White paper

3D lung tumor model with lung extracellular matrix to predict drug efficacy

Evelyn Aranda, Igal Germanguz, Jennifer C. Xiong, Natalia Kissel, Alexandra Nichols, John D. O'Neill

Cell-based assays are widely used to select the most promising anti-cancer drug candidates in early-stage drug development, however, nearly 90% of drug candidates still fail in clinical trials. Compared to conventional two-dimensional in-vitro monolayer culture systems used for drug testing, three-dimensional (3D) culture systems incorporating TissueSpec® extracellular matrix (ECM) hydrogel better recapitulate the physiologic cell–cell and cell–matrix interactions of the tumor microenvironment, enabling greater predictability of drug efficacy while maintaining workflow compatibility. The use of 3D lung tumor models with lung-specific ECM hydrogel is critical to identify targets for drug candidates and obtain more accurate and actionable results in early-stage drug development.

Introduction

Conventional in-vitro systems for pharmaceutical drug discovery utilize two-dimensional (2D) cell culture assays in which cells are maintained on polystyrene or other tissue culture plastic substrates. Such 2D systems, however, do not reflect the complex cellular microenvironments of human tissues, and as a result, 2D monolayer cultures experience loss of appropriate cell–cell interactions, high degree of polarization, excessive nutrition, and hyperoxygenation [1], and thus fail to provide the physiologic culture conditions needed to accurately assess drug response in humans. To address the limitations of conventional 2D in-vitro systems, three-dimensional (3D) cell culture systems that enable the natural spatial organization of cells more closely resembling that of human tissues are under development. 3D culture systems have repeatedly been shown to provide more physiologically accurate and predictive results compared to 2D culture systems. Furthermore, the high clinical trial attrition rate of drug candidates developed using 2D cell-based assays strongly motivates the use of 3D cell culture systems as a promising and valuable alternative (or complement) to conventional 2D systems in pharmaceutical drug development [2, 3].

Adoption of more physiologically relevant 3D culture systems in disease models, stem cell research, cancer biology, and tissue engineering continues to increase. In cancer research, advances in 3D culture systems have yielded tumor models that closely mimic the tumor microenvironment in vivo, resulting in better understanding of the mechanisms driving tumor growth and metastasis, and the opportunity to accelerate development of effective cancer therapies through more predictive drug screening tools [4].

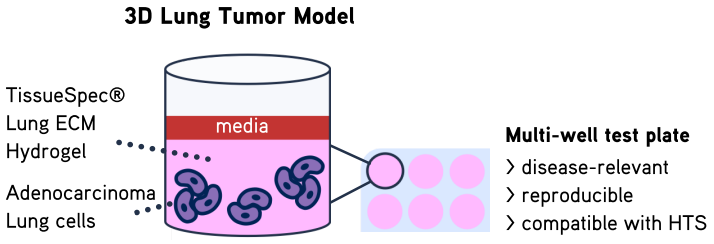


Figure 1. TissueSpec® Lung ECM Hydrogel in 3D lung tumor model. Tumor cell organization in 3D lung ECM hydrogel resembles lung tumors in humans, enabling significantly more predictive drug testing.

In lung cancer, tumor mass is comprised of dysregulated neoplastic cells within the tumor tissue micro-environment. Notably, the tumor microenvironment is fundamentally comprised of immune cells, fibroblasts,

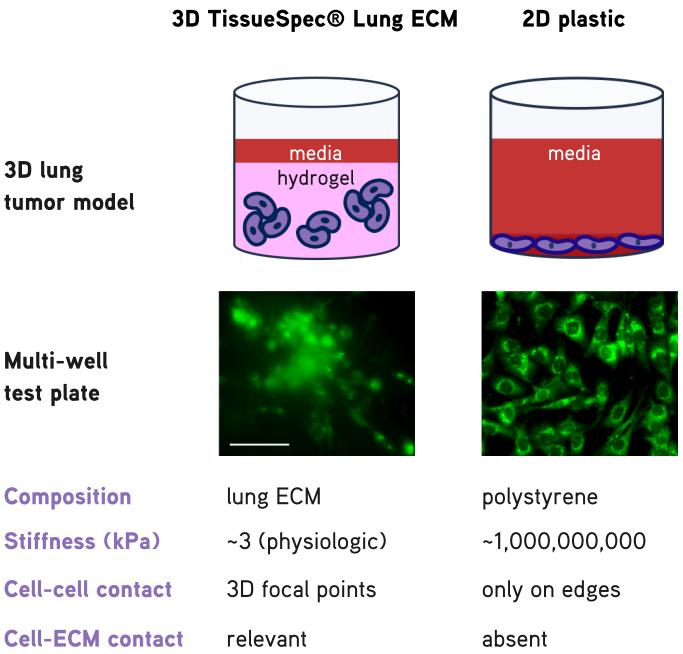
soluble factors (e.g., cytokines, hormones), and extracellular matrix (ECM), which plays a central role in tumor cell proliferation and metastasis [5]. Lung ECM is a bioactive component of the tumor environment and contains multiple categories of structural and signaling molecules, including collagens (type I, II, III, IV, VI, XIII, IX, XI, XVI), laminins (α5, β2, γ1), glycoproteins (fibrillin 1, nidogen), proteoglycans (heparan sulfate, hyaluronan), and matrix-associated growth factors. Furthermore, ECM has tissue-specific biophysical and mechanical characteristics that regulate cell activity and phenotype [6]. Since ECM is known to have important roles in tumorigenesis, in-vitro 3D models that integrate tissue-specific ECM recapitulate key features of the tumor tissue environment and open new opportunities in anti-neoplastic drug discovery.

Non-small cell lung cancer (NSCLC) remains the leading cause of cancer-related mortality worldwide [7], and new treatment options are critically needed. Here, we describe the use of TissueSpec® Lung ECM Hydrogel as a physiomimetic in-vitro substrate in 3D lung tumor models to assess anti-neoplastic drug response of non-small cell lung adenocarcinoma cells in a multi-well plate test platform (Figure 1). Compared to conventional 2D plasticware and other 3D substrates, TissueSpec® Lung ECM hydrogel is characterized by tissue-specific biochemical composition, mechanical stiffness, and native-like 3D topography of lung tissue (Figure 2). We evaluated the effects of three drugs with known anti-tumor effects: KPT-185, Erlotinib, and Etoposide [8-10]. Notably, the latter two drugs are approved for the treatment of advanced non-small cell lung carcinoma. We cultured lung adenocarcinoma cells (A549 or Jacket) in 3D TissueSpec® Lung ECM hydrogel, performed cell-based assays to quantify drug response, and compared results against standard cell culture substrates (tissue culture plastic without ECM and murine sarcoma extract Matrigel®).

Methods

Cell expansion

Human lung adenocarcinoma A549 cells (CCL-185, ATCC) were cultured using F12K Medium (30-2004, ATCC) and 10% fetal bovine serum. Human lung adenocarcinoma Jacket cells (CB030-000001, Cellaria) were cultured in Basal Renaissance Essential Tumor Medium (CM-0001, Cellaria) with RETM supplement, 25 ng/mL cholera toxin, and 5% heat-inactivated fetal bovine serum (SH3007103HI, HyClone). Both cell types were cultured under standard conditions (37˚C, 100% humidity, 5% CO2), and passaged at 80% confluency.



	3D TissueSpec® Lung ECM	2D plastic
Composition	lung ECM	polystyrene
Stiffness (kPa)	~3 (physiologic)	~1,000,000,000
Cell-cell contact	3D focal points	only on edges
Cell-ECM contact	relevant	absent

Figure 2. Physiologic features of TissueSpec® Lung ECM Hydrogel. Lung adenocarcinoma cells in 3D lung ECM hydrogel compared to cells on 2D surface of tissue culture plastic. Scale bar: 100 µm.

3D lung tumor model with TissueSpec® Lung Hydrogel

The 3D lung tumor models were prepared by mixing 5,000 lung adenocarcinoma cells with 40 µL TissueSpec® Lung ECM Hydrogel (MTSLG101, Xylyx Bio) at 4 mg/mL, or Matrigel® (354234, Corning) at 4 mg/mL in 96-well plates. Cells were incubated at 37˚C for 1 hour to allow substrates to gel. After incubation, 100 µL culture medium was added to each well.

Cells were also cultured directly on tissue culture plastic as a control without ECM. Tissue culture plastic without ECM and Matrigel® (a basement membrane extract from the Engelbreth-Holm-Swarm murine sarcoma) served as comparative substrates.

Drug treatment

KPT-185 (S7125, Selleckchem), Erlotinib (S7786, Selleckchem), and Etoposide (S1225, Selleckchem) were dissolved in DMSO to obtain 1 mM stock solutions. Cells were cultured for 24 hours, then treated with 100 µL media containing drug for 72 hours. Drug doses were 0.1, 0.5, 1, 5, and 10 µM. In parallel, as negative controls, A549 and Jacket cells were treated with media containing only DMSO at the same concentration of each drug preparation. After 72 hours, cell viability was assayed. All assays were performed with five technical replicates.

Cell proliferation assay

Cell proliferation was assessed using a Cell Proliferation Kit II (XTT, 11465015001, Sigma-Aldrich) according to the manufacturer's instructions. The assay reagent XTT is a second-generation tetrazolium dye that is reduced to a soluble orange-colored formazan derivative detectable in real-time. An Assay Protocol was established for use with TissueSpec® Lung ECM Hydrogels. Briefly, 50 µL XTT reagent was added to each well containing 100 µL culture medium. After 4 hours, absorbance was measured using a spectrophotometer at 492 nm and 690 nm as reference.

Data analysis

Absorbance values lower than control (DMSO) indicated reduction of cell viability. Half maximal inhibitory concentration (IC50) values were calculated for each drug using statistical analysis software (Prism, GraphPad) and nonlinear regression analysis.

Results

To demonstrate the utility of the 3D lung tumor model with lung ECM, the effects of three anti-cancer drugs (two currently used to treat non-small cell lung cancer) were investigated. KPT-185, Etoposide, and Erlotinib were prepared at a range of concentrations, and the viability of lung adenocarcinoma cells cultured in 3D TissueSpec® Lung ECM Hydrogel was evaluated and compared to cells cultured on 2D plastic without ECM.

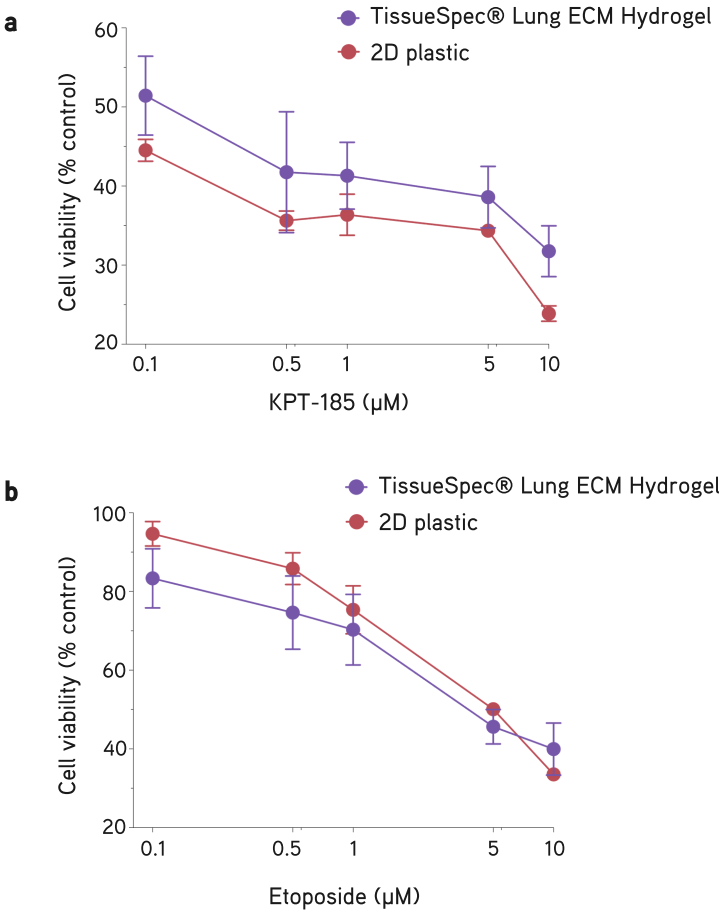


Figure 3. Sensitivity of lung adenocarcinoma cells to cancer drugs. Jacket cells treated with KPT-185 (a) or Etoposide (b). Data represent mean ± standard error mean of five technical replicates.

The effects of KPT-185 and Etoposide on Jacket lung adenocarcinoma cells cultured in 3D TissueSpec® Lung ECM Hydrogel and on 2D plastic were quantified. Cell viability in response to a range of KPT-185 doses (0.1 – 10 µM) is shown in Figure 3a.

KPT-185 induced dose-dependent growth inhibition of Jacket cells, which were more resistant in 3D TissueSpec® Lung ECM Hydrogel than on plastic without ECM for all doses evaluated. Etoposide showed dose-dependent growth inhibition in both substrates, however, contrary to the effects of KPT-185, Jacket cells were moderately more sensitive in 3D TissueSpec® Lung ECM Hydrogel than on 2D plastic in the range of 0.1 – 5 µM (Figure 3b). Notably, however, at 10 µM, the highest dose of Etoposide in this study, Jacket cells were more resistant in 3D TissueSpec® Lung ECM Hydrogel than on 2D plastic without ECM.

A549 cells have been used for decades as the gold standard cell model of lung adenocarcinoma for drug testing [11, 12]. Accordingly, we investigated the effects of Erlotinib on A549 and Jacket cells cultured in 3D TissueSpec® Lung ECM Hydrogel and on 2D plastic without ECM. Treatment of A549 cells with a range of doses of Erlotinib (0.1 – 10 µM) showed dose-dependent growth inhibition, with A549 cells more resistant in 3D TissueSpec® Lung ECM Hydrogel than on plastic. Similarly, Jacket cells showed a more resistant profile in 3D TissueSpec® Lung ECM Hydrogel than on 2D plastic, but only at concentrations above 5µM (Figure 4).

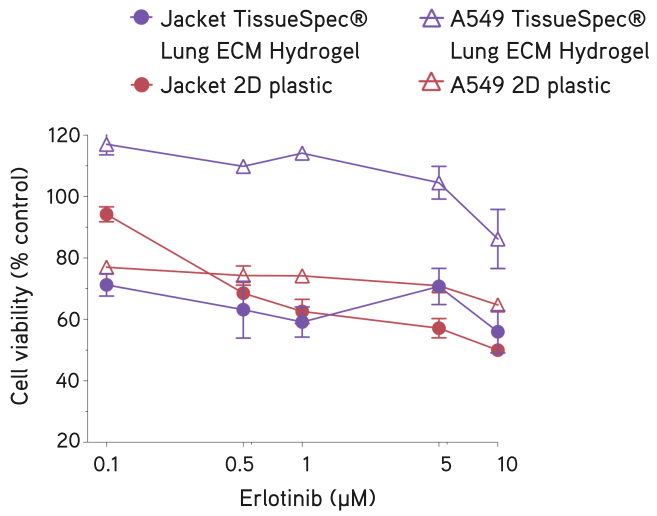


Figure 4. Effect of Erlotinib on lung adenocarcinoma cell viability. Viability of Jacket and A549 cells treated with Erlotinib. Data represent mean ± standard error mean of five technical replicates.

Based on these studies, half maximal inhibitory concentration (IC50) values of Erlotinib, KPT-185, and Etoposide, are reported in Table 1. The IC50 values of Erlotinib show that A549 cells were more resistant than Jackets cells in all substrates. Interestingly, A549 cells in 3D TissueSpec® Lung ECM Hydrogel had an IC50 value of 134.2 µM – more than 1800% higher than the IC50 value of Jacket cells in the same substrate (7.5 µM). Conversely, IC50 values of Etoposide show that Jacket cells in 3D TissueSpec® Lung ECM Hydrogel were slightly more sensitive (3.7 µM) than Jacket cells on 2D plastic (4.4 µM). A similar trend was observed with IC50 values of KPT-185, where Jacket cells were more resistant in 3D TissueSpec® Lung ECM Hydrogel (0.4 µM) than on plastic (0.2 µM).

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

KPT-185	**IC_{50}(µM)**	
Substrate	A549	Jacket
TissueSpec® Lung ECM	2.3	0.4
Plastic (no ECM)	8.8	0.2
Matrigel®	4.2	4.3

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

Table 1. IC50 values for A549 and Jacket cells. Data represent mean ± standard error mean of five technical replicates.

A common 3D lung tumor model involves culturing A549 cells in Matrigel®. Comparison of IC50 values of A549 and Jacket adenocarcinoma cells cultured in Matrigel® revealed that Jacket cells were more sensitive than A549 cells to Etoposide and KPT-185 (Table 1). Notably, IC50 values of cells in Matrigel® were considerably higher than IC50 values of cells in TissueSpec® Lung

ECM Hydrogel, consistent with previous studies where MCF-7 and MB-231 breast cancer cells were shown to be more resistant to doxorubicin when cultured in Matrigel® [13].

Notably, Jacket cells in TissueSpec® Lung ECM Hydrogel yielded highly consistent IC50 values. In two separate rounds of testing, Etoposide yielded IC50 values of 3.8 µM and 4.0 µM, and KPT-185 yielded the exact same IC50 value of 0.4 µM in both studies. By contrast, Jacket cells in Matrigel® yielded inconsistent, highly variable IC50 values across the same series of testing. IC50 values for Etoposide were 0.7 µM and 18.3 µM. Moreover, IC50 values for KPT-185 were 0.2 µM and 5.3 µM, suggesting that Matrigel® is highly inconsistent across tests and lots, and is therefore a poor choice of substrate for reproducible drug testing.

Overall, these data indicate that A549 cells are more resistant than Jacket cells to KPT-185, Etoposide, and Erlotinib in the 3D lung tumor model with TissueSpec® Lung ECM Hydrogel, and the other substrates evaluated. Furthermore, Jacket cells are more resistant to Etoposide and Erlotinib when cultured in a 3D environment compared to a 2D surface.

The difference in IC50 values of the three drugs in this study are attributable to the different cellular mechanism that each drug targets as well as expression of and access to each target. Erlotinib is an inhibitor of epidermal growth factor receptor (EGFR) tyrosine kinase, whereas Etoposide inhibits DNA topoisomerase II, which prevents DNA ligation and ultimately causes DNA strands to break [14]. KPT-185 induces apoptosis as a selective inhibitor of nuclear export (SINE) compound [10]. Additionally, variable rates of proliferation of different cell types and densities can significantly affect drug response. Another important consideration for drug testing in 3D culture systems is drug permeability through the ECM and cell membrane. Diffusion assays demonstrated that CellTracker Red CMTPX (686.2 g/mol) has a diffusion speed through TissueSpec® Lung ECM Hydrogel ≥ 1.3 mm/h.

Jacket cells were derived directly from a patient lung adenocarcinoma, and are therefore patient-specific, lack accumulation of genetic mutations through serial expansion and passaging, and likely represent a more predictive lung cancer model than A549 cells, a hypotriploid cell line. A 3D lung tumor model comprised of patient-specific lung tumor cells in TissueSpec® Lung ECM Hydrogel resembles the in-vivo human disease environment significantly more closely than conventional 2D or 3D models, and enables drug developers to obtain more physiologic cellular responses during drug testing.

Conclusions

3D lung tumor models with TissueSpec® Lung ECM Hydrogel from Xylyx Bio are a highly physiologic, disease-relevant culture system that supports cell–cell and cell–matrix interactions significantly more similar to the tumor tissue environment than the non-physiologic, stiff 2D surface of conventional tissue culture plastic. By closely mimicking the complex lung tissue environment, TissueSpec® Lung ECM Hydrogel offers an accurate and consistent 3D lung tumor model to evaluate mechanisms of action, identify drug targets, and gain relevant understandings of the drug sensitivity and resistance of any cell type. TissueSpec® Lung ECM Hydrogel recapitulates in-vivo conditions and drives in-vivo like cell response, thereby enabling more accurate and actionable results through multiple stages of the drug development pipeline.

References

1. Griffith, L.G. and M.A. Swartz, Capturing complex3D tissue physiology in vitro. Nat Rev Mol Cell Biol, 2006. 7(3): p. 211-24.

2. Pampaloni, F., E.G. Reynaud, and E.H. Stelzer, The third dimension bridges the gap between cell culture and live tissue. Nat Rev Mol Cell Biol, 2007. 8(10): p. 839-45.

3. Ryan, S.L., et al., Drug Discovery Approaches Utilizing Three-Dimensional Cell Culture. Assay Drug Dev Technol, 2016. 14(1): p. 19-28.

4. Kapalczynska, M., et al., 2D and 3D cell cultures - a comparison of different types of cancer cell cultures. Arch Med Sci, 2018. 14(4): p. 910-919.

5. Sounni, N.E. and A. Noel, Targeting the tumor microenvironment for cancer therapy. Clin Chem, 2013. 59(1): p. 85-93.

6. Walker, C., E. Mojares, and A. Del Rio Hernandez, Role of Extracellular Matrix in Development and Cancer Progression. Int J Mol Sci, 2018. 19(10).

7. Bray, F., et al., Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2018. 68(6): p. 394-424.

8. Pujol, J.L., et al., Etoposide plus cisplatin with or without the combination of 4'-epidoxorubicin plus cyclophosphamide in treatment of extensive small-cell lung cancer: a French Federation of Cancer Institutes multicenter phase III randomized study. J Natl Cancer Inst, 2001. 93(4): p. 300-8.

9. Gridelli, C., et al., Erlotinib in non-small cell lung cancer treatment: current status and future development. Oncologist, 2007. 12(7): p. 840-9.

10. Wang, S., et al., Antitumor effects of a novel chromosome region maintenance 1 (CRM1) inhibitor on non-small cell lung cancer cells in vitro and in mouse tumor xenografts. PLoS One, 2014. 9(3): p. e89848.

11. Guo, C.Y., et al., The prognostic value of PKM2 and its correlation with tumour cell PD-L1 in lung adenocarcinoma. BMC Cancer, 2019. 19(1): p. 289.

12. Huang, Q., et al., IL-17 Promotes Angiogenic Factors IL-6, IL-8, and Vegf Production via Stat1 in Lung Adenocarcinoma. Sci Rep, 2016. 6: p. 36551.

13. Lovitt, C.J., T.B. Shelper, and V.M. Avery, Doxorubicin resistance in breast cancer cells is mediated by extracellular matrix proteins. BMC Cancer, 2018. 18(1): p. 41.

14. Montecucco, A., F. Zanetta, and G. Biamonti, Molecular mechanisms of etoposide. EXCLI J, 2015. 14: p. 95-108.

Acknowledgments

We would like to thank David Deems and Dmitry Shvartsman of Cellaria Biosciences for insightful discussions of the experimental design and results.

Contact

To learn more about our ECM cell culture products or discuss partnering opportunities, please contact us at info@xylyxbio.com.

Xylyx Bio, Inc.
760 Parkside Avenue
Brooklyn, New York 11226, USA