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Effect of scaffold on drug sensitivity of multicellular spheroids. Which method is close to in vivo and suitable for HTS?

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Background, Method and Result

Background: Three-dimensional (3D) cell culture models are more widespread to be better models than two-dimensional (2D) cell culture models due to improved cellular signaling pathways, cell to-cell contact and cell morphology that resembles in vivo architecture. Different drugs screening and various biological responses between 2D and 3D cell culture models have been reported. However, very little information is available on cell function and/or drug sensitivity caused by differences in a methodology of three dimensional culture. Here, we compared drug responses of various cancer cells against distinctive anti-cancer drugs when grown in monolayer, scaffold 3D culture (Matrigel®, NanoCuluture Plate (NCP)) or scaffold-free 3D culture models (Low adhesion Round bottom plate /Low adhesion Flat bottom plate). We also explored which method is more closed to in vivo drug response and suggested which culture model is suitable for high-throughput screening for robust three-dimensional screening model.

Method: We compared the growth rate of lung cancer, prostate cancer, and breast cancer by ATP assay between scaffold type 3D model and scaffold-free type model. We examined drug sensitivities of lung cancer (A549), breast cancer (BT474, JIMT1, MCF7), hepatocellular cancer (HepG2), Pancreatic cancer (BxPC-3), and prostate cancer (DU145) cell lines under 3D cell culture conditions against several anti-cancer agents i.e. anti-metabolite agent (Gemcitabine, Methotrexate, 5-FU), anti-microtubule agent (Docetaxel), microtubule-stabilizing agent (Paclitaxel), molecular targeted agent (Temsirolimus, Afatinib, Gefitinib, Sorafenib). Furthermore, we analyzed the A549 gene expression differences among 3D culture methods (Matrigel®, NCP and Low adhesion Round bottom).

Result: Large variations in growth rate and drug responses were observed among the different cell culture models. Further, 3D scaffold culture models were more sensitive to drug than scaffold-free models. Especially Gemcitabine, Methotrexate, 5-FU, and Paclitaxel showed ineffective in 3D scaffold-free culture models compared to scaffold models. Additionally A549 cells expressed higher gene expression of JAK-STAT and Ras/MAPK signaling inhibitor genes while culturing in scaffold-free condition. Therefore it is suggested that the cells grown in scaffold-free culture models act too much toward growth suppression and anti-apoptosis.

3D cell culture methods fall into two major groups

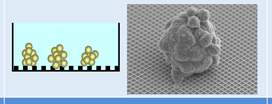
	Scaffold type		Scaffold-free type	
	Matrigel	NanoCulture Plate (NCP)	Low adhesion Round bottom/ Hanging drop	Low adhesion Flat bottom
				
suitable for HTS	-	+++	+++	+++
In vivo reproducibility	+++	?	?	?

Figure 1. 3D cell culture model
Matrigel, ECM-derived biomaterials, and NCP, the patterned surface mimicking ECM, are belong to a scaffold type 3D model. Low adhesion Round / Flat bottom plate and hanging drop plate are distinguished as a scaffold-free type 3D model.

Matrigel's pros and cons
Pros: Spheroids in Matrigel reflects on in vivo characteristic, indicating that the gene expression of cells grown in Matrigel model more closely resembles the gene expression of the xenograft model.
Cons: Matrigel is not well suited for HTS because of complicate handling and the lot-to-lot variation.

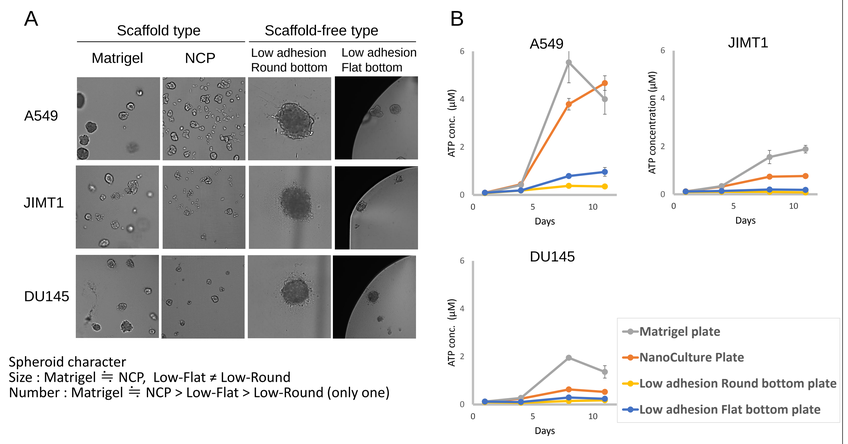
Study of scaffold effects on cellular characteristics under 3D culture conditions

Comparison of scaffold type 3D model with scaffold-free type 3D model in-

Step 1	Spheroid Morphology and Proliferation	Figure 2
Step 2	Sensitivity to Anti-Cancer Drug	Figure 3 and Table 1
Step 3	Gene Expression	Table 2
Step 4	Discussion: Signaling pathway	Figure 4

Which type of model close to in vivo?
Which one is better model for screening of anti-cancer drugs?

Cell proliferation rate on scaffold type 3D model is higher than on scaffold-free type 3D model



Spheroid character
Size : Matrigel ≒ NCP, Low-Flat ≠ Low-Round
Number : Matrigel ≒ NCP > Low-Flat > Low-Round (only one)

Figure 2. Spheroid morphology and proliferation
Three cell lines (A549, JIMT1, DU145) were seeded in 375 cells/well in each 384-well plate and cultured 12 days. (A) All cell lines developed multicellular spheroids in each plate. Spheroids size and morphology were different between scaffold and scaffold-free type. (B) The growth rate was different between scaffold and scaffold-free type. These cell lines spheroids on scaffold type model grew faster than in scaffold-free type.

Spheroids in scaffold-free type model show less drug sensitivity

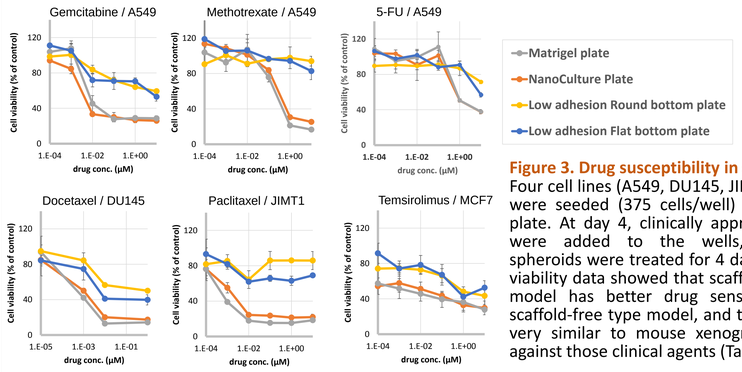


Figure 3. Drug susceptibility in each type
Four cell lines (A549, DU145, JIMT1, MCF7) were seeded (375 cells/well) in 384-well plate. At day 4, clinically approved drugs were added to the wells, and the spheroids were treated for 4 days. The cell viability data showed that scaffold type 3D model has better drug sensitivity than scaffold-free type model, and the extent is very similar to mouse xenograft models against those clinical agents (Table 1)

Table 1. IC50 of anti-cancer drugs in scaffold and scaffold-free type 3D model

Anti-cancer drugs and xenograft model studies				IC50 (nM)			
				Scaffold		Scaffold-free	
Drug	Action	Cell line	Reference	Matrigel	NCP	Low adhesion Round bottom	Low adhesion Flat bottom
Gemcitabine	DNA synthesis inhibitor	A549	1	7	9	>10,000	>10,000
Methotrexate	TMP and purine base synthesis inhibitor	A549	2	670	520	>10,000	>10,000
5-FU	TMA synthesis inhibitor	A549	3	1,407	1,316	>10,000	>10,000
Docetaxel	Anti-microtubule agent	DU145	4	1	1	>10,000	8
Paclitaxel	Microtubule-stabilizing agent	JIMT1	5	1	3	>10,000	>10,000
Temsirolimus	mTOR inhibitor	MCF7	6	3	23	907	723
Afatinib	Tyrosine kinase (HER1/EGFR and HER2/neu) inhibitor	BxPC-3	7	89	1,369	8,069	6,166
Gefitinib	EGFR tyrosine kinase (HER1/EGFR) inhibitor	BT474	8	686	662	504	671
Sorafenib	Tyrosine kinase inhibitor Angiogenesis inhibitor, VEGF inhibitor	HepG2	9	3,744	2,476	8,589	5,354

Red: > 10 times more than Matrigel

The gene expression of spheroid in NCP most closely is similar to Matrigel models. Scaffold-free type is not.

Signaling pathway		Gene	NCP Fold change to Matrigel	Low adhesion Roud bottom Fold change to Matrigel
JAK-STAT	Cytokine	IL6	1.9	3.5
	IL6 receptor	IL6R	1.2	6.6
	JAK	JAK1	2.1	3.9
	STAT	STAT1	2.5	6.3
	SHP1	PTPN6	1.5	2.4
	SOCS	SOCS5	0.7	3.0
		SOCS2	1.4	59.0
Ras-MAPK	SPRED	SPRED1	0.9	3.0
	SPROUTY	SPYR1	0.4	2.3
		SPRY4	3.3	51.4
PI3K-AKT	PI3K	PIK3CB	2.5	5.6
		PIK3R1	1.6	3.4
		PIK3R3	1.2	2.6
	AKT	AKT3	1.3	2.2

Table 2. Comparison of gene expression of JAK-STAT, Ras-MAPK and PI3K-AKT signaling pathway on various 3D models in A549
Gene expression in JAK-STAT / Ras-MAPK/PI3K-AKT signaling pathway were evaluated by qRT-PCR. And gene expression of A549 spheroids on NCP and round-bottom were compared with Matrigel model. NCP showed similar gene expression pattern to Matrigel. On the other hand, all of 14 genes increased in round-bottom 3D model. This data exposed that NCP, the patterned surface mimicking ECM, belongs to scaffold type 3D model group.

Scaffold-free 3D culture condition may inhibit cell proliferation activity, and turned the cells into anomalously tough

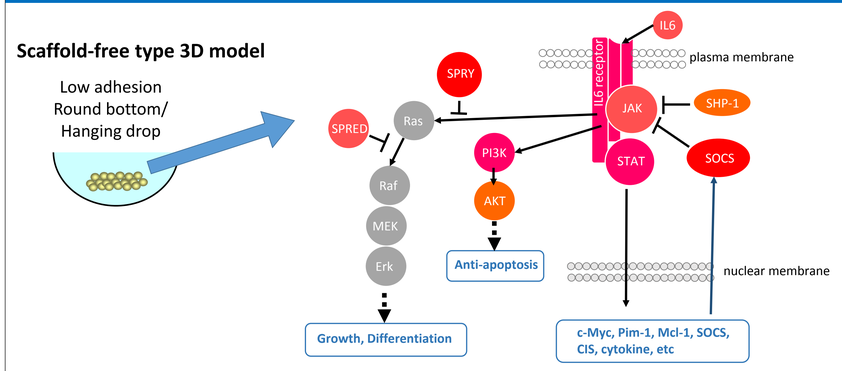


Figure 4. JAK-STAT, Ras-MAPK and PI3K-AKT signaling pathway in spheroid on scaffold-free.
JAK-STAT, Ras-MAPK and PI3K-AKT signaling pathway are upregulated in scaffold-free 3D model.
These results may raise some hypothesis that 1) cells proliferation are inhibited by upregulating SPRY and SPRED, 2) cells obtain high anti-apoptotic character due to PI3K up regulation, 3) negative feedback inhibition of JAK by upregulating SOCS causes a proliferation inhibition, and 4) finally, cells grown in scaffold-free type 3D model show chemo-resistance as compared with both scaffold type 3D model and cell line xenograft model.

Conclusion

- Spheroids cultured on NCP showed similar characteristic to spheroids cultured on Matrigel.
- Scaffold-free 3D culture condition exaggerated cellular character i.e. lower growth rate, chemo-resistance against clinical anti-cancer drugs, upregulating gene expressions which are relevant to inhibit proliferation and anti-apoptosis.
- In the drug screening assay, scaffold-free 3D culture models might show false-negative data due to the cellular character which is different from in vivo .
- Therefore It is obvious that NCP, scaffold type 3D model, is suitable 3D model for high-throughput drug screening

Reference

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