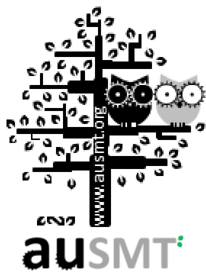

Microfluidic Tumor-mimicking In Vitro Cell Culture Methods

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(**Received** 10 November 2016; **Accepted** 22 November 2016; **Published on line** 1 December 2016)
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DOI: 10.5875/ausmt.v6i4.1314

Abstract: In vitro cell culture models are extensively used for cancer research and cancer drug development. Unlike traditional cell culture methods which can only create simple cell culture conditions, microfluidics allow for the creations of more complex in-vitro cell culture models to mimic tumor microenvironments. This mini-review highlights some of the most promising applications of microfluidic techniques in creating tumor-mimicking in-vitro cell culture models for cancer research.

Keywords: microfluidics, cell culture, cancer

Introduction

Cancer is one of the leading causes of death, leading to nearly 600,000 deaths in the US alone in 2016.[1] Despite significant advances in cancer drug research, only 8% of selected in-vitro drug candidates ever enter Phase I clinical trials. The high failure rates of cancer drug discovery is partially due to the fact that the outcome of pre-clinical in-vitro drug testing cannot accurately predict the drug's in-vivo efficacy.[2]

Conventional cell culture techniques are based on growing a monolayer of cells on a rigid plastic substrate in petri dishes or well plates, as shown in Fig. 1a. This 2D culture method is simple to perform, but cell morphology, gene/protein expression, and behavior may differ in vivo, resulting in biased experimental results.[2] This flat culture model is being gradually replaced with 3D cell culture models [3] to better mimic cell microenvironments in vivo.[4] There are two general formats of 3D cell culture molds: 1) cells are embedded in a hydrogel to mimic physical environment and cell-ECM (extracellular matrix) interaction (Fig. 1b),[5] and, 2) cells are aggregated into a spheroid using a suspension culture of cells in a low attachment well, hydrogel or hanging drop (Fig.1c).[6] In addition to 3D culture formatting, co-culturing of different cell types to simulate cell-cell interaction has also been used to mimic tumor environments containing cancer cells, other supporting cells (e.g., stromal cells and fibrblasts) and immune cells (e.g., macrophages and lymphocytes). Cell co-culturing can also provide experimental conditions allowing for investigation of cell-secreted soluble factors and direct cell-cell contact. The conventional transwell assay is the most widely used method for cell co-culture experiments. In a transwell assay, a porous membrane is used to separate the well space into top and bottom portions, where the two cultured cell types can share the same medium containing molecules released from the cells (Fig. 1d). [7] The transwell assay is also used for cancer cell migration and invasion tests.[8] Overall, these conventional cell culture tools are readily available and simple to use, but are not particularly well suited for creating more complex experimental conditions to mimic in vivo tumor microenvironments. This review will focus on introducing microfluidic-based cell culture strategies which aim to

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provide more tumor-like in-vitro models for cancer studies.

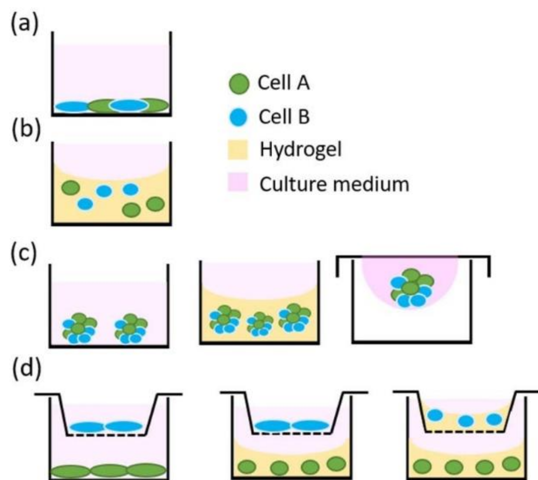

Figure 1. Conventional cell culture and co-culture methods. (a) 2D co-culture model. (b)3D co-culture in hydrogel model. (c) 3D cell spheroid formation by low attachment surface (left), hydrogel (middle), and hanging drop (right) methods. (d) 2D-2D (left), 2D-3D (middle) and 3D-3D (right) co-culture by transwell method.

Microfluidic devices

In recent years, microfluidic technologies have emerged as powerful tools for biological research by allowing for in-vitro cell experiments under precisely controlled conditions. In microfluidic systems, micro-scale channels can be generated in various geometries and along with cell stimulating perfusions to mimic complex in-vivo cell environments. Due to their small sizes, microfluidic systems require smaller sample and reagent volume, and provide high-throughput and easy observation during experiments. For cancer research, the use of microfluidic technologies has driven the development tof a new class of approaches to mimic tumor microenvironments and invasions, and for in vitro metastasis analysis for drug discovery. The use of microfluidic devices for tumor-mimicking cell cultures in cancer research are described below.

Cells embedded in gel

These cell models require the use of natural or synthetic gels as a scaffold. Depending on the composition, the gels can have different chemical/physic properties for simulating the tumor microenvironment for cell-ECM interaction.[9] Li et al. developed a micro-scaffold array chip on which hepatocellular carcinoma and non-small lung cancer cell were embedded in a collagen gel for anti-cancer drug cytotoxicitity testing.[10] They showed that cells embedded in ECM were more resistant to cancer drugs than cells in a 2D monolayer culture. Xu et al. developed another 3D cell culture platform (Fig. 2a) integrated with a concentration gradient generator (CGG) and used to co-culture lung cancer and stromal cells-BME (cell–basement membrane extract) cells to compare the drug sensitivity of co-cultured and mono-culture cancer cells. The co-cultured lung cancer cells showed lower rates of apoptosis, indicating that the co-cultured stromal cells increased the drug resistance of the lung cancer cells.[11] They also used primary cells from lung cancer patients for individualized treatment testing, highlighting the benefit of using a miniaturized device for handling scarce samples (e.g., tumor biopsies) and valuable reagents. The ability of microfluidic devices to precisely manipulate small numbers of cells was also applied to study cancer stem-like cells in a 3D collagen gel culture model (Fig. 2b). In this study, single TA7D breast cancer cells were encapsulated under different conditions in a gel-island by a vacuum-assisted operation method to study proliferation rates. It was found that the Notch+ (stem-like) cells were more drug resistant.[12] Microfluidic devices can use micro-structures to precisely control the spatial locations of cells. Huang et al. used a microfluidic device with a post array to load multiple ECM (collagen and matrigel) with breast cancer cells or macrophages to different channels to create a parallel and continuous cell migration testing platform. In their device, they observed that the macrophages proliferated and invaded the adjacent cancer cell area, indicating that the breast cancer cells can secrete cytokines and soluble factors which were diffused through hydrogel into the adjacent area to induce macrophage migration.[13] Liu et al. fabricated a microchannel device filled with hydrogel to culture and study the interaction of fibroblasts and cancer cells (Fig. 2c). They found that the invasion of salivary gland adenoid cystic carcinoma (ACC) cells could be induced by the co-cultured carcinoma-associated fibroblasts, but not the established fibroblast cells.[14] Mi et al. co-cultured MDA-MB-231 breast cancer cells and HMEpiC normal breast cells in a gel-containing microchannel array device (Fig. 2d) for metastasis studies and anti-metastatic drug screening on cancer cells with different degrees of aggressiveness (mild, moderate, severe).[15] Using a microfluidic device, it is possible to co-culture more than two types of cells at the same time. Liu et al. simulated a bladder cancer microenvironment by co-culturing four cell types (fibroblasts, bladder cancer cells, macrophages and endothelial cells) in 3D matrigel. In their device, the chambers that contain the four different cell types were connected by microchannels (Fig. 2e). It was observed that the macrophages were activated (shown by their Arg-1 marker expression) and invaded the adjacent chamber of cancer cells.[16] The above studies highlight the capability of microfluidic techniques to precisely control the spatial arrangement of cells in in-vitro cancer cell

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experiments.

Moreover, microfluidic techniques are also a powerful tool for manipulating the properties of gels to mimic the physical/chemical properties of ECM in tumors. For example, Pedron et al. used microchannels and diffusive mixing to create a gel with a stiffness gradient to culture brain tumor cells (Fig. 3a). They observed that when cultured in the stiffer region of the gel, the brain tumor cells had a greater spread morphology and an increased proliferation rate compared to when they were cultured in the soft region. [17] Microfluidic techniques can be used to control the spatial and temporal distributions of biomolecules secreted from cells cultured in a gel. Truong et al. used a microfluidic device to culture cancer cells which were exposed to an epidermal growth factor (EGF) concentration gradient environment in gel (Fig. 3b). The results showed that the cancer cells migrated toward the EGF source region. [18]

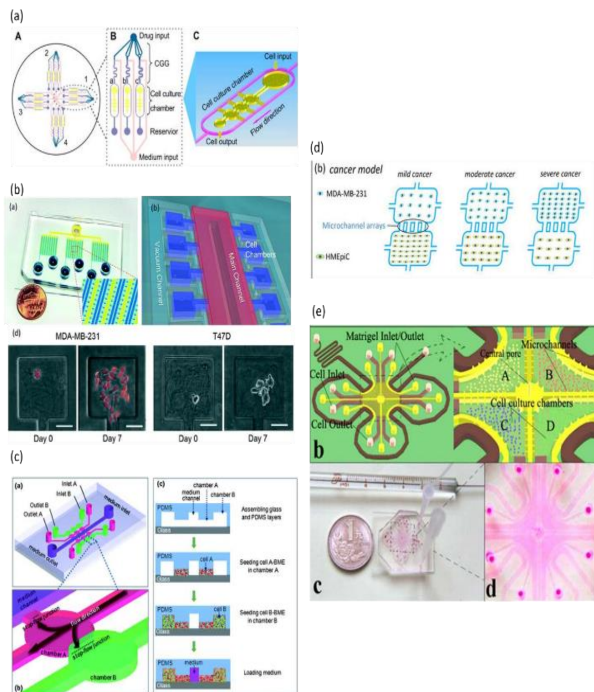

Figure 2.Cultured cells embedded in gel in microfluidic devices. (a) 3D cell culture device with integrated concentration gradient generator (CGG) for anti-cancer drug sensitivity testing. (b) 3D single cancer cell culture in a device containing gel islands. Interconnecting cell-containing gel in microchannels for cancer cell co-culture and invasion studies. (d) Micro-chambers connected by microchannels to study the effect of paracrines on cancer cells. (e) A microfluidic chip capable of co-culturing four cell types to mimic bladder tumor for cancer studies.

Cell spheroids

Tumor sphere models are widely used for cancer research studies, most of which focused on drug screening[19, 20] and cancer stem-like cell research.[21] There are two major methods for culturing spheres in microfluidic chips: 1) culturing pre-formed spheroids and, 2) culturing spheroids from single cells in microfluidic devices.[22-25] As shown in Fig.4a, Ruppen et al. developed a spheroid trapping device which used flow resistance difference to trap individual cancer spheroids in a microchannel for chemodrug resistance testing.[26] The same group used a microfluidic channel device to form cell spheroids from single cells (Fig.4b). They compared the chemosensitivity on primary lung adenocarcinoma epithelial cells (PLETCs)-formed spheroids to that of spheroids formed from PLETCs and primary pericytes (PCs) co-culture. Their results showed that the presence of pericytes increases the resistance of PLETCs to drug toxicity.[27] Patra et al. injected a cancer cell suspension into a microfluidic device to generate cancer cell spheroids of different sizes, and demonstrated that spheroid size affected drug treatment response.[28] McMillan et al. developed another microfluidics-based method to generate glioma spheroids in droplets in microchannels and used the resulting spheroids for testing chemo- and radio-treatments. Their method was based on using a T-junction microchannel design to produce cell containing aqueous droplets in oil, each of which was individually trapped in a specific location of the microchannel. After one-day culture, the encapsulated cells aggregated and successfully formed spheroids (Fig. 4c).[29] Chen et al. developed a high-throughput microfluidic chip for growing multiple cancer spheroids from a single cancer stem-like cell. They found that the spheroids formed from Notch+ stem-like cells were larger than those formed from Notch- cells. They also found heterogeneities in gene expression among these single cell-derived tumor spheroids. [30]

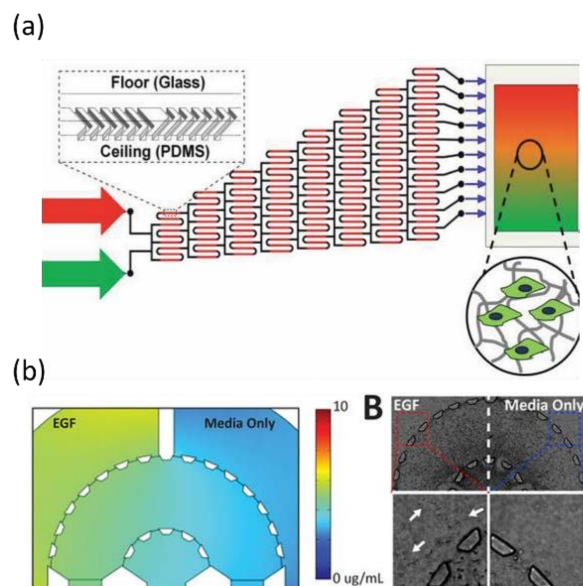

Figure 3. Microfluidic device for creating gels with physical and chemical gradients. (a) Gel with stiffness gradient for studying brain tumor cell invasiveness. (b) Gel containing EGF gradient for studying breast cell migration.

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In addition to forming cancer spheroids, microfluidic techniques can also be used to manipulate spheroids for different applications. As shown Fig. 5a, Chen et al. created a co-culturing device which allowed for simultaneous co-culturing of both suspended and adherent cell types. The co-culture was demonstrated with T47D breast cancer cells and primary cancer associated fibroblasts (CAF) on chip for 14 days.[31] Kim et al. developed a microfluidic device which used a gravity-driven flow to culture pre-formed spherical microtissues under continuous perfusion. To test the chip, rat liver and colorectal tumor microtissues were cultured in interconnected chambers on the chip in the presence of the pro-drug cyclophosphamide to test the impact of liver cells on the tumor microtissues' growth (Fig.5b).[32] A hanging drop platform was also developed for liver and tumor spheroids co-cultured under a continuous perfusion flow (Fig. 5c).[33]

Challenge and conclusion

In summary, microfluidic cell culture techniques are advantageous because they use smaller sample and reagent volumes, can precisely handle cells and fluids and can create more complex experimental conditions to

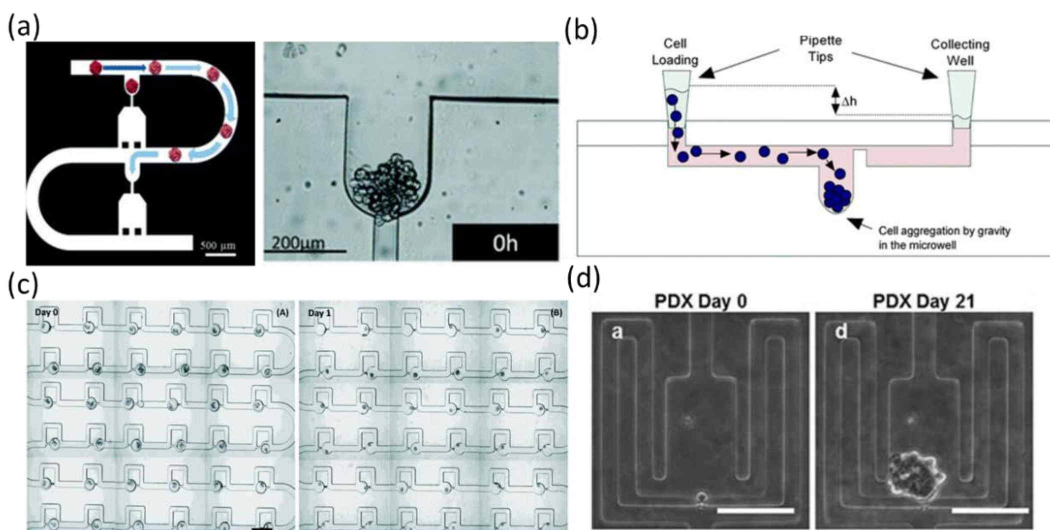

Figure 4. Tumor spheroids formation in microfluidic device. (a) Trapping of pre-formed spheroids in microchannel. (b) Forming spheroids in the micro-well using gravity to gather individual cells in suspension. (c) Forming an array of spheroids encapsulated in medium droplets in oil. (d) Forming single cancer stem-like cell-derived spheroids in a microfluidic device.

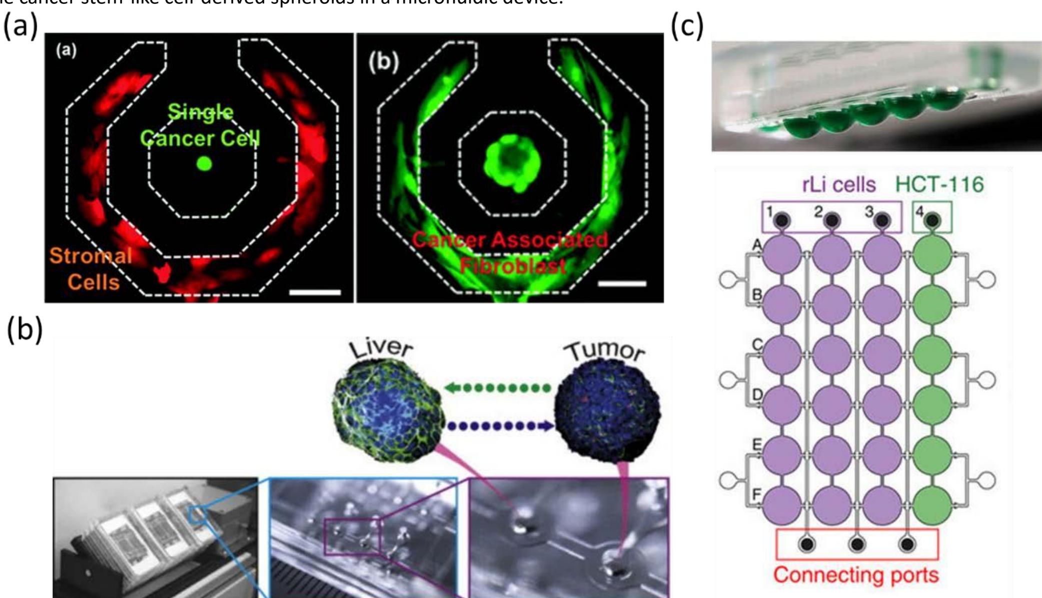

Figure 5. Co-cultures of tumor spheroids and other cell types in microfluidic devices.(a) 2D adherent cells and 3D cell spheroid co-culture for cancer stem-like cell studies. (b) Drug testing on off-chip preformed micro-tissues in a microfluidic device. (c) A microfluidic hanging drop device for culturing spheroids of cancer and liver cells for cancer drug testing.

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mimic tumor microenvironments. This mini review highlights the state-of-the-art uses of microfluidic techniques for tumor-mimicking cell culture experiments. However, the use of microfluidic devices is still limited due to the difficulty of analyzing cells on-chip using non-optical-based methods. Therefore it is necessary to develop cell detection components and integrate them onto the cell culture devices for on-chip cell analysis, or to develop micro-to-macro interfaces to allow for easy cell retrieval from the microfluidic devices for analysis. This will unleash the full potential of microfluidic technologies in tumor cell culture models

Acknowledgement

This work was supported by a grant from the Ministry of Science and Technology (105-2628-E-400 -001 -MY2).

Reference

[1] R. L. Siegel, K. D. Miller, and A. Jemal, "Cancer statistics, 2016," *CA: a cancer journal for clinicians*, vol. 66, no.1, pp. 7-30, 2016.
doi: 10.3322/caac.21332

[2] F. Pampaloni, E. G. Reynaud, and E. H. Stelzer, "The third dimension bridges the gap between cell culture and live tissue," *Nature reviews Molecular cell biology,* vol. 8, no. 10, pp. 839-845, 2007.
doi: 10.1038/nrm2236

[3] R. Edmondson, J. J. Broglie, A. F. Adcock, and L. Yang, "Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors," *Assay and drug development technologies*, vol. 12, no. 4, pp. 207-218, 2014.
doi: 10.1089%2Fadt.2014.573

[4] D. F. Quail and J. A. Joyce, "Microenvironmental regulation of tumor progression and metastasis," *Nature medicine,* vol. 19, no. 11, pp. 1423-1437, 2013.
doi: 10.1038%2Fnm.3394

[5] S. R. Caliari and J. A. Burdick, "A practical guide to hydrogels for cell culture," *Nature methods*, vol. 13, no. 5, pp. 405-414, 2016.
doi: 10.1038/nmeth.3839

[6] L. B. Weiswald, D. Bellet, and V. Dangles-Marie, "Spherical cancer models in tumor biology," *Neoplasia,* vol. 17, no. 1, pp. 1-15, 2015.
doi: 10.1016%2Fj.neo.2014.12.004

[7] L. Goers, P. Freemont, and K. M. Polizzi, "Co-culture systems and technologies: taking synthetic biology to the next level," *Journal of The Royal Society Interface*, vol. 11, no. 96, p. 20140065, 2014.
doi: 10.1098/rsif.2014.0065

[8] N. Kramer, A. Walzl, C. Unger, M. Rosner, G. Krupitza, M. Hengstschläger, et al., "In vitro cell migration and invasion assays," *Mutation Research/Reviews in Mutation Research*, vol. 752, no. 1, pp. 10-24, 2013.
doi: 10.1016/j.mrrev.2012.08.001

[9] M. W. Tibbitt and K. S. Anseth, "Hydrogels as extracellular matrix mimics for 3D cell culture," *Biotechnology and bioengineering*, vol. 103, no. 4, pp. 655-663, 2009.
doi: 10.1002%2Fbit.22361

[10] X. Li, X. Zhang, S. Zhao, J. Wang, G. Liu, and Y. Du, "Micro-scaffold array chip for upgrading cell-based high-throughput drug testing to 3D using benchtop equipment," *Lab on a Chip*, vol. 14, no. 3, pp. 471-481, 2014.
doi: 10.1039/C3LC51103K

[11] Z. Xu, Y. Gao, Y. Hao, E. Li, Y. Wang, J. Zhang, *et al.*, "Application of a microfluidic chip-based 3D co-culture to test drug sensitivity for individualized treatment of lung cancer," *Biomaterials,* vol. 34, no. 16, pp. 4109-4117, 2013.
doi: 10.1016/j.biomaterials.2013.02.045

[12] Z. Zhang, Y.-C. Chen, Y.-H. Cheng, Y. Luan, and E. Yoon, "Microfluidics 3D gel-island chip for single cell isolation and lineage-dependent drug responses study," *Lab on a Chip*, vol. 16, no. 13, pp. 2504-2512, 2016.
doi: 10.1039/C6LC00081A

[13] C.-P. Huang, J. Lu, H. Seon, A.-P. Lee, L. A. Flanagan, H.-Y. Kim, *et al.*, "Engineering microscale cellular niches for three-dimensional multicellular co-cultures," *Lab on a Chip,* vol. 9, no. 12, pp. 1740-1748, 2009.
doi: 10.1039/B818401A

[14] T. Liu, B. Lin, and J. Qin, "Carcinoma-associated fibroblasts promoted tumor spheroid invasion on a microfluidic 3D co-culture device," *Lab on a Chip*, vol. 10, no. 13, pp. 1671-1677, 2010.
doi: 10.1039/c000022a

[15] S. Mi, Z. Du, Y. Xu, Z. Wu, X. Qian, M. Zhang, et al., "Microfluidic co-culture system for cancer migratory analysis and anti-metastatic drugs screening," *Scientific Reports*, vol. 6, Article number: 35544, 2016.
doi: 10.1038/srep35544

[16] P.-F. Liu, Y.-W. Cao, S.-D. Zhang, Y. Zhao, X.-G. Liu, H.-Q. Shi, et al., "A bladder cancer microenvironment simulation system based on a microfluidic co-culture model," *Oncotarget*, vol. 6, no. 35, p. 37695, 2015.
doi: 10.18632/oncotarget.6070

[17] S. Pedron, E. Becka, and B. A. Harley, "Spatially gradated hydrogel platform as a 3D engineered

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tumor microenvironment," *Advanced Materials,* vol. 27, no. 9, pp. 1567-1572, 2015.
doi: 10.1002/adma.201404896

[18] D. Truong, J. Puleo, A. Llave, G. Mouneimne, R. D. Kamm, and M. Nikkhah, "Breast Cancer Cell Invasion into a Three Dimensional Tumor-Stroma Microenvironment," *Scientific Reports,* vol. 6, no. 34094, 2016.
doi: 10.1038/srep34094

[19] C.-H. Lee, C.-C. Yu, B.-Y. Wang, and W.-W. Chang, "Tumorsphere as an effective in vitro platform for screening anticancer stem cell drugs," *Oncotarget,* vol. 7, no. 2, pp. 1215-1226, 2016.
doi: 10.18632/oncotarget.6261

[20] F. Hirschhaeuser, H. Menne, C. Dittfeld, J. West, W. Mueller-Klieser, and L. A. Kunz-Schughart, "Multicellular tumor spheroids: An underestimated tool is catching up again," *Journal of Biotechnology,* vol. 148, no. 1, pp. 3-15, 2010.
doi: 10.1016/j.jbiotec.2010.01.012

[21] Y. Zhang, B. Sun, X. Zhao, H. Sun, W. Cui, Z. Liu, *et al.*, "Spheres derived from the human SN12C renal cell carcinoma cell line are enriched in tumor initiating cells," *Journal of Experimental and Clinical Cancer Research,* vol. 35, no. 1, p. 163, 2016.
doi: 10.1186/s13046-016-0442-8

[22] K. Kwapiszewska, A. Michalczuk, M. Rybka, R. Kwapiszewski, and Z. Brzózka, "A microfluidic-based platform for tumour spheroid culture, monitoring and drug screening," *Lab on a Chip,* vol. 14, no. 12, pp. 2096-2104, 2014.
doi: 10.1039/c4lc00291a

[23] Y.-C. Chen, X. Lou, Z. Zhang, P. Ingram, and E. Yoon, "High-throughput cancer cell sphere formation for characterizing the efficacy of photo dynamic therapy in 3D cell cultures," *Scientific reports,* vol. 5, no. 12715, 2015.
doi: 10.1038%2Fsrep12175

[24] A. Y. Hsiao, Y. S. Torisawa, Y.-C. Tung, S. Sud, R. S. Taichman, K. J. Pienta, *et al.*, "Microfluidic system for formation of PC-3 prostate cancer co-culture spheroids," *Biomaterials,* vol. 30, no. 16, pp. 3020-3027, 2009.
doi: 10.1016/j.biomaterials.2009.02.047

[25] Y. Fan, D. T. Nguyen, Y. Akay, F. Xu, and M. Akay, "Engineering a Brain Cancer Chip for High-throughput Drug Screening," *Scientific reports,* vol. 6, no. 25062, 2016.
doi: 10.1038/srep25062

[26] J. Ruppen, L. Cortes-Dericks, E. Marconi, G. Karoubi, R. A. Schmid, R. Peng, *et al.*, "A microfluidic platform for chemoresistive testing of multicellular pleural cancer spheroids," *Lab on a Chip,* vol. 14, no. 6, pp. 1198-1205, 2014.
doi: 10.1039/c3lc51093j

[27] J. Ruppen, F. D. Wildhaber, C. Strub, S. R. Hall, R. A. Schmid, T. Geiser, et al., "Towards personalized medicine: chemosensitivity assays of patient lung cancer cell spheroids in a perfused microfluidic platform," *Lab on a Chip*, vol. 15, no. 14, pp. 3076-3085, 2015.
doi: 10.1039/C5LC00454C

[28] B. Patra, C.-C. Peng, W.-H. Liao, C.-H. Lee, and Y.-C. Tung, "Drug testing and flow cytometry analysis on a large number of uniform sized tumor spheroids using a microfluidic device," *Scientific reports,* vol. 6, no. 21061, 2016.

[29] K. S. McMillan, M. Boyd, and M. Zagnoni, "Transitioning from multi-phase to single-phase microfluidics for long-term culture and treatment of multicellular spheroids," *Lab on a Chip,* vol. 16, no.18, pp. 3548-3557, 2016.
doi: 10.1038%2Fsrep21061

[30] Y.-C. Chen, P. N. Ingram, S. Fouladdel, S. P. McDermott, E. Azizi, M. S. Wicha, et al., "High-Throughput Single-Cell Derived Sphere Formation for Cancer Stem-Like Cell Identification and Analysis," *Scientific reports*, vol. 6, no. 27301, 2016.
doi: 10.1038/srep27301

[31] Y.-C. Chen, Z. Zhang, S. Fouladdel, Y. Deol, P. N. Ingram, S. P. McDermott, et al., "Single cell dual adherent-suspension co-culture micro-environment for studying tumor–stromal interactions with functionally selected cancer stem-like cells," *Lab on a Chip*, vol. 16, no. 15, pp. 2935-2945, 2016.
doi: 10.1039/c6lc00062b

[32] J.-Y. Kim, D. A. Fluri, R. Marchan, K. Boonen, S. Mohanty, P. Singh, *et al.*, "3D spherical microtissues and microfluidic technology for multi-tissue experiments and analysis," *Journal of biotechnology,* vol. 205, pp. 24-35, 2015.
doi: 10.1016/j.jbiotec.2015.01.003

[33] O. Frey, P. M. Misun, D. A. Fluri, J. G. Hengstler, and A. Hierlemann, "Reconfigurable microfluidic hanging drop network for multi-tissue interaction and analysis," *Nature communications,* vol. 5, no.4025, 2014.
doi: 10.1038/ncomms5250

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