Scientific Research Publishing

Journal of Biosciences and Medicines, 2018, 6, 36-43
http://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

The 3D Cell Culture System in the Study of Tumor-Applications and Prospects

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How to cite this paper: Yang, H.Z. and Jiao, J.Q. (2018) The 3D Cell Culture System in the Study of Tumor-Applications and Prospects. *Journal of Biosciences and Medicines*, **6**, 36-43.
https://doi.org/10.4236/jbm.2018.69004

Received: August 14, 2018
Accepted: September 11, 2018
Published: September 14, 2018

Abstract

Compared with 2D tumor cell culture, 3D tumor cell culture can better simulate the microenvironment of signal transduction between cells and extracellular matrix. As one of the best cell models in tumor research, it has been widely used in the study of cancer cell morphology, nanotechnology drug delivery system, and anticancer drug screening. The main theme of this paper is to review the previous research of 3D cell culture applying to tumors *in vitro* and the prospects for the applications of 3D cell culture system.

Keywords

3D Cell Culture System, 2D Cell Culture System, *In Vitro* Tumors

1. Introduction

2-dimensional (2D) culture means a method of culturing tutors by building models outside the body in two dimensions. Regarding as the 3-dimensional (3D) culture, it is also a way to culture tissues but one more dimension than 2D culture. *In vitro*, tumoris anartificial tissue, is established outside of bodies as a model to culture *in vitro* tumors for better tumor research.

The previous work on the three-dimensional (3D) cell culture system has indicated that the morphology and function of isolated cells in 3D culture are closer to the real growth state of these cells *in vivo* comparing the traditional two-dimensional (2D) cell culture [1]. Due to the highly abnormal graphic and mechanical constraints compelled on cells in 2D cultures could alter cellular interactions with the extracellular matrices (ECM) and the signaling pathway; 2D cell cultures cannot simulate the cellular properties of normal tissues. In addition, 2D cell culture oversimplifies the various processes that Nano-medicine reaches its targeted site *in vivo*.

#Contributed equally, signature by the alphabetical order of names.

1.1. Features of 3D Cell Culture

Tumor cells are closer to the tumor microenvironment under 3D culture conditions, showing more characteristics of tumors in the body. The 3D culture of tumor cells can form a hollow tissue necrosis area similar to that of the tumor tissue in the body, which is often significantly different from the surrounding nutrient and oxygen supply areas [2]. More importantly, the growth of tumor cells in 3D culture is relatively slower than that of monolayer cells, and is therefore more representative of the physiological growth of tumor cells [3]. In addition, it has been found that tumor cells have obvious advantages in morphology, signal transduction, cell proliferation, growth and differentiation, and response to therapeutic stimulus under 3D conditions [3].

1.2. Advantages and Disadvantages of 3D Cell Culture

Shown in Table 1, by comparing the effects of 2D and 3D culture on some aspects of cell growth, it is clear that 2D culture has an inevitable limitation in application, especially in the study of microenvironment and cell interaction. As we all known, human beings live in a three-dimensional world. Human body tissues are also a complex 3D configuration. Various substances act to connect and transmit information, thereby regulating various functions of cells. 3D culture is also based on the above reasons, and has the advantage that 2D cannot be compared. In 2D cell culture, the microenvironment of monolayer growth is not sufficient to fully mimic the conditions of growth *in vivo*. The data obtained under the 2D model cannot explain the research very clearly, so in order to obtain the information in the 3D culture as much as possible, it is necessary to have enough samples to cultivate a large number of 3D culture models, and it is difficult to analyze the data.

Although 3D culture technology has many advantages, which are beyond 2D, classical 2D culture still occupies a large proportion in the *in vitro* culture mode. On the one hand, after more than 30 years of development and improvement of

Table 1. The comparison of 2-dimensional culture and 3-dimensional culture.

Cellular and microenvironmental features	2-dimensional culture	3-dimensional culture (advantages)
Cell morphology	Typically flattened and unconstrained spreading on the substrate	Depending on the adhesion ligands used, cells can be spherical, spindle shaped or exist as multicellular aggregates
Cytoskeletal structure	Display thick bundles of well-developed ventral stress fibers	Few thin stress fibers found near the cell cortex
Gene and protein expression	Similarities with *in vivo* counterparts are lacking	Generally more *in vivo*-like
Cell differentiation	Two-dimensional inducts cell differentiation	Three-dimensional space is closer to the intracellular microenvironment

2D culture, it has formed a standardized research mode. On the other hand, 3D is still a relatively new technology. Although 3D culture has been applied in some researches, it is more in the experimental stage. This phenomenon is due to some technical difficulties in the development of 3D culture technology.

1) It is difficult to establish 3D culture materials. Natural materials with suitable diameters often have some uncontrollable residues such as growth factors and unknown impurities, which will affect the final experimental data to varying degrees. At present, many materials are synthesized from known components. Some researchers have used synthetic materials to establish 3D cultures, such as Puramatrix [4], synthetic hydrogel system [5] etc. Applied in 3D culture studies, but there is still no standardized synthetic material. Therefore, it is important to develop suitable scaffold materials for constructing 3D culture.

2) Since 3D culture is one more layer than 2D culture, the amount of information is much larger than 2D culture, so when extracting a large amount of effective information, a higher level of technology such as high permeability and high resolution chemical or fluorescent labeling is required. At present, automated processes have been applied to laboratories in the inoculation of primary cells in 3D culture to improve research efficiency, which tackles these problems in some degree. So this is also one of the difficulties that the current research needs to overcome.

2. Application of 3D Cell Culture

My partner and I grabbed the main idea of the article, as shown in **Figure 1**. We searched all major databases for meaningful articles with the keywords "tumor" and "3D technology". Then we read all articles carefully to write articles.

As an effective means of drug and biological research, three-dimensional (3D) cell culture systems have received extensive attention. The main advantages of biological effects in 3D culture model for tumor research shows in three aspects.

2.1. Establishing an Ideal Research Tumor Model

The tumor microenvironment is vital for tumor cells to survive. It is well known

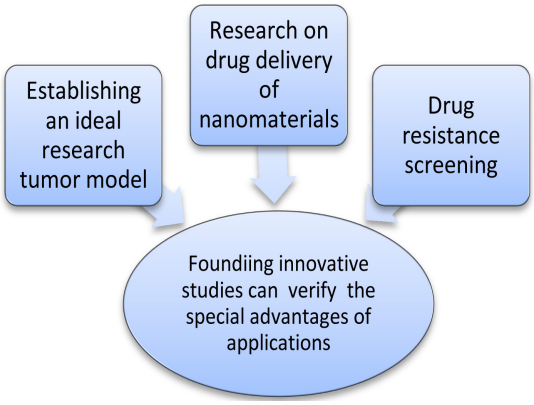

Figure 1. The choice of papers.

that it contains a series of complex mixtures, all kinds of cells and ECM such as oligopeptides and enzymes. There are some mutual stimulation and tumor cells. Although two-dimensional culture is still a reliable culture model reflecting cell proliferation and migration, there are still many deficiencies in the interaction between tumor cell growth, proliferation, metastasis and invasion. In addition, single-layer planar cultured tumor cells are rearranged through cell stents to obtain artificial cell polarity, resulting in abnormal gene and protein expression of tumor cells [6].

In contrast, when cells are cultured in 3D, due to their 360˚ surround interaction, their internal procedures are initiated to restore their former memories in their native tissue structure. Previous experiments by Sheff D have suggested when epithelial cells grow in a 3D cell culture model, they can "intuitively" reorganize themselves into apical and basal lateral orientations, which have characteristic columnar arrangements [7]. The cavity side of these epithelial cells can be arranged perpendicular to or parallel to gravity or any other direction between these two extremes, and the entire "tissue" structure is functionally notified. This is the reason why the results of the 3D culture experiment are closer to the microenvironment in the body. 3D cultured cells form dense tissue clusters and have spatial degrees of freedom to form more diverse cell orientations, partly due to they are not attached to a single planar substrate, and this freedom causes cells to reassemble lost natural interactions. Therefore, 3D cell culture as a model for *in vitro* study of tumors can simulate the microenvironment of tumors in the body and be used for the study of tumor cell behavior *in vivo* [8]. Studies have shown that the tumor microenvironment is related with malignant progression of breast cancer, Hyaluronan (HA), as the main part of the extracellular matrix, plays a critical role in tumor angiogenesis, invasion, and metastasis. Some scholars have established a 3D culture system through covalently cross-linked HA. This environment facilitates completely distinct cellular behavior compared to 2D culture systems. The malignancy and drug resistance of tumor cells and differentiation ability and expression of stemness genes is strongly enhanced when cultured in 3D scaffolds. It is very beneficial to study the role of HA with different molecular weights (MWs) on breast cancer cell invasion [9].

2.2. Research on Drug Delivery of Nanomaterials

At present, in the 2D environment, the use of a single *in vitro* cell to optimize the biological behavior in the body is quite limited; there have been many failures *in vivo* three-dimensional biological and physicochemical behavior by using *in vitro* cell assays. Therefore, the utilization of 3D cell culture systems for nano-medicine screening is expected to take on the role of the bridge to close up the gap between 2D cell culture work and the pre-clinical stages of animal works. Experiments have proved that nano-materials have the potential ability of 3D models for drug delivery, so it might be promising for the *in vitro* prediction of

3D behavior of drugs and drug carriers [10].

It's limited use of traditional 2D cell culture techniques. The cell culture method should use a supporting scaffold equivalent to the cytoplasmic matrix. Researchers demonstrate the advantages of novel nano-structured 3D grids fabricated using electro-spinning technique, as scaffolds for cultures of neoplastic cells. The results show that the fibers allow for a dynamic growth of HeLa cells, forming multi-layer structures of symmetrical and spherical character. And it is proved that grids can be used to examine the cell ultrastructure *in situ* [11].

2.3. Drug Resistance Screening

In the most recent years, 3D fine cell culture technology have shown incomparable advantage in the field of drug research and development, especially the evaluation of drug safety [12] and the study of new drug screening [13]. With the development of 3D culture technology *in vitro*, researchers have gradually realized that because cells form a 3D environment together with the ECM in the body, the planar biological characteristics of cells in 2D planar culture *in vitro* are significantly different from those in the body. So the experimental results are different from the actual results. *In vitro*, it is an inevitable trend to construct a 3D tumor model to substitute the traditional 2D tumor model for drug screening.

The most promising drugs do not have further clinical trials, in part because many 2D experiments fail to reflect the 3D interaction between solid tumor cells and the matrix [14]. Most scholars are currently working on establishing a universal 3D cancer culture and screening device to ensure a high degree of flexibility in selecting well-developed 3D culture methods and screening methods for any cell sample. Previous experiments by Miriam Widder, a modified 384-well-device for investigations of tumor biology was established, through higher reproducibility and the ability for high-throughput analyses, the screening and treatment of cancer cells have a good effect [15]. Now, the most popularly used 3D tumor model is a multicellular sphere. Using this model, the spherical diameter, cytotoxic effects, and drug penetration rates can be analyzed, and different anti-tumor drugs can be screened and studied [16]. Godugu [17] has constructed a bulging spherical sieve selection model based on alginate; though the analysis of the evaluation, cytotoxicity, apoptosis, and the infiltration of nanoparticles into tumor cells *in vitro*, the anti-swollen tumor drugs were selected for screening and research during the use of different materials.

3. Design and Results of Other Related Experiments

At present, some scholars have made various attempts to apply 3D technology, as shown in **Table 2**, which is of great reference significance for the development of our future medical industry.

4. Future Prospects

To sum up, 3D cell culture shows incomparable advantages in simulating tumor

Table 2. Other relevant results of the experimental.

Experimental model	Solved disease	Experimental material	The experimental results	Reference
3D human-tissue models	Atherosclerosis	Paclitaxel (PTX)	It suggested excellent biological characteristics and potential treatment effects of the nanoparticles optimized *in vitro*	[1]
3D model set up by crosslinking HA with alginate	Breast cancer	Hyaluronan (HA)	The invasion and migration abilities of 4T-1 and SKBR3 cells are significantly enhanced by the presence of HA35	[2]
nanostructured 3D grids fabricated using electro-spinning technique	Screening of new drugs	Electrospun scaffolds	The scaffolds are transparent and thus allow observation of viable cells in time; standard fluorescence or colorimetric methods such can be used for evaluation of cells	[6]
Inflamed mucosa model (colon)	Chronic IBD (colon)	Budesonide/ fluoreceinamine	Prolonged anti-inflammatory effect was observed for 4 days	[18]
3D skin model	Skin	Squalene	Superior defensive and anti-wrinkle actions of squalane loaded fullerene-C60 was reported	[19]

microenvironment, nanodrug delivery and drug screening. At present, a variety of bio-simulated 3D tumor models have been widely used, such as the 3D culture resistance model of breast cancer cells [20], the 3D culture model of OSCC-3 [21], the 3D skin model [22], and the 3D blood and brain barrier model *in vitro* [23].

However, it is still has many flaws. Many models still do not accurately reflect the pathological characteristics of the initial disease and some tumor models are only morphologically similar to native tumor tissues, lacking complex cell signals and complex biological behaviors. With the development of 3D cell culture technology, tumor models are becoming more and more complex. They urgently need to participate in multiple disciplines such as mathematical modeling, tumor genomics, and system biology. The researcher will gradually overcome the shortcomings of 3D models and show great potential for pre-clinical research advantages.

Fund Project

Innovation and Entrepreneurship Training Graduate Project (2018162).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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