Drug sensitivity assessment between scaffold and scaffold-free 3D models: close to in vivo and suitable for HTS?

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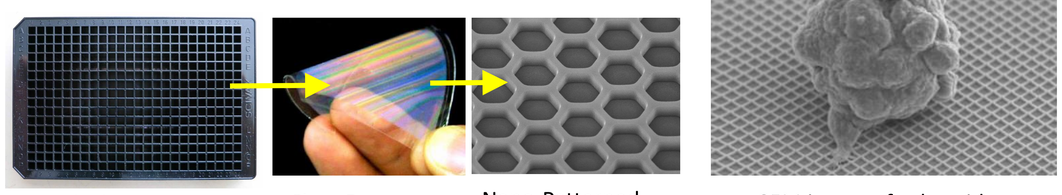
Background

Three-dimensional (3D) cell culture models are better models than two-dimensional (2D) cell culture models due to its enriched cellular signaling pathways, cell to-cell contact, more representative of tissue morphology, and projecting of drug responses in vivo. Diverse drugs screening and several biological responses between 2D and 3D cell culture models have been reported. However, very little information is available on cell function and/or drug susceptibility caused by a difference in methodology of three dimensional culture. Here, we compared drug responses of different cancer cells against distinctive anti-cancer drugs when grown in monolayer, scaffold 3D culture (Matrigel, NanoCulture Plate (NCP)) or scaffold-free 3D culture models (ultra-low attachment round bottom plate), and also confirmed which method is suitable for high-throughput screening for robust three-dimensional screening model.

Nanoclture Plate and Categorizing of 3D cell culture methods

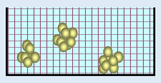
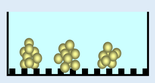
NanoCulture Plate



Black Cases | Clear Bottom Film | Nano-Patterned Surface

SEM image of spheroid on micro-structures

Table 1. Categorizing 3D cell culture methods

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

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

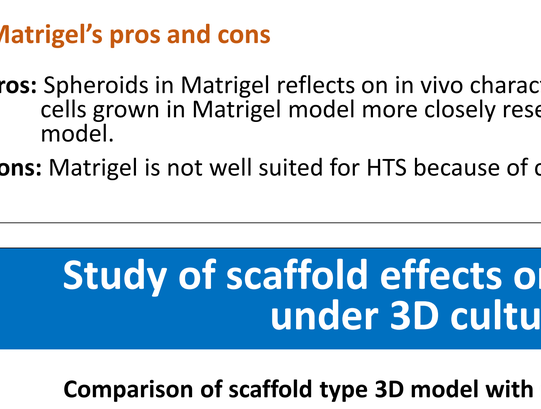
Comparison of scaffold type 3D model with scaffold-free type 3D model in-

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

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

Result 1: Cell proliferation rate on scaffold type 3D model is higher than on scaffold-free type 3D model

A

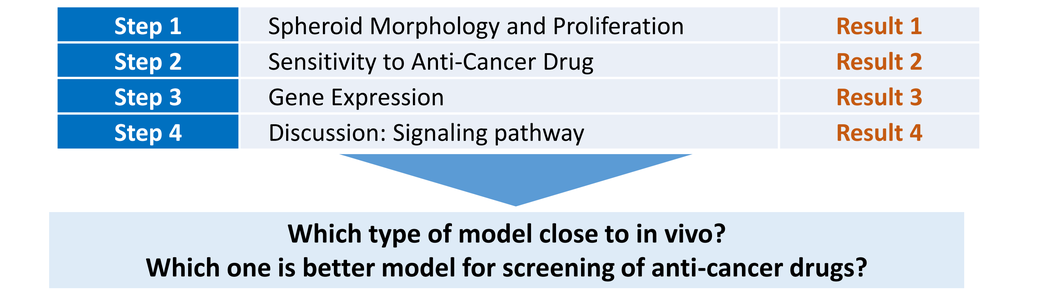


Spheroid character

Size
Matrigel ≒ NCP, Low-Flat ≠ Low-Round

Number
Matrigel ≒ NCP > Low-Flat > Low-Round (only one)

B



Result 2: Scaffolds in scaffold-free type model showed less drug sensitivity

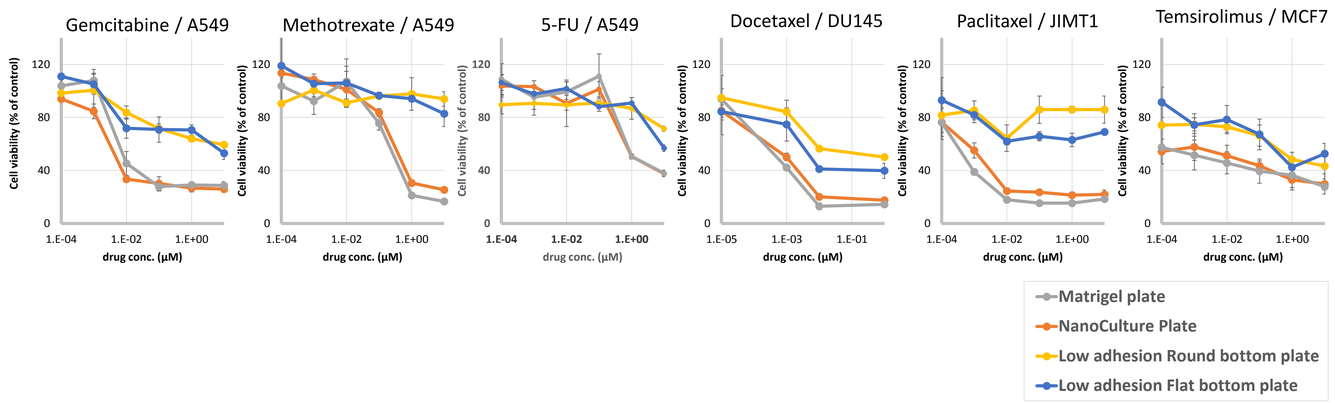


Table 2. IC50 of anti-cancer drugs in scaffold and scaffold-free type 3D model

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

1. Anticancer Drugs. 2004 Jun;15(5):503-12.
2. Biochem Pharmacol. 2011 Feb 15;81(4):510-7
3. BMC Cancer. 2010 May 18;10:211
4. Interview form
5. Mol Cancer Ther. 2014 Apr;13(4):890-901
6. Br J Cancer. 2011 Nov 8;105(10):1554-62
7. Br J Cancer. 2011 Nov 8;105(10):1554-62
8. Cancer Res. 2001 Dec 15;61(24):8887-95.
9. Cancer Biol Ther. 2011 Mar 1;11(5):524-34.

Result 3: The gene expression of spheroid in NCP most closely is similar to Matrigel models. Scaffold-free type is not.

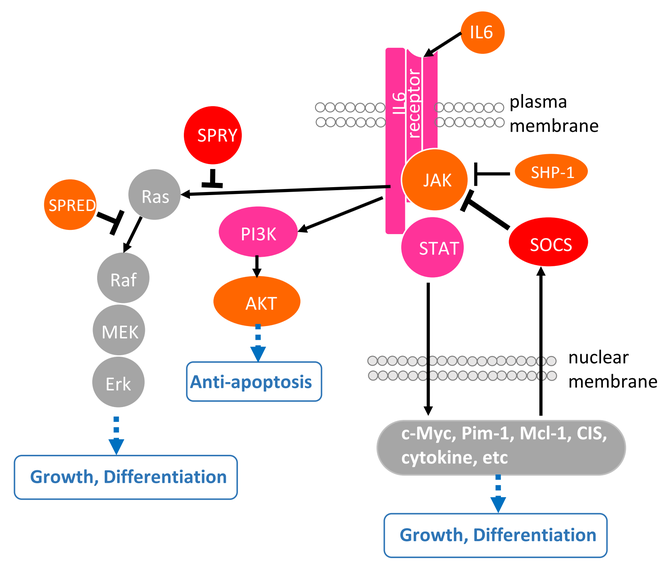
Table 3. Change of gene expression pattern as compared with Matrigel (fold change)

Signaling pathway		Gene	NCP	Low adhesion Round bottom
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
	SPRY	SPRY1	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	Gene AKT3	1.3	2.2

Result 4: Scaffold-free 3D culture condition may inhibit cell proliferation activity, and turned the cells into anomalously tough

Scaffold-free type 3D model

Low adhesion Round bottom/ Hanging drop



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