TissueSpec® Bone, Liver, and Lung ECM substrates enable disease-relevant in-vitro models of pre-metastatic tissue microenvironments

E. Aranda[1], E. Gadee[1], I. Germanguz[1], J.C. Xiong[1], N. Kissel[1], A. Nichols[1], D.P. Daly[1], D. Shvartsman[2], D. Deems[2], J.D. O'Neill[1]*

[1] **Xylyx Bio**, Brooklyn, New York, USA [2] Cellaria, Wakefield, Massachusetts, USA * Corresponding author: john@xylyxbio.com

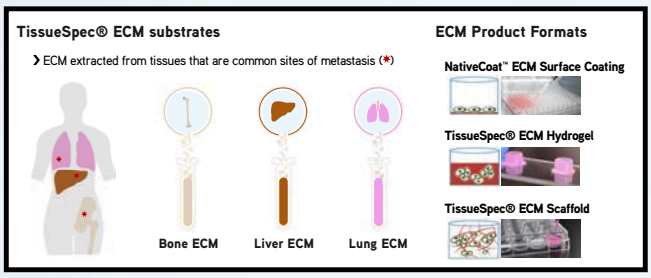
TISSUE-SPECIFIC ECM RECAPITULATES THE TISSUE ENVIRONMENT AT METASTATIC SITES

Problem: *In-vitro* metastasis models lack pre-metastatic niche ECM components

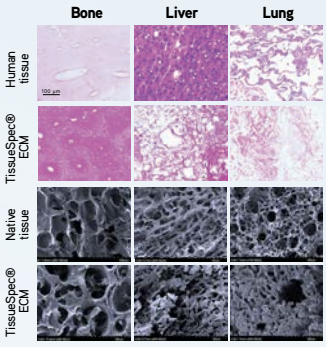
Current *in-vitro* models of metastasis fail to recapitulate the critical cell–matrix interactions involving the pre-metastatic extracellular matrix (ECM) of liver, lung, and bone tissues.

Solution: TissueSpec® Bone, Liver, and Lung ECM substrates

Tissue-specific ECM have biochemical and mechanical features that enable disease-relevant *in-vitro* modeling of metastatic processes in bone, liver, and lung tissue microenvironments.



TissueSpec® ECM have the tissue-specific properties of human tissues



Elastic modulus (kPa)

Tissue	TissueSpec® Hydrogel	Human tissue*
Bone	22	10^5
Liver	20	7 – 15
Lung	6	3 – 6

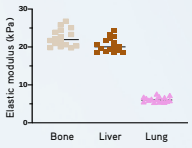
* Skardal *et al. J Mech Behav Biomed Mater.* 7:307-316 (2013)

Matrisome profiles by mass spectrometry (partial list)

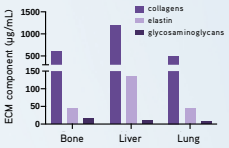
			TissueSpec® ECM		
Protein category	Protein	Gene	Bone	Liver	Lung
Collagen	type I alpha 1 chain	COL1A1	*	*	*
	type IV alpha 2 chain	COL4A2		*	*
	type V alpha 2 chain	COL5A2	*	*	*
	type VI alpha 5 chain	COL6A5		*	
Proteoglycan	Hyaluronan and proteoglycan link protein 1	HAPLN1			*
	Heparan sulfate proteoglycan 2	HSPG2	*	*	*
Glycoprotein	Elastin	ELN	*		*
	Laminin, gamma 1	LAMC1		*	*
	Periostin	POSTN	*		*
	Tenascin C	TNC	*		
	Fibronectin 1	FN1	*		

ECM component (µg/mL)	Bone ECM	Liver ECM	Lung ECM
collagens	580 – 620	1100 – 1300	400 – 530
elastin	40 – 50	120 – 150	40 – 50
glycosaminoglycans	10 – 20	5 – 15	3 – 5

Biomechanical stiffness

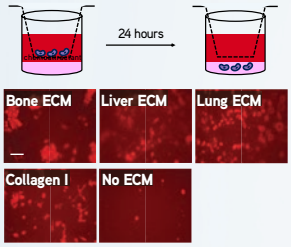


Biochemical composition



INVASION & MIGRATION ASSAYS

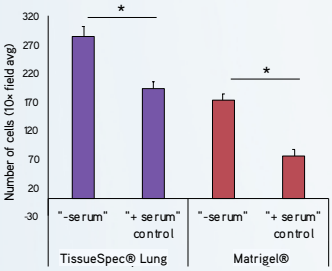
Migration assay



› **Methods:** TissueSpec® Bone, Liver, or Lung ECM Hydrogels, collagen I gel, or plastic (no ECM) were added to the bottom of wells as chemoattractants. Lung adenocarcinoma cells (Jacket, Cellaria) were then cultured on transwell inserts with 8 µm pores. After 24 hours, migration was assessed.

› **Results:** Adenocarcinoma cells showed greater migration toward ECM substrates, and organized differently in each tissue-specific ECM. Notably, clusters formed in TissueSpec® Bone ECM. Scale bar: 100 µm

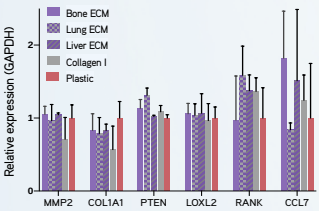
Invasion assay



› **Methods:** Lung adenocarcinoma cells (A549) were cultured on transwell inserts coated with TissueSpec® Lung ECM Hydrogel or Matrigel®. Media with 10% serum was added to lower compartments, and media without serum (or with serum as control) was added to upper compartments. After 24 hours, invasion was quantified by crystal violet stain.

› **Results:** Lung adenocarcinoma cells cultured in TissueSpec® Lung ECM Hydrogel exhibited significantly greater motility & invasiveness than cells cultured in Matrigel®. *$p<0.05$.

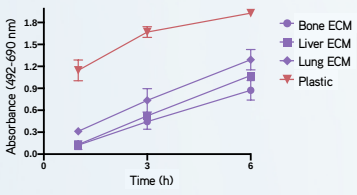
Metastasis-related gene expression



› **Methods:** Metastatic breast cancer cells (BT-549) were cultured on NativeCoat™ ECM, collagen, or plastic for 24 hours. Gene expression was normalized to BT-549 cells cultured on plastic.

› **Results:** Cells cultured NativeCoat™ Bone, Liver, and Lung ECM expressed higher levels of RANK mRNA. Cells cultured on NativeCoat™ Bone ECM expressed higher levels of CCL7 mRNA.

Proliferation in pre-metastatic ECM

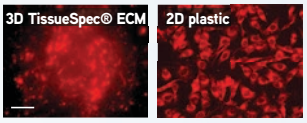


› **Methods:** Breast cancer cells (BT-549) were cultured in Bone, Liver, or Lung ECM Hydrogels, and on plastic for 24 hours. Proliferation was assessed by XTT assay.

› **Results:** Breast cancer cells cultured in TissueSpec® ECM Hydrogels had different rates of proliferation. Cells cultured in Lung ECM showed higher activity than cells in Bone and Liver ECM Hydrogels.

3D APPLICATIONS

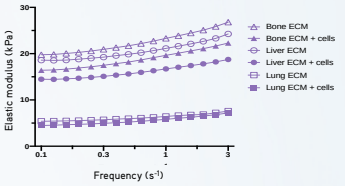
3D models of metastases



› **Methods:** Jacket cells were labeled with CellTracker Red CMTPX and cultured in 3D TissueSpec® Lung ECM Hydrogel and on 2D plastic (no ECM). Scale: 100 µm

› **Results:** 3D TissueSpec® Lung ECM enables cell-cell contact and cell-ECM interactions and show 3D aggregations of cancer cells.

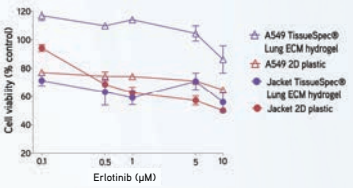
ECM remodeling

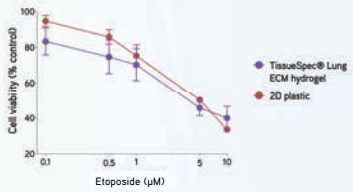


› **Methods:** Rheometric testing was conducted on TissueSpec® Bone, Liver, and Lung ECM Hydrogels with and without $5x10^5$ Jacket lung adenocarcinoma cells after 48 hours.

› **Results:** Bone and liver ECM hydrogels had reduced elastic moduli, whereas modulus of lung ECM did not change.

Drug response assays





› **Methods:** A549 cells and Jacket cells were cultured in TissueSpec® Lung ECM Hydrogel, Matrigel®, or 2D plastic. After 24 hours, drugs were added (DMSO as control). Cells were cultured for 72 hours, then XTT assay was measured viability. IC_{50} values were obtained via non-linear fit (GraphPad).

› **Results:** Cells cultured in TissueSpec® Lung ECM Hydrogel exhibited distinct drug resistance profiles which may indicate more predictive physiological response. Jacket cells had lower IC_{50} versus A549 cells.

Erlotinib	IC_{50} (µM)	
Substrate	A549	Jacket
TissueSpec® Lung ECM	134.2	7.5
Plastic (no ECM)	36.4	5.2
Matrigel®	57.5	8.7

Etoposide	IC_{50} (µM)	
Substrate	A549	Jacket
TissueSpec® Lung ECM	16.6	3.7
Plastic (no ECM)	40.3	4.4
Matrigel®	35.5	15.8

Physiologically relevant
TissueSpec® Bone, Liver and Lung ECM Hydrogels contain the full milieu of proteins & growth factors present in pre-metastatic niche ECM of human tissues.

More accurate, predictive results
TissueSpec® ECM Hydrogels provide ideal conditions for maintaining cell phenotype, leading to more accurate results compared to other substrates.

Standardized experiments
TissueSpec® ECM Hydrogels demonstrate consistent composition profiles across different lots, resulting in reproducible studies.

Clinically translatable
TissueSpec® ECM Hydrogels facilitate downstream clinical translation because they contain tissue-specific ECM from medical grade swine tissues.

XYLYX
the cell environment company

SUMMARY

TissueSpec® ECM Hydrogels contain pre-metastatic niche ECM components and are compatible with 3D applications and assays.