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Synthetic Hydrogels Support 3D Mammary Duct Morphogenesis

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Advised by: Prof. David Beebe

20170311 EPA Satellite Meeting at SOT

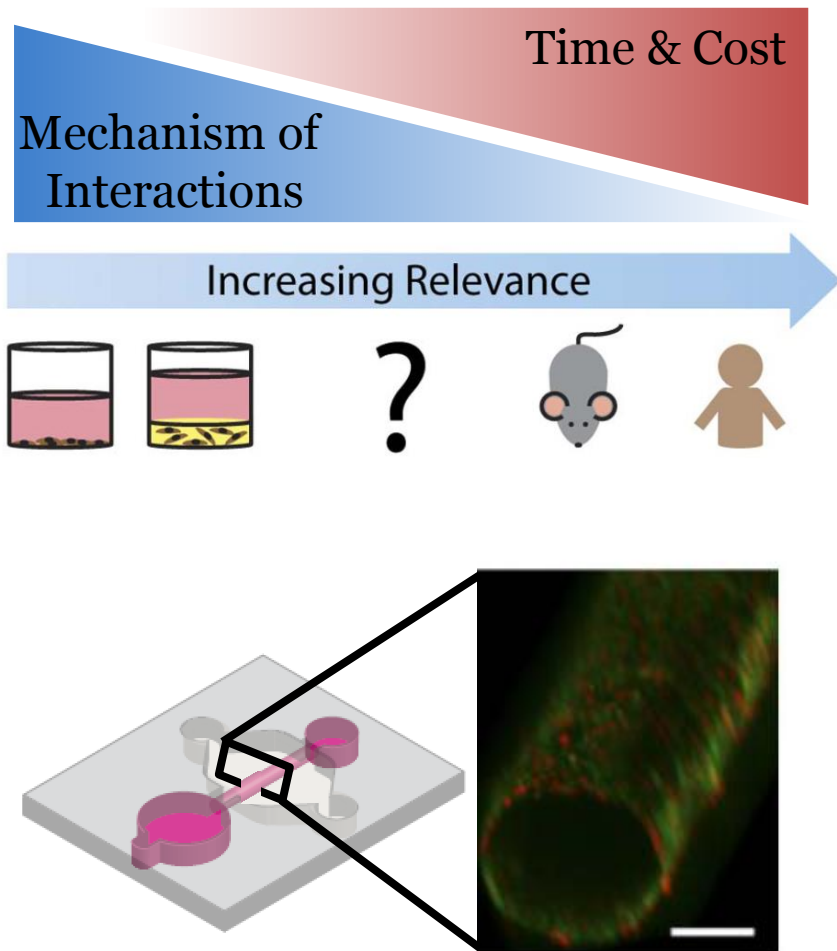


Toxicity Screening Models

Estrogen receptor (+)
breast cancer progression
is linked to toxicant
exposure from the
environment.

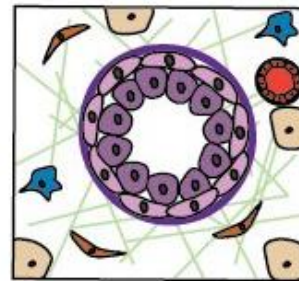
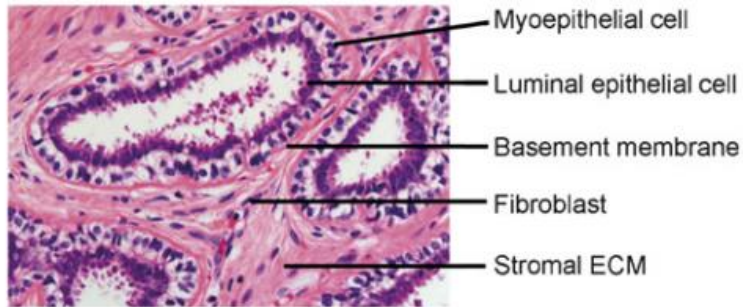
ToxCast Library

ER Agonist	Inactive
Pyridaben	Isoxaflutole
Fenpyroximate (Z,E)	Dimethomorph
Rotenone	Aldicarb
2,2-Bis(4-hydroxyphenyl)- 1,1,1-trichloroethane	Acetochlor
Bisphenol A	Cyfluthrin
Propargite	Diclofop-methyl
Dilenzquat metilsulfate	Prometon
Flumetralin	Fenpropathrin
Methoxychlor	Imazaquin
Cyazofamid	Esfenvalerate

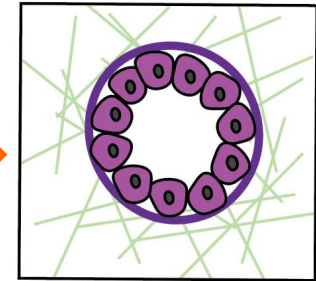


Development of a Robust 3D Model

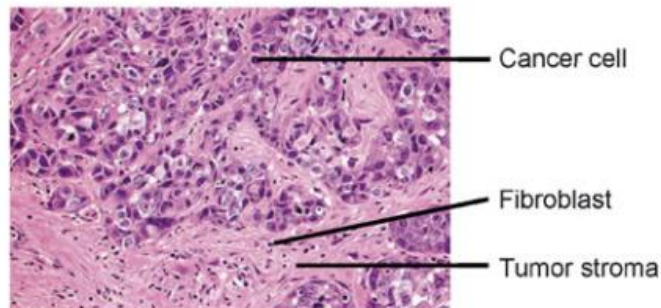
Normal Breast



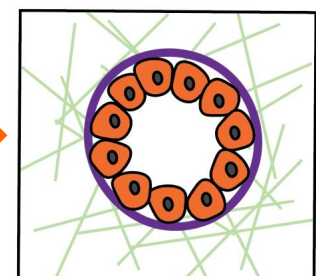
Simplify



Breast Cancer



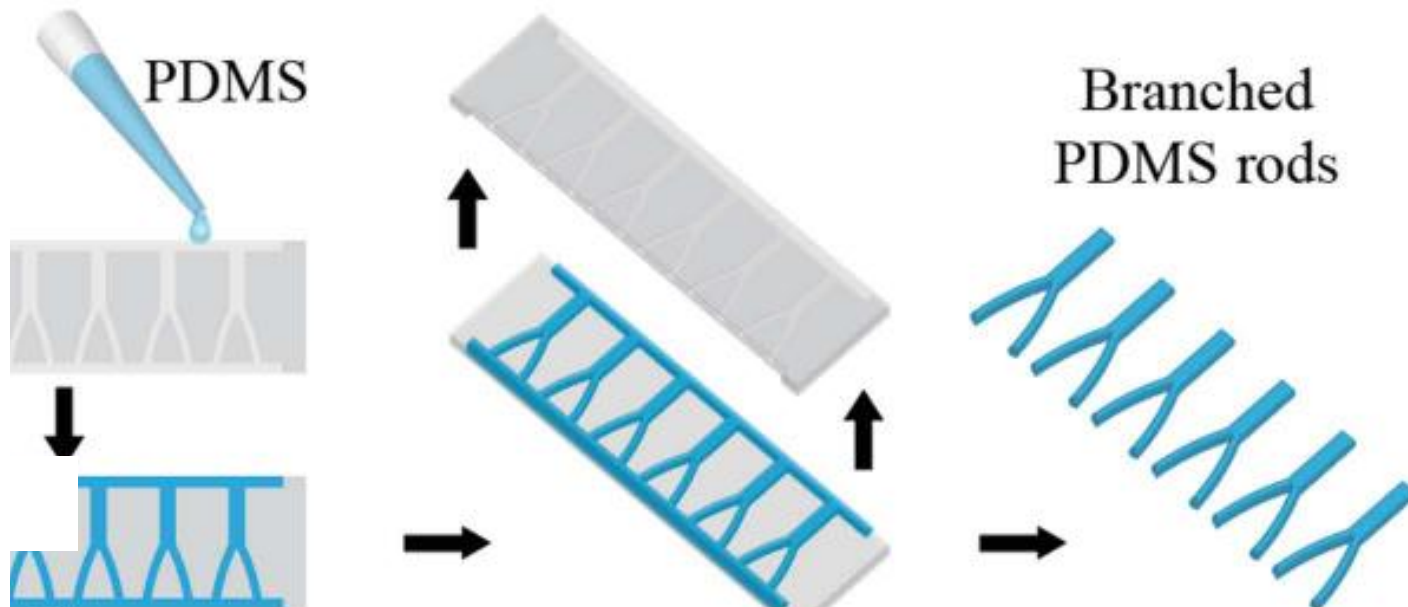
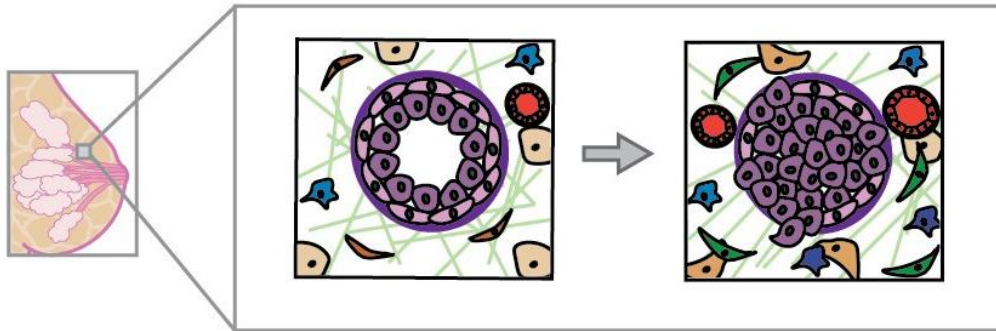
Simplify



- ✓ Start with a simple model of epithelium on collagen 1 matrix.
- ✓ Use an adverse outcome pathway to map disease progression and make decisions regarding complexity of the model.
 - ✓ Model normal duct and ER+ breast cancer duct.



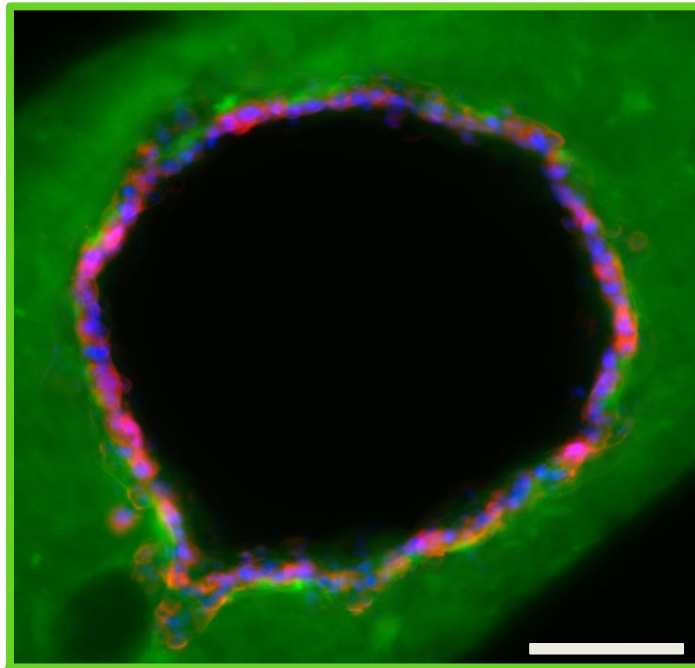
Engineering the Model: LumeNEXT



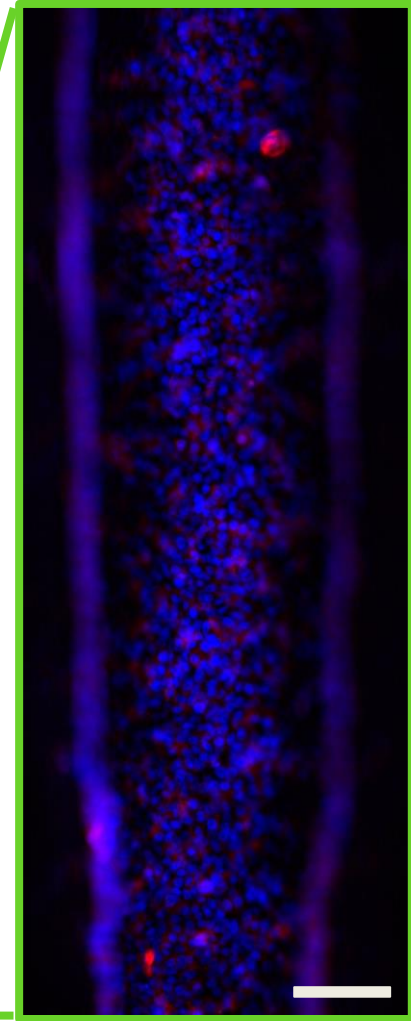
Jimenez-Torres, et. al. 2015.



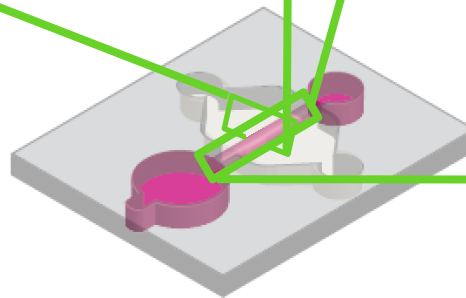
Engineering the Model: LumeNEXT



MCF12a
Nuclei F-Actin AF488 Collagen 1



MCF12a
Nuclei F-Actin



Cell Types

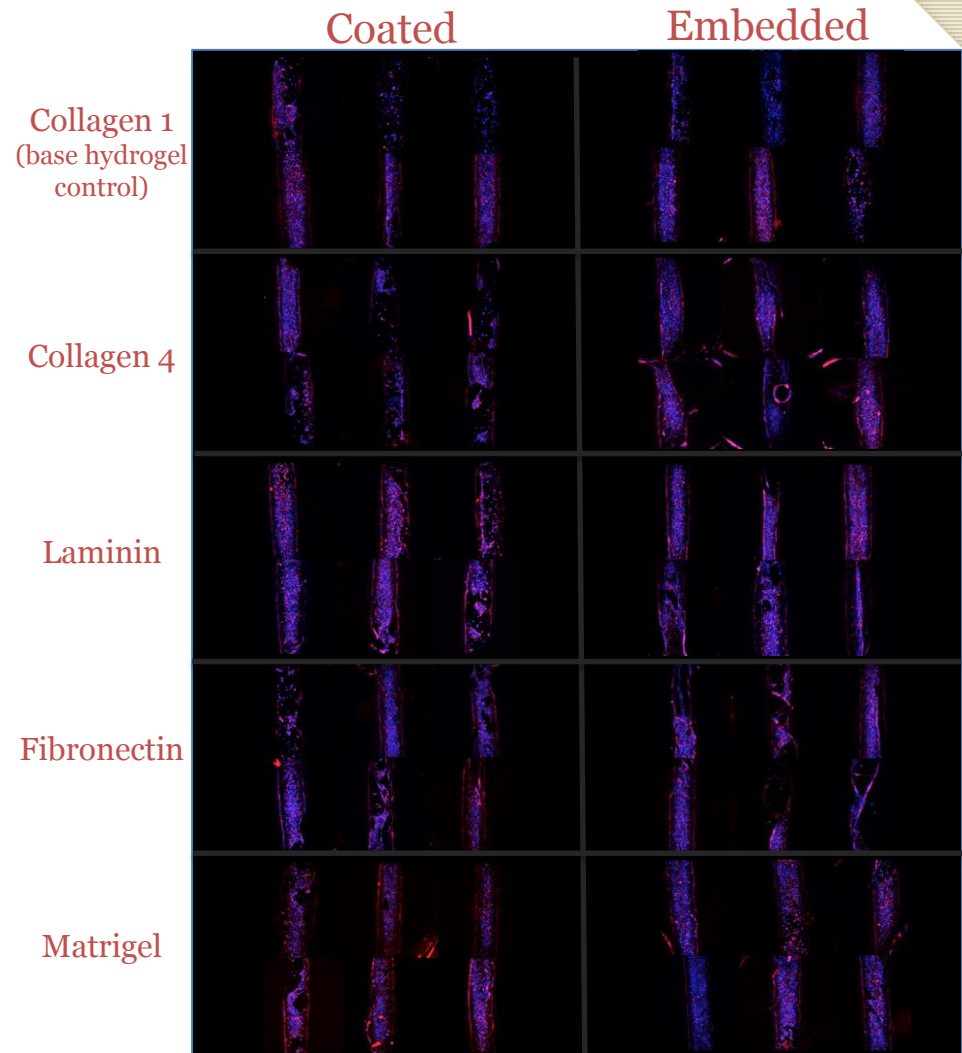
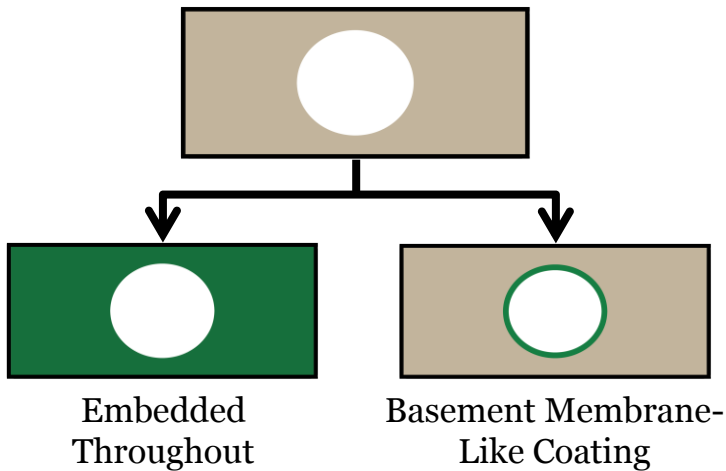
Normal	MCF12a
Tumorigenic	MCF7



Model Optimization: Extracellular Matrix

ECM Proteins
Collagen 1
Collagen 4
Laminin
Fibronectin
Matrigel

Base Hydrogel =
6mg/mL Collagen I



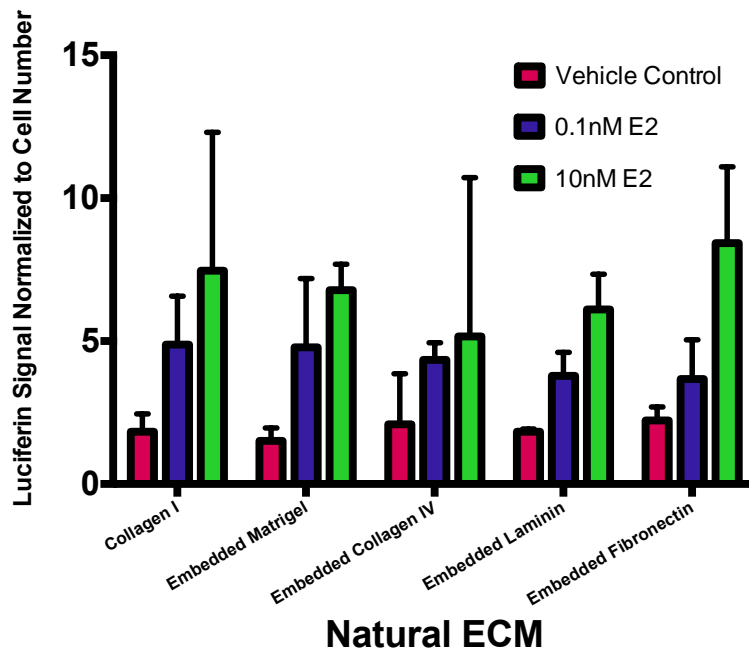
M. Livingston & M. Morgan et. al. In Prep

6 Duct Models for Each Condition

Model Optimization: Extracellular Matrix



Response to Estradiol Treatment in Natural-Hydrogel Duct Model

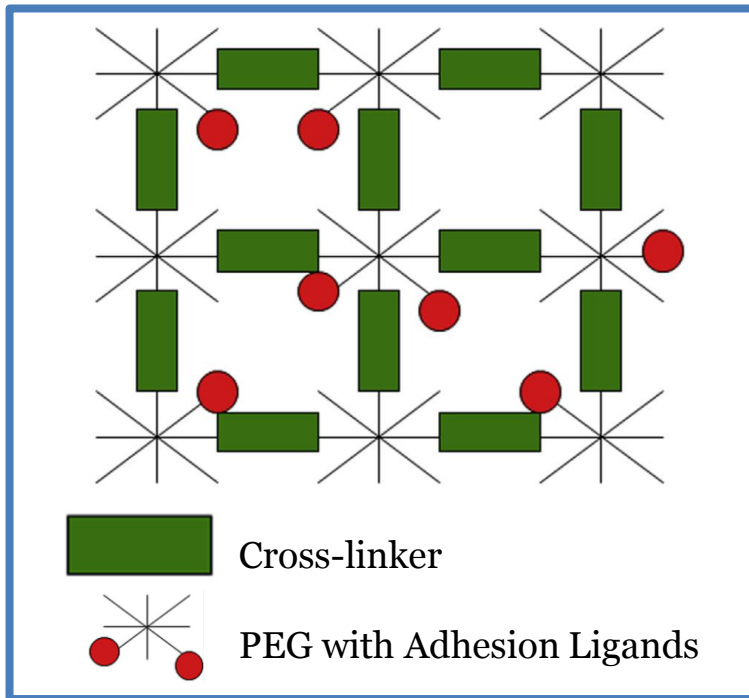


* No significance between ECM conditions with respect to vehicle or E2 treatment

- Mammary duct organotypic models:
 - Confluent monolayer
 - High viability (ave. >90%)
 - Respond to estradiol treatment
- High variance and low sensitivity in **naturally** derived hydrogel ductal models
- **Hypothesis:** A more chemically defined **synthetic** hydrogel will promote more reproducibility and higher sensitivity in readouts and model response.



Increasing Sensitivity with Poly(ethylene glycol) (PEG) Hydrogels



Modular
reaction in
one simple
step.

Screen PEG Gels for Cell Adhesion

Variable	Screened Condition
PEG Concentration	Low, Medium, High
Cross-link Density	70%
Adhesion Ligand	CIKVAV & Linear CRGDS Each at varying concentrations.
Cross-linker	MMP Cleavable & Non-Degradable

152 Conditions in 2D 384
well-plate format

Cell Lines: MCF12a,
MCF7, HMF



Cell Adhesion on 2D PEG-based Hydrogel

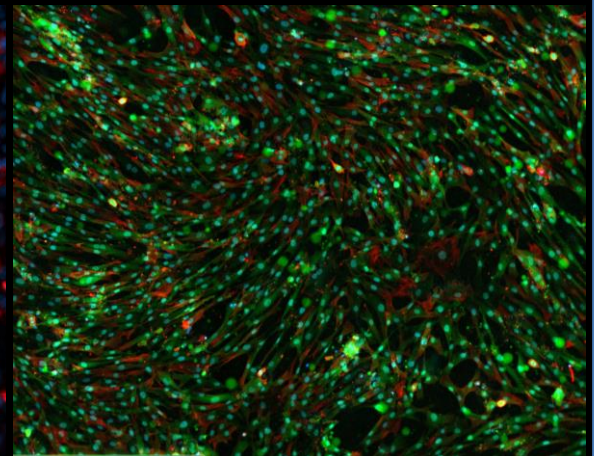
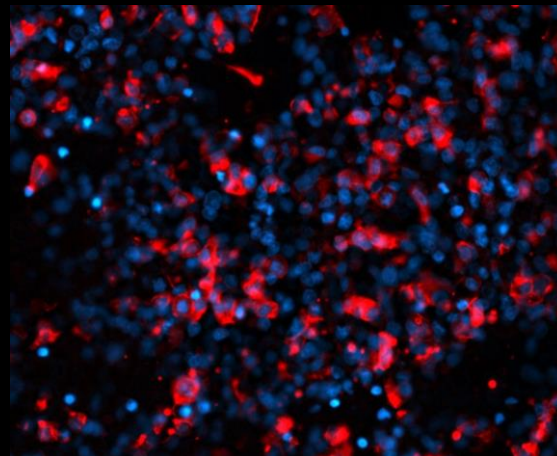
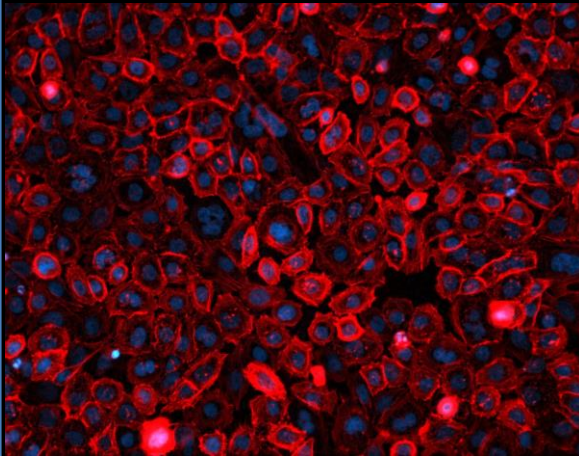


Score of 5 in Three Cell Lines at 20X

MCF12a

MCF7

Human Mammary Fibroblasts



F-Actin

Nuclei

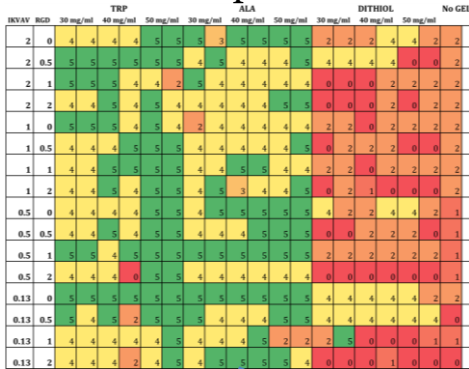
HMF-GFP

Adherence of 3 cell lines is possible on 2D PEG hydrogels.

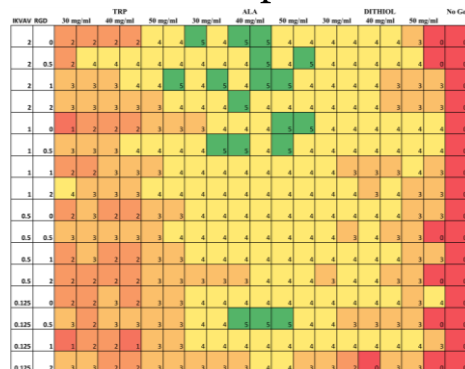


2D Adherence Results

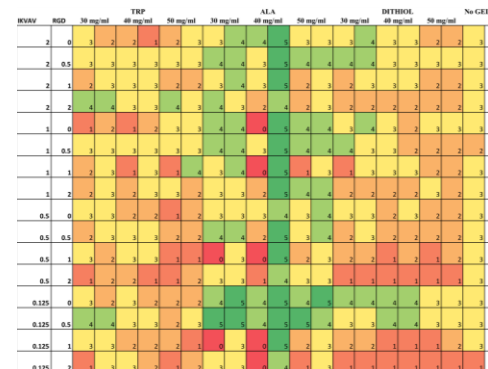
MCF 12a
Normal Epithelial



MVLN (MCF7)
Cancerous Epithelial



Human Mammary
Fibroblast



Cleavable

Non-Cleavable

Subjective Scoring

0	No Attachment
1	Minimal Coverage
2	Coverage w/ Clusters
3	Reduced Coverage
4	Full Coverage w/ Clusters
5	Full Monolayer

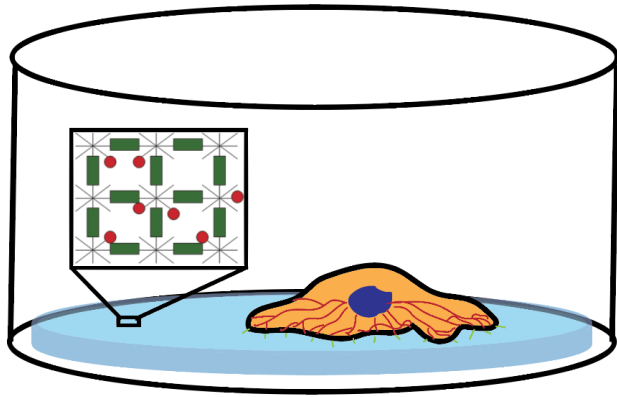
IKVAV RGD		30 mg/ml			40 mg/ml			50 mg/ml			ALA			50 mg/ml			30 mg/ml			40 mg/ml			50 mg/ml		
		30	40	50	30	40	50	30	40	50	30	40	50	30	40	50	30	40	50	30	40	50			
2	0	3	2.667	2.667	2.333	3.667	4.333	3.667	4.667	5	4	4	3	3.333	3	3.667	3	1.333	1.667						
2	0.5	3.333	4	4	4	4	4	4.333	3.667	4.667	4	4.667	4	4	3.667	3.667	2.333	2.333	1.667						
2	1	3.333	3.667	3.667	3.667	3.333	4	4.333	3.667	4.667	3.667	3.667	2	2.333	2.333	2.667	2.333	2.333	1.667						
2	2	3.667	3.667	3.667	3.333	4	3.66	4	3.667	3.667	4	3.667	2	2	2	2.333	1.667	2.333	1.667						
1	0	2.333	3	2.667	2.667	3.667	3.33	3	4	2.667	4.333	4.333	4.333	3	3.333	2.333	2.667	3	3	1.667					
1	0.5	3.333	3.333	3.333	4	4	4	4.333	4	4.333	4.333	4.333	2.667	3	3	2.667	2	2	1.333						
1	1	2.667	3	3	3.667	3	4.33	3.667	4	3	4.667	3	3.667	2.333	2.667	2	2.667	2.667	2.333	1.667					
1	2	3.333	3.333	3.333	3.333	4	3.66	3.667	4	3	4.333	4	4.333	2	2.667	2	2	2	1.667	1.667					
0.5	0	3	3.333	2.667	2.667	3	3.33	3.667	4	4	4.333	4	4.333	3.667	3	2.667	3.667	3	2.333	1.333					
0.5	0.5	3	3.333	3.667	3.333	3.333	3.66	4	4	3.333	4.667	4	4.333	2	2	2.667	2.333	2.333	0.667	1.333					
0.5	1	3.333	3.333	3	3.333	3	3	4	3	4.667	3.667	4	4.667	2.667	2.667	2.333	2.667	2	2.333	1.333					
0.5	2	2.333	2.667	2.667	1	3	3.33	3.333	3.333	2.667	4	3.667	3.667	1.333	1.667	1.667	1.333	1.333	0.333	1.333					
0.125	0	3.333	3	3.667	3	3.333	3.33	4.333	4.667	4.333	4.667	4.333	4.667	4	4	4	4	3.333	3	1.667					
0.125	0.5	4	3.333	3.667	2.667	3.333	3.66	4.667	4.333	4.333	4.667	5	4.333	3.667	3.333	3.667	3.667	3.333	2.333	1					
0.125	1	2.667	3	2.667	2.333	3	2.667	3.667	2.667	2.667	2.667	5	2.667	3.667	1.667	1.667	1.667	1.667	2	1.333					
0.125	2	2.667	3.333	3	2	2.667	3.33	3.333	3.667	4.333	3.333	3.333	3.333	1.333	1	0.333	1.667	1.333	0.333	0.333					

Cells have higher adherence to **MMP cleavable** gels, specifically the gel with the **alanine** cross linker.



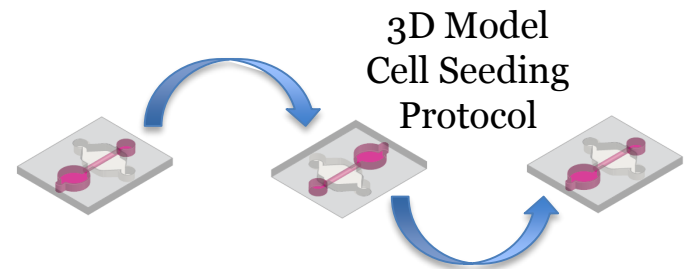
2D Screen Informed 3D Model

PEG-Hydrogel Choices



- 2D PEG-hydrogel screen for adhesion indicated:
 - Cells adhere to a cleavable substrate
 - Lower concentrations of adhesion promoting peptides are suitable for 2D conditions
 - PEG concentrations (stiffness) did not have a significant impact on adhesion

- Thinking forward to 3D duct model:
 - Requires stronger and faster adhesion
 - Increased concentration of Linear RGD
 - Included cyclic confirmation of RGD peptide

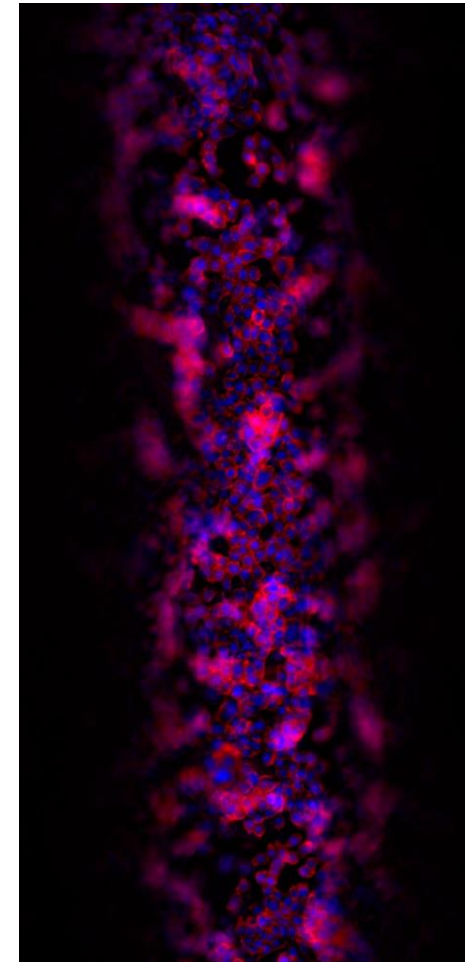


Results: 3D PEG-based Duct Model Screen



Adhesion Promoting Peptide Sequences Found in Collagen

		Cyclic RGDS					Linear RGDS					
		0mM	1mM	2mM	3mM	4mM	5mM	0mM	1mM	2mM	3mM	4mM
Adhesion Promoting Peptide Sequences Found in Laminin	IKVAV	0mM	1mM	High Adhesion				Low Adhesion				
		1mM										
		2mM										
		3mM										
		0mM	1mM	High Adhesion				Low Adhesion				
AG73		1mM										
		2mM										
		3mM										
		0mM	1mM	High Adhesion				Low Adhesion				



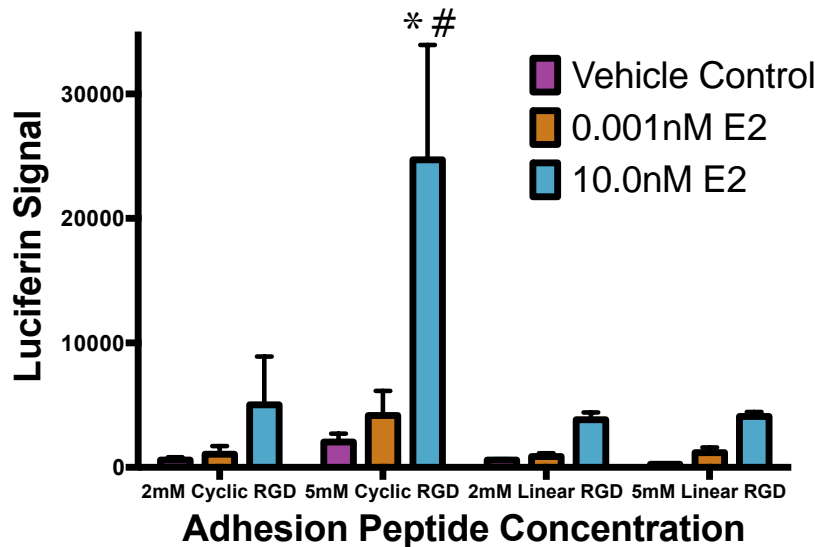
MCF12a
Nuclei F-Actin

2mM CyclicRGD Adhesion Promoting Peptide
70% Cross-linked

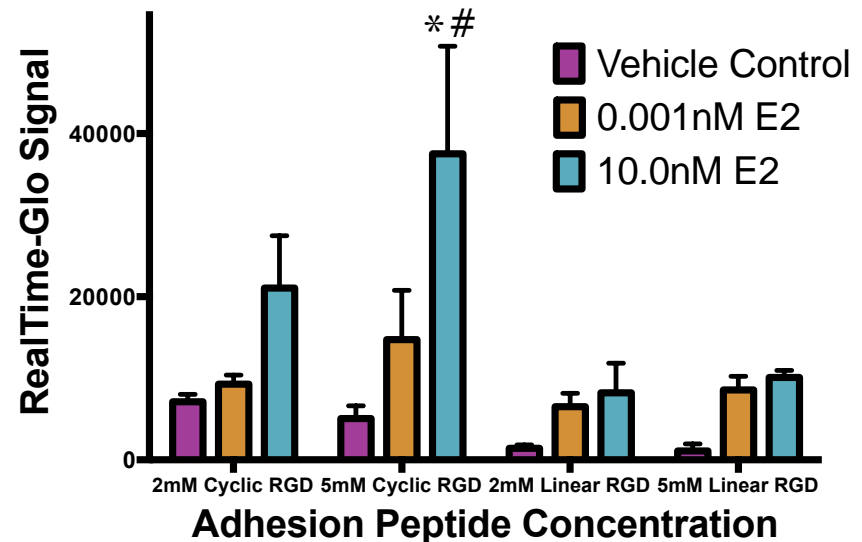


PEG-Based Mammary Duct Models Respond to Estradiol Treatment

Estrogen Response Element Activity in PEG-Based Duct Models



Cell Number in PEG-Based Duct Models

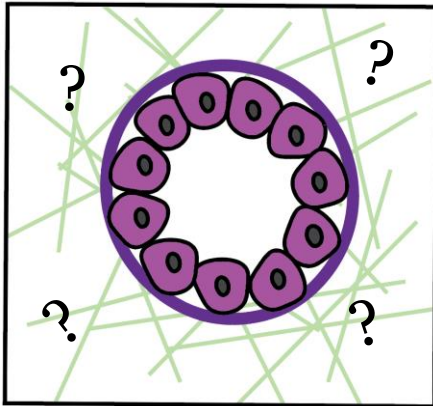


- Cyclic RGD adhesion promoting peptide shows higher ERE activity and cell number than its linear RGD counterpart
- PEG-based duct model shows promise for providing higher sensitivity as a 3D organotypic screening model

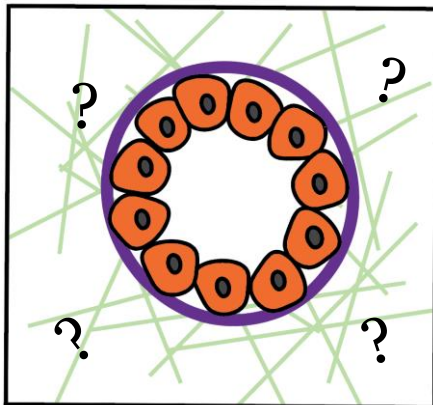
Concluding Comments



Normal Duct



Cancerous Duct



- We have a viable organotypic model of the human mammary duct made of **natural** and **synthetic** hydrogel matrices
- Both **natural** and **synthetic** models respond to the addition of estradiol
- 3D Duct Models are compatible and can be multiplexed with several endpoints:
 - Quick throughput: ERE Luciferase & RealTime Glo
 - Automated Image Processing: Phenotype, Cell Adhesion, IF Staining

Acknowledgements



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