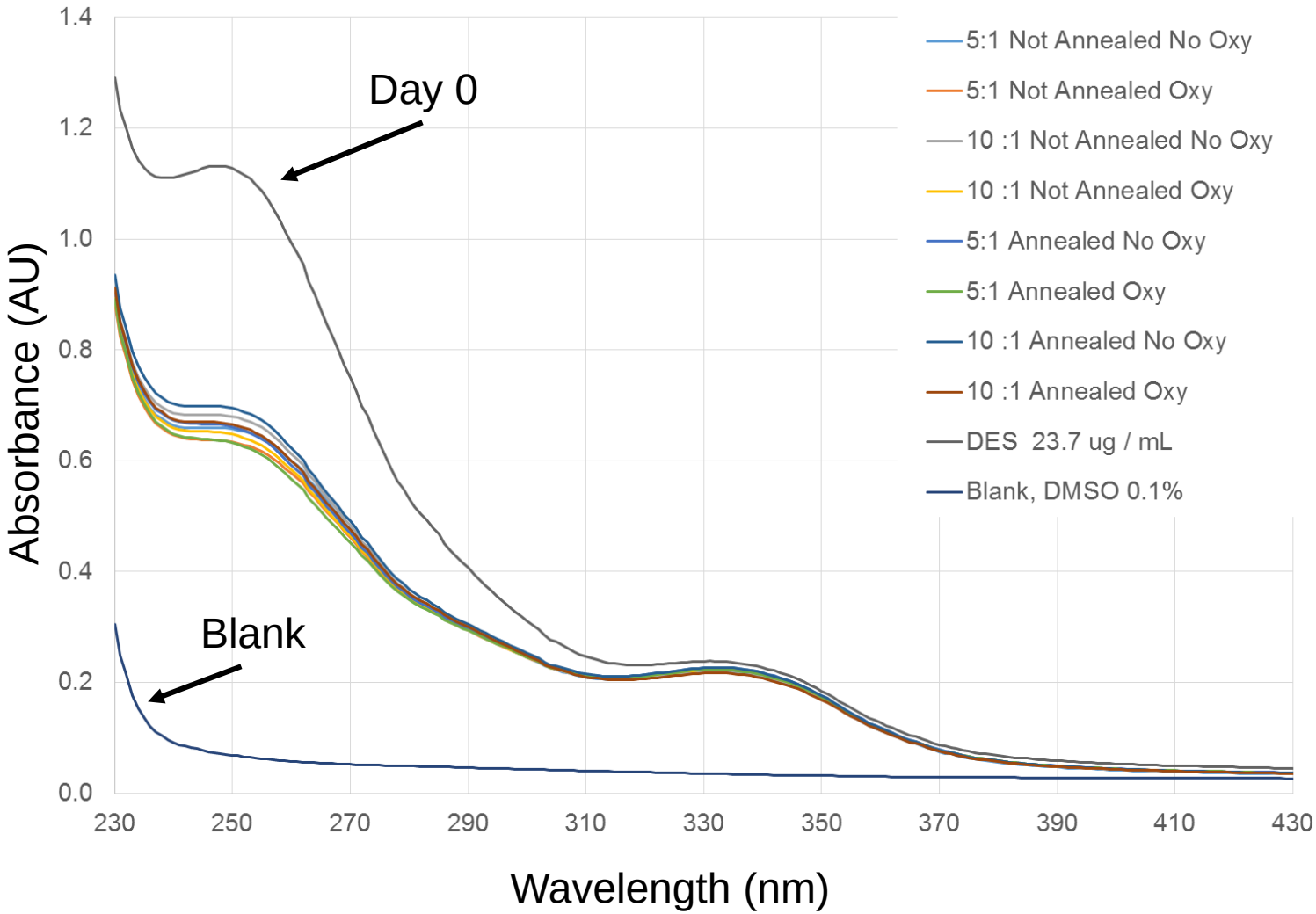
Approach:

- PDMS pucks (naïve, oxidized, annealed, normal and high density) were soaked in 2 mL of compound solution for 216 h (9 days)
- 300 µL aliquots were taken at 48, 96, and 216 h from control and sample vials
- Absorbance spectra was taken using BioTek Neo 2 plate reader (230 nm – 400 nm)
- Solvents used: DI water or DI water with 0.1% DMSO for Log P > 2
- N = 3

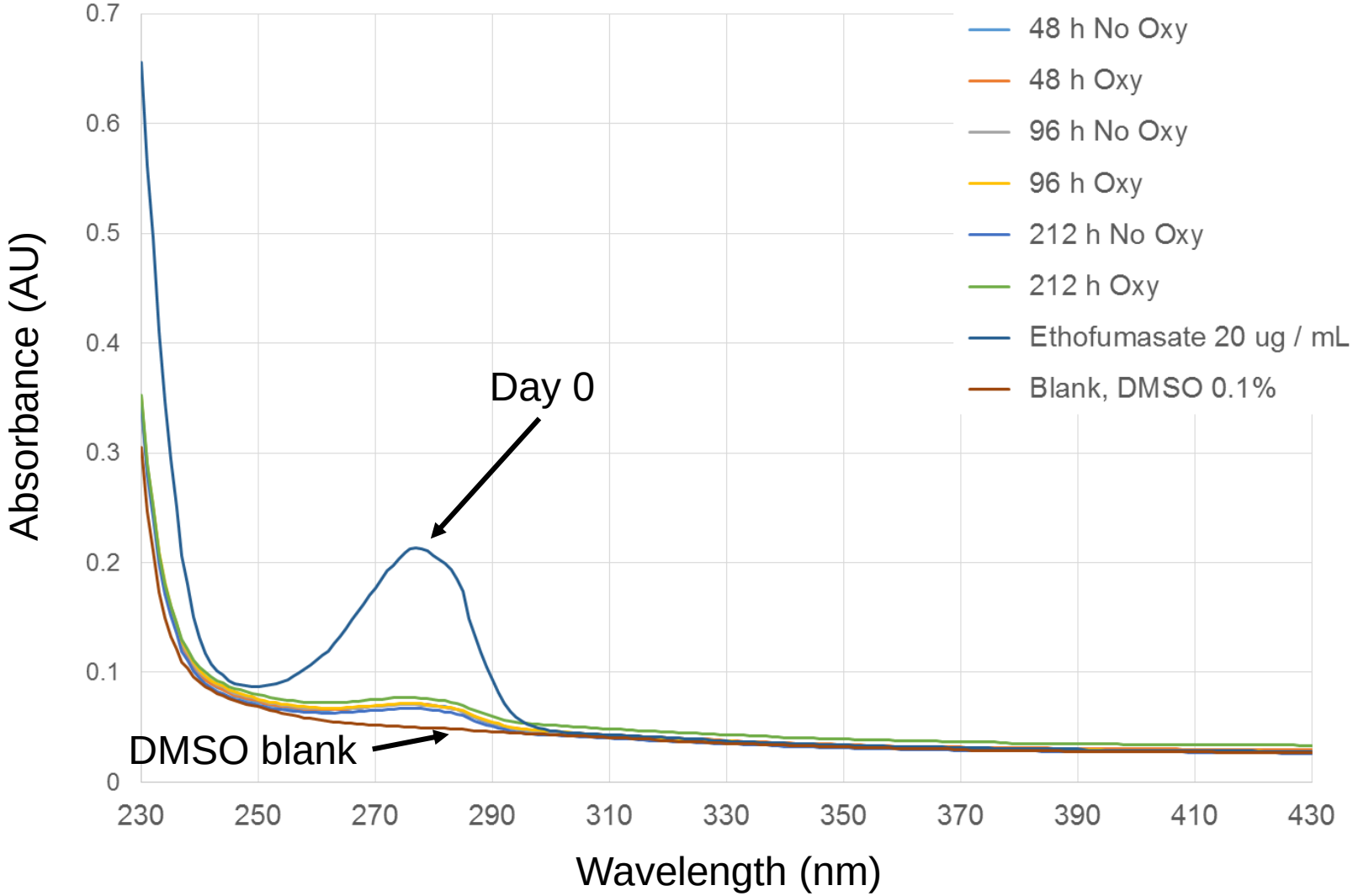
- There were no changes in Acetamiprid concentration in free solution as a function of PDMS soak or PDMS treatment
- Similar results were generally observed for compounds with Log P values <2



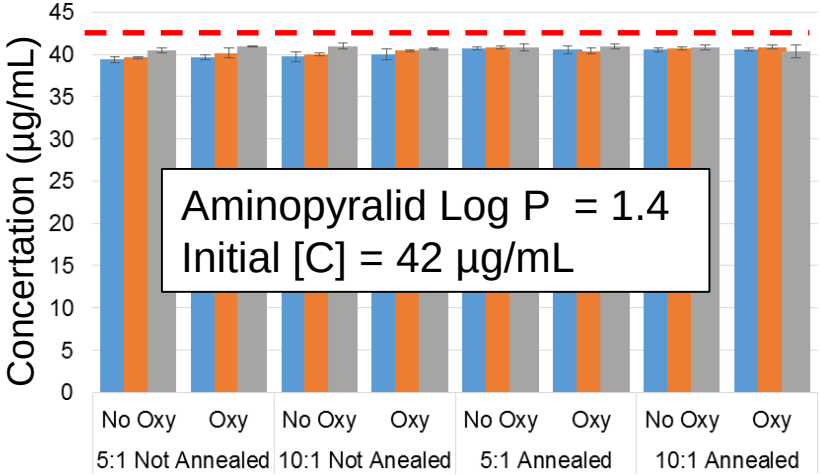
Changes in Diethylstilbestrol spectra after soaking PDMS +/- surface and temperature treatments for 48 h

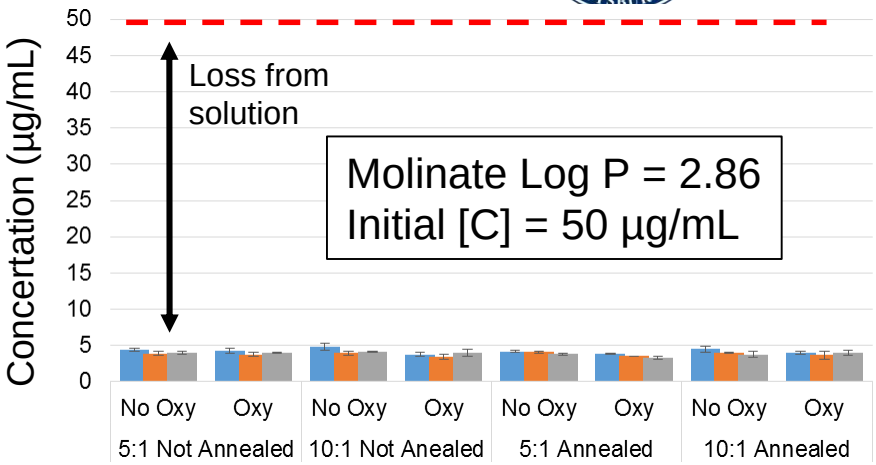


Changes in Ethofumisate spectra after soaking annealed PDMS +/- surface treatments for 48, 96, and 212 h

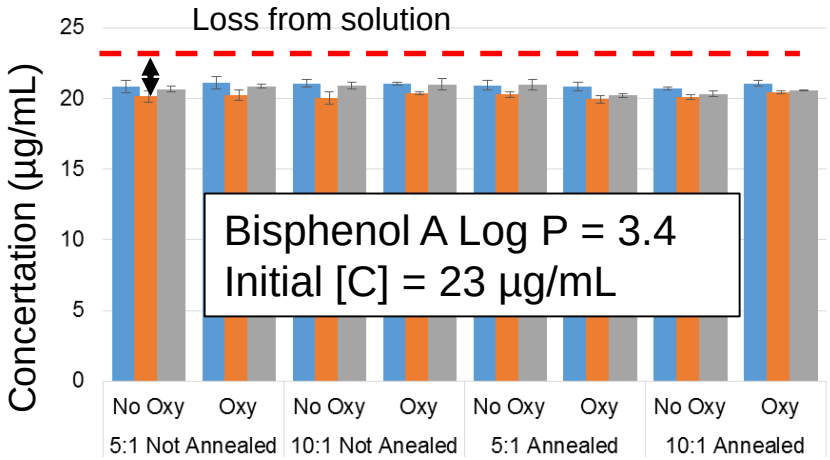


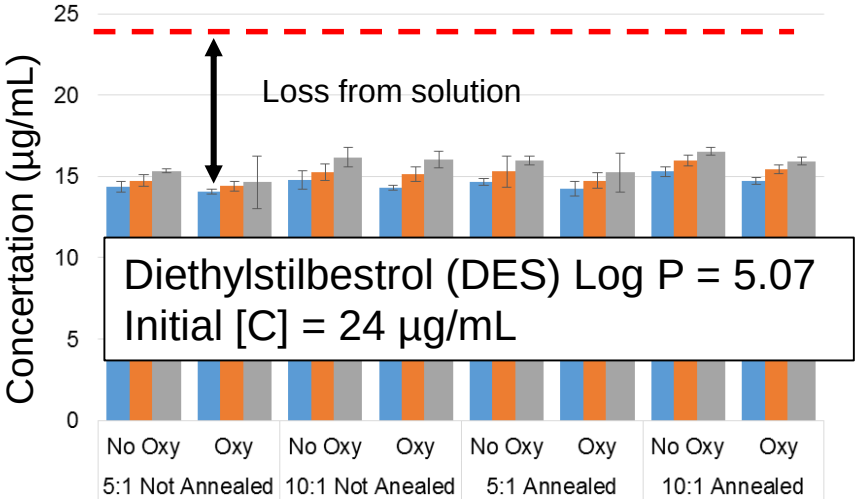
Monitoring toxicant interactions with multiple formulations of PDMS





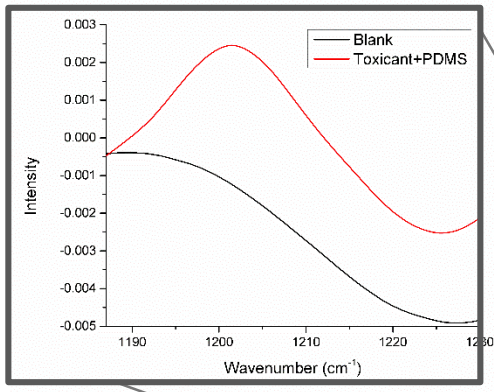
48 h 96 h 216 h



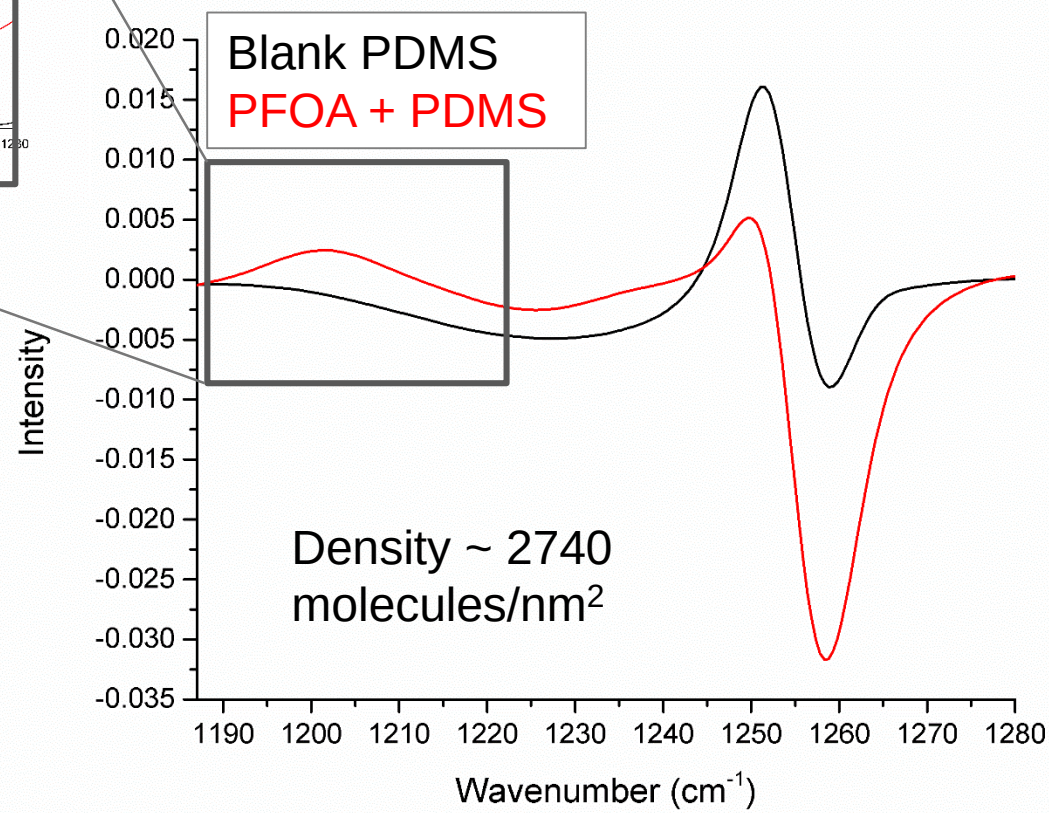


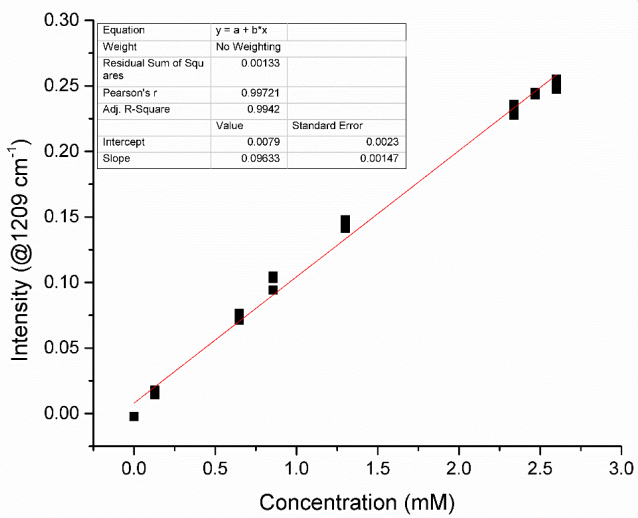
It appears that considering Log P values (for Log P >2) as a single predictor of interaction between the compound and a microfluidic surface is not always appropriate.

PFOA Adsorption to PDMS Surface Observed by ATR



Normalized ATR spectrum of PFOA on PDMS surface



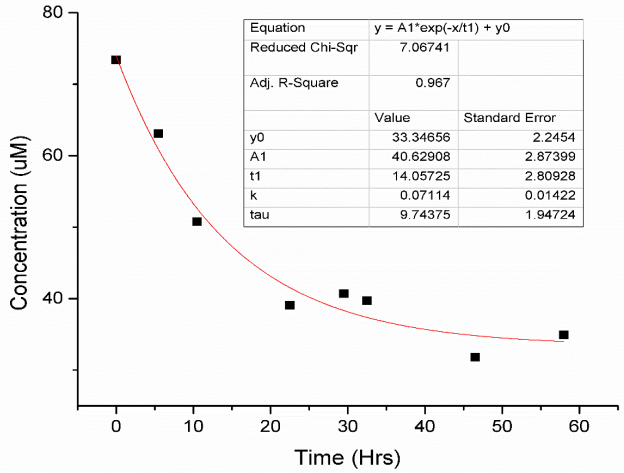


Determining toxicant "OFF" rates: Ethofumesate

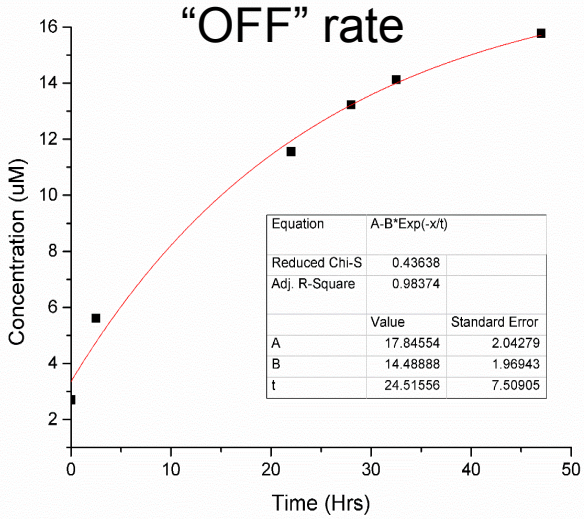
	Naïve PDMS	Oxidized PDMS
On rate		
Half-Life of Absorption (h)	7.4 ± 0.4	8.7 ± 0.3
Equilibrium Loss from Solution (molecules)	1.13 ± 0.04 x10^{17}	9.4 ± 0.1 x10^{16}
Equilibrium Surface Absorption (molecules/nm^2)	370±10	314 ± 3
Off rate		
Half-Life of disassociation (h)	8.1 ± 0.7	7 ± 1
Equilibrium Disassociation (molecules)	3.1 ± 0.5 x10^{16}	4.0 ± 0.1 x10^{16}

Starting concentration = 70 uM

"On" rate



"OFF" rate



Compounds tested to date		
Toxicant	**Log P**	**Interaction with PDMS**
Secoisolariciresinol diglucoside (SDG)	-2.93	None
Foramsulfuron	0.17	None
Acetaminophen	0.20	None
Imazaquin	0.29	None
Acetamiprid	0.80	None
Sulfentrazone	0.99	None
Formetanate HCl	1.03	None
Hexazinon	1.17	None
Aminopyralid	1.40	Weak
Ethofumesate	2.70	Strong
Entacapone	2.80	Moderate
Molinate	2.86	Strong
Genistein	3.08	Moderate
Bisphenol A (BPA)	3.40	Weak
Propiconazole	3.72	Strong
Tolcapone	4.00	Moderate
Indomethacin	4.30	None
Diethylstilbestrol (DES)	5.07	Moderate
Oxadiazon	5.33	Strong
Nonylphenol	5.90	Moderate
Perfluorooctanoic acid (PFOA) by ATR	6.30	Weak
Tamoxifen*	7.10	N/E
Vitamin D_3	7.50	Weak

Conclusions So Far

- UV-Vis is an excellent way to perform quick screening of multiple PDMS surface treatments
- There was no loss detected of the tested compounds with Log P < 1 for all PDMS modifications.
- Log P its own (if it is >2) is not always the best predictor of molecule interaction with the surface.
- For compounds that adsorb to PDMS surface majority of this interaction usually happens within **first 48 h**.
- We can extract “On” and “Off” rates to be used for modeling exposure profiles
- We saw little difference in PDMS between 10:1 and 5:1 formulations using both UV-Vis and ATR

SPECIAL THANKS

Vanderbilt:

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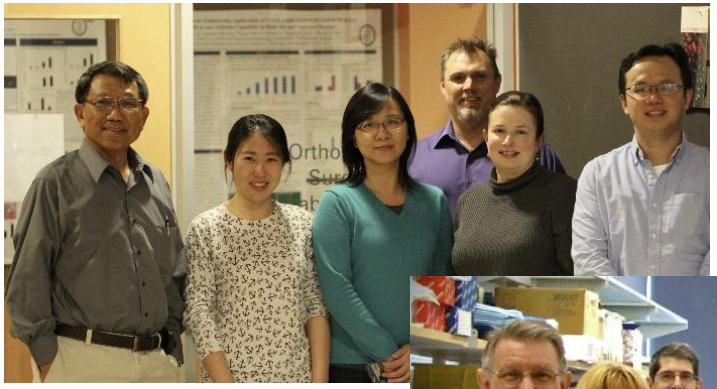
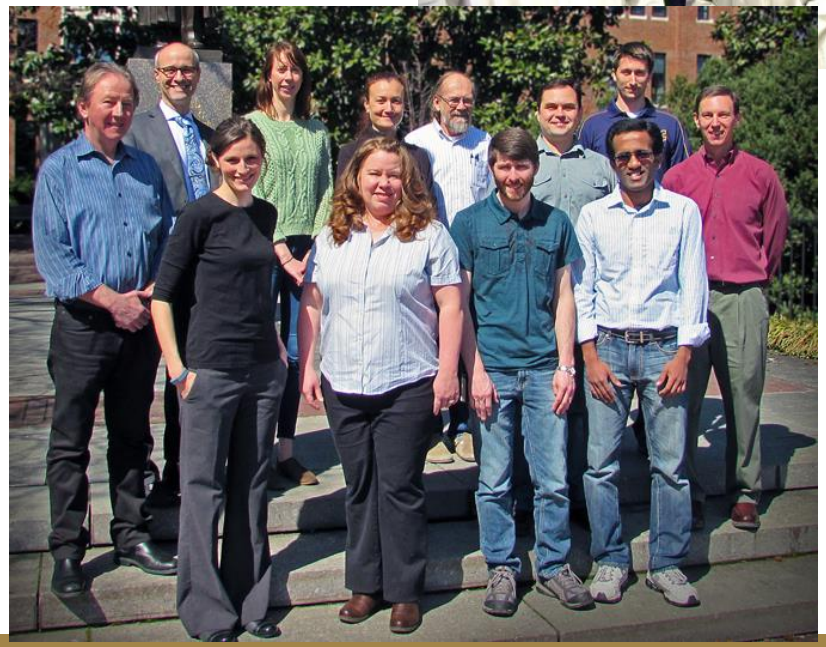


Pitt Crew

VU Crew