

Mini Review

The Relevance of Using 3D Cell Culture, in Addition to 2D Monolayer Culture: The New Frontiers *In vitro* Models for Stem Cells and Cancer Drug Discovery

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Abstract

While two-dimensional (2D) cell culture is still a dominant method in many biological studies, three-dimensional (3D) culture systems have gained significant attention in stem cell, cancer research, and tissue engineering. For the past few years, numerous examinations have shown the 3D cell culture advantages in offering more physiologically relevant data for *in vivo* trials compare to results collecting from 2D cultures. In this review, we discuss the challenges in developing an accurate *in vitro* model that mimics the complexity of living tissues in order to recapitulate the phenotype of cells within a tissue and their interaction with their microenvironment. The evolution and progression of some of the 3D cell culture such as microfluidic chambers, organ-on-chips as well as rolls and their features are another focal points of this review.

ABBREVIATIONS

TRACER: Tumour Rolls for Analysis of Cellular Environment and Response; ECM: Extra Cellular Matrix

INTRODUCTION

Cancer is a genetic disease followed by a somatic invasion that is induced by multiple factors including genetic and epigenetic. The progress of such invasion is influenced by cell-cell or tissue-cell communication. As cancer cells population accumulate over time, they undergo profound changes, enabling them to survive within their microenvironment. These adaptations are characterized, for instance, changing the metabolic behaviour, the selection pressures exerted by therapeutic reagents and the development of metastases in the advanced stages [1]. Moreover, it is shown that only in the proper context of the microenvironment, and oncogenes induce malignant phenotype. Thus, tissue architecture has a profound effect on cell phenotype that even dominates the cellular genotype [2]. As a result, numerous studies have been investigating tumour-microenvironment interaction as a potential target for therapeutic intervention and understanding cancer development. Consequently, the challenge would be

developing an accurate *in vitro* model that mimics the complexity of living tissues to establish collective properties of cells within a tissue and their interaction with their microenvironment [1,2].

TWO-DIMENSIONAL (2D) CELL CULTURE

Traditional 2D cell culture is still the most established *in vitro* test platform in cell-based assays and drug screening. In this model, cells adhere on a scaffold such as glass or more commonly polystyrene plastic flasks and grow in a flat and stretched arrangement which permits all the cells receiving parallel amounts of nutrients and growth factors from the medium [3].

It is demonstrated when tumor cells are removed out of their natural niche, the cells accept their new *in vitro* environment by changing their physiology at the transcriptional and translational levels. The assessment of gene expression levels among cancer cell lines cultured in 2D *in vitro*, shows approximately 30% variance of gene expression compare to cancerous cells sited in their natural environment [3]. Comparing to corresponding tissue origins, while many genes responsible for proliferation are often upregulated, the expression of some other genes that limit the growth are repressed in 2D-adopted cell culture [3].

To this date, the standard procedure of drug compounds screening starts with 2D cell culture-based tests, followed by animal model tests to clinical trials. However, many of the clinical trials fail and it is suggested that the percentage of these failures is due to the data collected from the 2D monolayer culture tests which do not effectively mimic the *in vivo* microenvironment [3]. To improve cellular function and behavior in 2D cell culture, scientists apply technologies such as nano-patterning to bio-mimic the topographical features of the ECM. Nonetheless, the accuracy of results is still under investigation. As a result, more emphasis is being placed on 3D culture models with over 900 original publications now on PubMed [1-7].

THREE DIMENSIONAL (3D) CELL CULTURE

As opposed to 2D monolayer cell cultures, in 3D microenvironments, cells aggregate or spheroid on a scaffold or suspension medium. Spatial organization and cell morphology in 3D cultures mimic tumour natural shape and impact cell-cell interactions and cell-ECM interactions. 3D spheroids are embraced cells in numerous stages, including proliferating, quiescent, apoptotic, hypoxic, and necrotic cells [3]. Such cellular heterogeneity architecture resembles tumors in *in vivo* tissues in a form that the outer layers of the spheroid are highly exposed to the medium and mainly contained of viable and proliferating cells while the quiescent or hypoxic core cells receive less nutrient, oxygen and growth factors from the medium. As a result, the signal transduction, gene expression and cellular behavior in 3D spheroids resemble *in vivo* tumour-microenvironment composition [3].

For the past few years, numerous experiments show the importance of the bidirectional interactions of cancer cells with adjacent non-malignant cells [2,3-5]. As cancer treatments are directing toward targeted therapy, tremendous effort has been put into developing of 3D culture systems, recapitulating the *in vivo* microenvironments and optimizing of such systems in cell-based assays in drug discovery, cancer cell biology and stem cell study [1-5].

With recent advances in biotechnology and introduction of different methods of tissue engineering, microfluidic platforms have been introduced as a promising technique to recapitulate *in vivo* microenvironment in a 3D-culture method. Microfluidic systems deliver features including the ability to control the spatiotemporal distribution of cellular signals and analyzing cell differentiation and function. In addition, employing these systems requires fewer cells and smaller quantities of reagents as well as performing multiple assays at the same time. Moreover, microfluidic devices provide opportunities for high-resolution real-time imaging [4,6].

Organs-on-chips are microfluidic devices for culturing living cells in micrometer-sized compartments. The simplest model is a single microfluidic chamber containing one kind of cultured cell (e.g., hepatocytes or kidney tubular epithelial cells) and displays function of one tissue type. More complicated Organs-on-chip systems contain two or more microchannels, coupled by porous membranes, lined on opposite sides by different cell types to reconstruct the interfaces and interaction between different tissues (e.g., lung alveolar-capillary interface or blood-brain

barrier) [6]. By taking advantage of the ability to install several microenvironments on the same chip, several studies have developed cancer-on-chip experiments and show the impact of specific types of ECM on tumor-cell morphology and growth [6]. However, despite the successful demonstration of several studies that show organs-on-chips can mimic specific organ-level physiology, the field is still in its infancy and under investigation. For instance, timing wise, microfluidic chips are effective for examining physiological and disease developments that occur in a relatively short time frame (less than ~1 month). In addition, when applying analytical approaches (e.g., mass spectroscopy), conventional systems produce more tissue mass compare to microfluidic chips, which require larger experimental samples and thus easier to recover larger numbers of cells for analysis from larger systems [6].

In a recent experiment, Rodenhizer et al., have recapitulated the spatial characteristics of cell-cell interactions and tissue gradients using an engineered bio-composite sheet (strip) that mimic three-dimensional tumour [1]. The strip can be rolled and unrolled rapidly, allowing segregation of cells from specific tumour sites and perform spatial mapping of cellular metabolism. The engineered tumour rolls for analysis of cellular environment and response (TRACER) receive oxygen and nutrients from the medium only by diffusion from the culture medium adjacent to the TRACER [1]. Hence, the gradient of oxygen and nutrients is analogous to what is seen in tumour cellular composition with lower levels in each layer at gradually increasing distances from a blood vessel. After the desired culture time, TRACER would be unrolled rapidly into the thin strip for snap-freezing or fixing cells for snapshot analysis. Also, live cells can be recovered from different sections of the thin bio-composite strip for secondary analyses involving live cells [1]. In addition to assessment of metabolic adaptation of tumour cells, TRACER facilitates experiments in a wide spectrum of applications including drug screenings; understand the impact of new anti-hypoxic drugs on cancer cells and formulation of effective combination therapies.

As a step toward a more realistic *in vitro* assay, Aref et al., have developed and demonstrated a 3D microfluidic system, and accompanying image analysis process to characterize the statistics of anti-metastatic drug responses. A majority of cancers are of epithelial origin, and the progression of carcinoma has been hypothesized to involve epithelial-mesenchymal transition (EMT). EMT has also been implicated in the formation of tumor-initiating or cancer stem cells and in drug resistance. They have demonstrated a tumor microenvironment model based on a microfluidic device (Figure 1) capable of (1) recapitulating the physical and biochemical contexts that allow for the manifestation of EMT of cancer cells in 3D, in the presence of human endothelial cells; and (2) quantitatively monitoring the EMT inhibitory effect of drugs. Results confirm the importance of growing cells in 2D vs. 3D and that other cell types, in this case, endothelial cells, can significantly alter the levels of drug required to inhibit EMT (Figure 2), they also demonstrate the use of this system for obtaining a mechanistic understanding of cell signaling in early metastasis. These studies, therefore, offer a new approach in drug screening with the potential to better replicate the *in vivo* microenvironment [7].

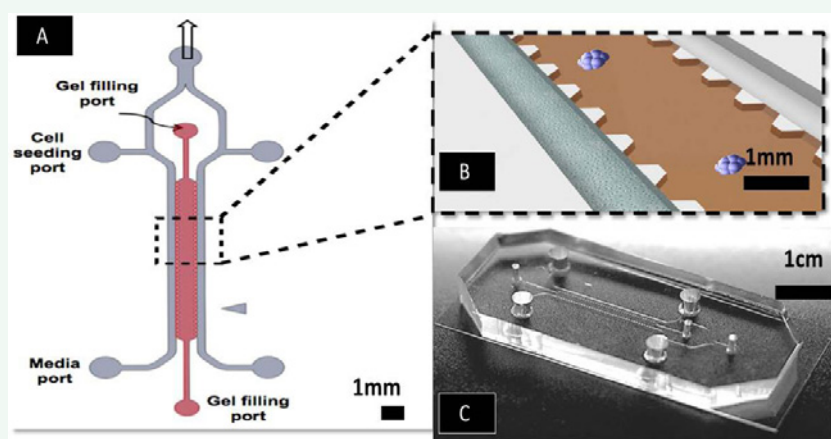


Figure 1 Schematic and photograph of a 3D co-culture microfluidic device. (A) Schematic diagram of device layout depicts the inlets for injecting cells, filling collagen, and replenishing medium. (B) Enlarged view of gel region and the HUVEC-lined channel. Cytokines in conditioned medium from the HUVEC monolayer diffuse into the gel region triggering spheroids to undergo EMT. (C) Photograph of the PDMS-molded device bonded on a glass.

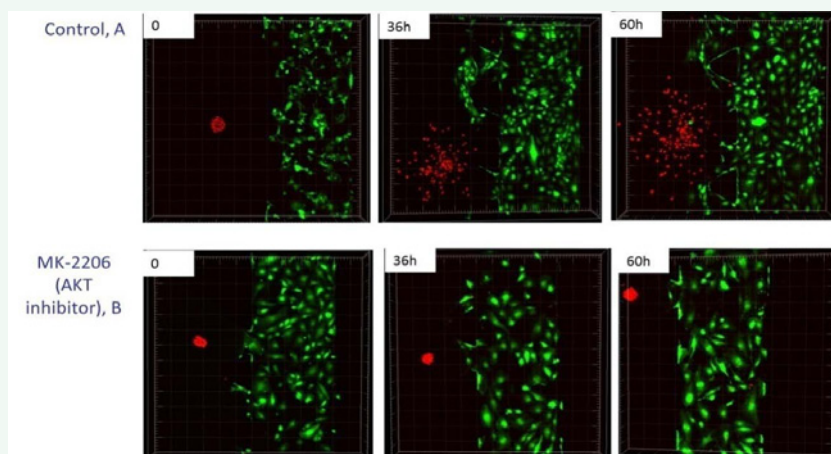


Figure 2 Fluorescent images in time-series showing A549 cell dissemination in the 3D collagen gel. (A) Control condition in the presence of a HUVEC monolayer in the side channel, i.e. 3D co-culture. (B) AKT-targeted drug (300 nM of MK-2206) applied in the presence of a HUVEC monolayer. Red: nuclei of A549 cells; green: HUVEC.

DISCUSSION

Numerous studies have shown the importance of the local environment architecture and signaling from neighboring cells to initiate, progress and drug resistance of cancer stem cells. Although both 2D and 3D cell cultures are practical for exploring cellular sensitivity to drugs, toxins and biomaterials, only 3D cultures display a hierarchical structure and cellular heterogeneity that can carefully mimic *in vivo* cell morphology and function (proliferation, differentiation, gene expression, etc.), cell-cell interactions and diffusion barriers. Hence, 3D models are the better candidate for replication of physiological conditions of *in vivo* cellular responses to external stimuli compared to the 2D monolayer [3]. Subsequently, in the past few years, huge variations of 3D cell culture systems have been designed for diverse research purposes. However, despite remarkable progress in engineering and developing the 3D cell culture models, there are still challenges that should be addressed

before these systems can be widely accepted in the industry. The maturity of the technology and the cost still remain as the main concerns in making this transition from 2D to 3D models. Much effort is still required to guarantee reproducibility, compatible readout techniques and data analysis in order to establish consistent and validated 3D cell culture models [3].

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