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REVIEW

The application of three-dimensional cell culture in clinical medicine

Qiao Chen · Youbin Wang

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Abstract Three-dimensional cell culture technology is a novel cell culture technology, which can simulate the growth state of cells in vivo by scaffolds or special devices. Cells can form tissues or organs in vitro. It combines some advantages of traditional cell experiments and animal model experiments. Because of its advantages, it is widely used in clinical medical research, including research on stem cell differentiation, research on cell behavior, migration and invasion, study on microenvironment, study on drug sensitivity and radio-sensitivity of tumor cells, etc. In this paper, the evolution and classification of three-dimensional cell culture are reviewed, also the advantages and shortages are compared. The application of three-dimensional cell culture in clinical medicine are summarized to provide an insight into translational medicine.

Keywords In vivo · Three-dimensional cell cultures · Scaffold-based techniques · Scaffold-free techniques · Clinical application · Translational medicine

Q. Chen · Y. Wang (✉)
Department of Plastic Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Shuaifuyuan No 1, Dongcheng District, Beijing 100730, China
e-mail: wybenz@sina.com

Introduction

Three-dimensional (3D) cell culture is a novel cell culture method, which is applied to the researches of tumor cells and stem cells. The cells in vivo live in a microenvironment composed of adjacent tissue cells and the surrounding matrix. There are fibroblasts, immune cells, blood vessels and lymphatic vessels, and extracellular matrix in the surrounding matrix (Nazareth et al. 2007). Microenvironment is an important place for reaction, so its stability can ensure normal cell proliferation, differentiation, metabolism and functional activities (Behonick and Werb 2003). Cells can release cytokines into microenvironment through autocrine and paracrine to maintain survival conditions. Microenvironment, through the changes of metabolism, secretion, immunity, structure and function, conducts the changes of the environment in the whole body or in the distance, limiting and influencing the occurrence and development of regional cells (Mantovani et al. 2008). 3D culture technology is an in vitro culture method simulating the internal microenvironment. It constructs the external matrix skeleton and microenvironment of cell growth artificially, provides a culture condition similar to the internal environment, and enables cells to grow in a 3D way in vitro (Ovsianikov et al. 2012).

After the concept of three-dimensional cultivation technology was put forward, there were many related studies. One research direction is the selection and

creation of scaffold materials. Finding a scaffold material with good biocompatibility and convenient preparation will be a breakthrough. In the field of tissue engineering, scaffold manufacturing technology based on electrospinning and 3D printing has achieved significant progress in replacing damaged tissues and organs at the laboratory level (Mabrouk et al. 2020). Related researches on nutrient dissemination in a three-dimensional culture environment have also attracted much attention. Nutrients (such as glucose, protein) needed by the cells in the scaffold to maintain growth. Different from the distribution of nutrients in conventional liquid media (Suhaimi et al. 2015a, b), in general, the diffusion coefficient of glucose increases with the increase of the pore size of the material (Suhaimi et al. 2015a, b). Mastering the diffusion characteristics can predict the distribution of nutrients in the cultured tissues, facilitating deeper studies of metabolism (Suhaimi and Das 2016).

The mechanical response, extracellular matrix, movement and migration, proliferation, differentiation, and gene expression of cells in the three-dimensional culture system and the two-dimensional system are different (Souza et al. 2018). The study of the differences in biological characteristics between the three-dimensional culture and the two-dimensional culture is also necessary. It is generally accepted that these differences are cell line specific (Duval et al. 2017). For example, liver cells and neuronal cells cannot survive well under traditional 2D culture methods (Ardalani et al. 2019; D'Aiuto et al. 2019). The ability of mesenchymal stem cells (MSCs) to develop into neurons, myoblasts and osteoblasts can be changed according to the different culture conditions of the matrix (Haugh et al. 2018). Before carrying out relevant three-dimensional research, it is often necessary to verify that the cell has not changed the characteristics related to the research purpose due to the culture environment. Researchers need to choose a suitable cell culture method according to the purpose of the experiment, rather than just believing that three-dimensional culture is better.

From the perspective of clinicians, there is no systematic review of the application of 3D cell culture in clinical medicine. This article focuses on the application of three-dimensional culture in stem cell differentiation-related research, tumor cell behavior research, migration and invasion, tumor microenvironment research, tumor cell drug sensitivity research, and tumor cell radiosensitivity research in recent years, and deepen researchers' understanding of three-dimensional cells. This research can provide ideas for technology applications and translational medicine.

Advantages of three-dimensional culture over conventional technology

In the traditional two-dimensional culture technology, cells need to rely on the mechanical support of the bottom of the culture dish, then grow into monolayer cells attached to the bottom. Cells get even nutrition and oxygen in the surrounding environment, and finally achieve even and homogeneous growth (Duval et al. 2017). Under 3D culture conditions, cells show more real distribution and access to oxygen and nutrients, and the interaction between cells can also be reflected in the culture environment (Kapalczynska et al. 2018). Compared with the traditional two-dimensional culture technology, 3D culture technology can not only retain the shape of cells in vivo, but also reflect the intuitive and controllable conditions of cell culture, which is an ideal model for cell migration and differentiation. Compared with the partial polarization of cells in two-dimensional culture, it can provide a more accurate description of cell polarization (Baker and Chen 2012) (Fig. 1). The 3D environment allows cells to survive for up to 300 days and maintain healthy non-cancerous growth, which makes them more suitable for long-term research such as the long-term effects of drugs (Table 1). It is an experimental technology between animal experiments and two-dimensional cell culture.

Classification and evolution of three-dimensional culture technology

In 1968, Boiron invented and reported the 3D culture technology, and since then, the 3D culture technology began to develop rapidly (Boiron et al. 1968). The 3D culture can be divided into two kinds: the technique with scaffold and the technique without scaffold (Fig. 1). Cell scaffold technology can be divided into solid scaffolds and gel scaffolds according to the different scaffold materials. Moreover, scaffolds free

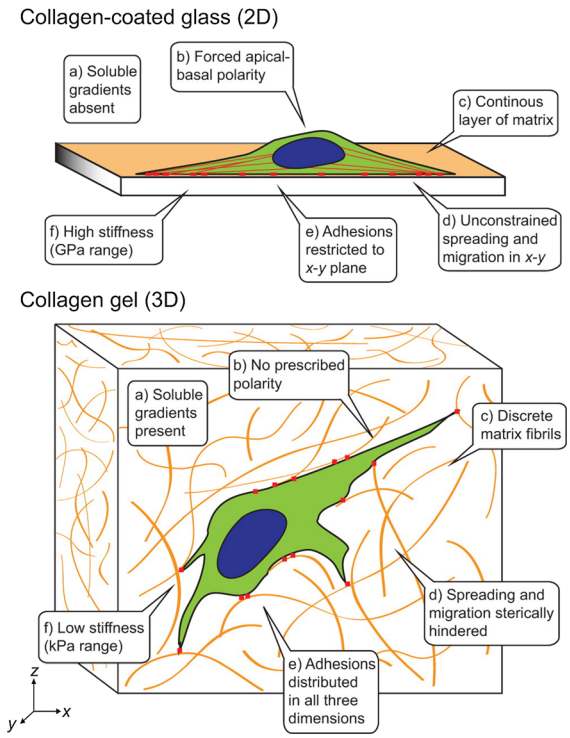

Fig. 1 Adhesive, topographical, mechanical, and soluble cues in 2D and 3D. The cues encountered by a cell are strikingly different between an ECMcoated glass or plastic surface (2D) and a typical 3D ECM, such as collagen

can also achieve 3D cell culture. The principles are well explained by the schematic diagram (Fig. 2).

According to the application of external forces, 3D cell culture types are divided into static 3D culture (Jianmin et al. 2001) and dynamic 3D culture (McKee et al. 2017; Yang et al. 2017).

Solid scaffolds

Solid materials use precise nanostructured materials as scaffolds, such as RGD peptide chain cross-linked sodium alginate (Dou et al. 2013), a scaffold network of carbon fibers to maintain cell growth (Liu et al. 2012). The cells grow between the complex nest-like scaffolds, which are also adherent to the wall in essence, but there are certain 3D structure and the interaction between cells. The preparation of scaffolds requires high requirements. It is usually necessary to use an electron microscope to detect the pore diameter of the scaffolds before the experiment, and only the scaffolds that meet the pore diameter can be tested in the next step. Whether the scaffold itself has an effect on cell survival also needs to be verified by experiments. Therefore, it is widely used in materials science and biomedical engineering.

Gel scaffolds

Gel scaffolds are a 3D skeleton composed of cross-linked polymers, including natural materials, such as chitin, collagen, hyaluronic acid and other ECM

Table 1 Comparison of three-dimensional cell culture, two-dimensional cell culture and animal model

Comparison	Three-dimensional cell culture	Two-dimensional cell culture	Animal model
Supporting force	All directions	From the bottom	All directions
Cell morphology	Multilayer, spherical	Single layer	Multilayer, spherical
Maturity	Tissue, organoid	Cell	Tissue, organ
Nutrition and oxygen	Less inside and more outside, tumor-like environment	Uniform	Uniform
Cellular interaction	Have	Less	Have
Cellular polarization	Three-dimensional	Two-dimensional	Three-dimensional
Growth time	Up to 300 days	A few days	Equal to animal life
Standardization	General	High	General
Price	High	Low	Medium
Freedom of design	High	Low	Low
Downstream experiment	General, need to extract the cell from the medium	Easy	General, need to surgically treat animals

components, such as (Liu et al. 2016), polyester degradable polymers (Gümüşderelioğlu and Türkoğlu 2002). Polystyrene and polycaprolactone which can generate scaffolds similar to bone matrix structure are very popular in bone regeneration research (Patel et al. 2019). Chitin hydrogel has high cell compatibility, but poor mechanical properties. Chitin hydrogel infused into poly ε-caprolactone (PCL)/ nano-hydroxyapatite (nHA) scaffold with good mechanical properties. Results showed that it effectively promotes vascularization and osteoporosis, and it would be a promising application for bone regeneration. The collagen gel and glycosaminoglycan from animal origin have good biocompatibility, so they are also commonly used materials (Murray and Spector 2001). Special molecules and factors can also be added to the hydrogel according to specific experimental needs. Researchers achieved the substantial expansion of hematopoietic stem and progenitor cells in the degradable zwitterionic hydrogel which induced an inhibition of excessive reactive oxygen species (ROS) production via suppression of O + 2-related metabolism (Bai et al. 2019). The preparation procedure for gel scaffolds is relatively simple. It was easy to realize standardization by paying attention to the control of pH value and the temperature. However, gel scaffolds are usually not transparent enough to perform real-time imaging, but rather need to separate cells to successfully imaging (Zanoni et al. 2016). Hydrogels are not conducive to drug delivery in the study of hydrophobic drugs (Hoare and Kohane 2008). Therefore, the hydrogel is not a universal solution. Researchers need to decide on the skeleton properties according to specific experimental purposes.

Scaffold free

The principle of scaffold free culture is to prevent tumor cells from adhering to the wall by various physical methods, to suspend tumor cells in the culture medium, and to promote cell aggregation and growth to form tumor cell spheres. The culture methods include low adhesion cell culture plate (Eiraku and Sasai 2011), suspension drop culture plate (Weeks et al. 2013), rotating bioreactor (Samuelson and Gerber 2013), magnetic suspension (Haisler et al. 2013) and 3D printing (Zhao et al. 2014). On the low adhesion culture plate, the cells could not adhere to the

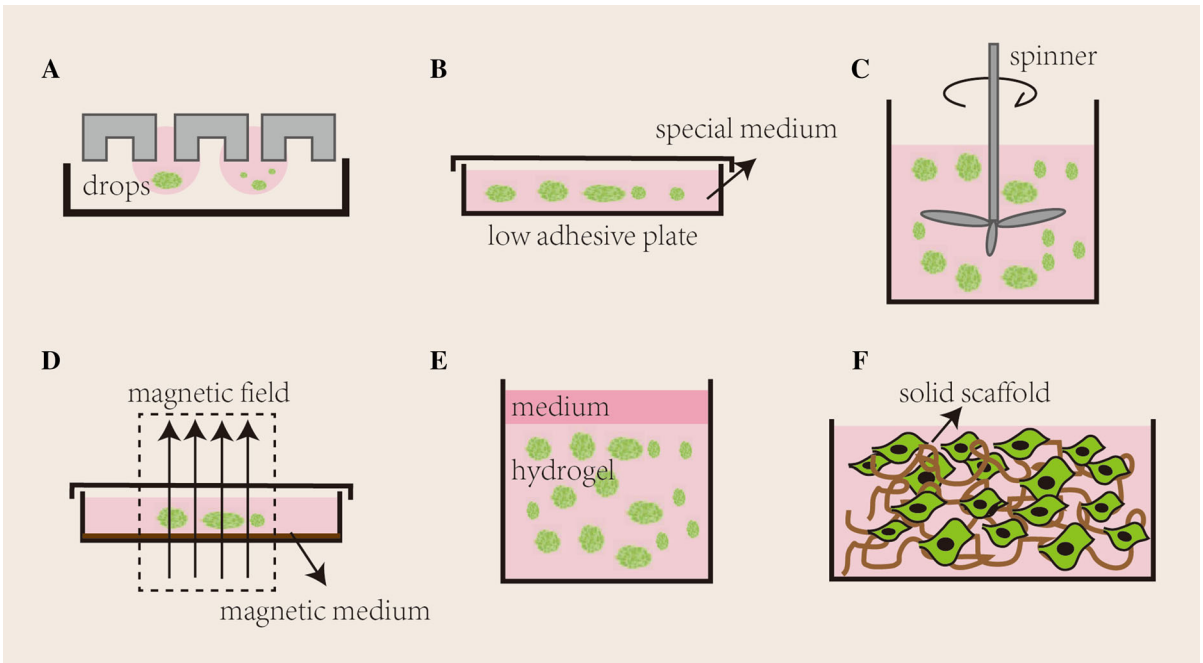

Fig. 2 Classification of three-dimensional cell culture techniques. The technology can be divided into three categories according to the scaffolds: gel scaffolds, solid scaffolds and scaffold free. The classic device pattern and cell morphology are shown in the figure above. **a** suspension drop culture plate. **b** low adhesion cell culture. **c** rotating bioreactor. **d** magnetic suspension. **e** Gel scaffolds. **f** solid scaffolds

wall, some cells died correspondingly, while the cells without death initially formed a round sphere. Then cells gradually form smaller spheres, which can screen out more aggressive tumor cell subcategories (Cordes et al. 2017). The culture medium in the suspension culture plate presents the shape of water drop and has no support, rather than the cylindrical shape on the traditional culture plate, so that the cells cannot be subjected to other forces except gravity, and finally realize the spherical growth, in the form of 96 well plate, it is convenient to realize the drug screening. Magnetic suspension is characterized by the presence of magnetic iron oxide and other substances in the culture medium, so that the external magnetic field can act on the whole culture medium to achieve suspension. The advantage of scaffold free culture is that cell detection is more convenient, most cells do not need to be separated from the medium, and can be directly used for the next step of experiments.

Static three-dimensional culture and dynamic three-dimensional culture

According to the application of external forces, 3D cell culture types are divided into static 3D culture (Jianmin et al. 2001) and dynamic 3D culture (McKee et al. 2017; Yang et al. 2017). Gravity is the only mechanical influence factor in the static 3D culture process. In order to achieve spherical growth in static culture, a special medium will be used, which contains no serum but high concentration of growth factors. At the same time, use a low-adhesion substance, such as 1.5% agar, to coat the culture dish to prevent cells from adhering to the surface to promote the formation of spherical cells (Derakhti et al. 2019). However, the stiffness of the culture medium can be used as an influencing factor to interfere with cells in a static system (Xu et al. 2019). The softer hydrogel helps mesenchymal stem cells differentiate into osteogenic cells (Žigon-Branc et al. 2019). Stress that can affect cells can be added in the dynamic 3D culture according to the needs of research. At present, it mainly includes shear force, tension and pressure (Kanazawa et al. 2014). The same cell line grows differently under static and dynamic culture environment. Stem cells from human exfoliated deciduous teeth can achieve better differentiation into hepatocytes in a dynamic environment (Huang et al. 2020). Researchers can choose different experimental method according to the specific purpose.

Application of three-dimensional culture

The clinical application of 3D culture can be divided into four categories: cell behavior, microenvironment, drug sensitivity, radiation sensitivity, and stem cell differentiation. Search for relevant keywords on pubmed, and the number of studies obtained (Table 2 and Fig. 3). Except for radiation sensitivity, hundreds of articles are published every year, which shows the popularity of related research.

Research on cell behavior

In 3D culture, cells have the ability to move in all directions, which has a great advantage in the study of cell behavior. In particular, for the study of tumor invasion and metastasis, the growth mode of cell clusters in the 3D culture system is poly condensation, and the cells in the central area are lack of oxygen and sufficient supply of nutrients, which is similar to the environmental characteristics of the cells in the central area of solid tumors in vivo, and better reflects the biological characteristics of tumor cells (Lewis et al. 2017). In 3D environment, the whole process of tumor

Table 2 The number of three-dimensional cell culture clinical studies published on Pubmed in the past five years

Years	Cell behavior	Microenvironment	Drug sensitivity	Radiation sensitivity	Stem cell differentiation
2015	271	131	62	18	320
2016	316	175	75	11	376
2017	359	168	105	9	433
2018	403	214	121	13	476
2019	366	223	118	11	454

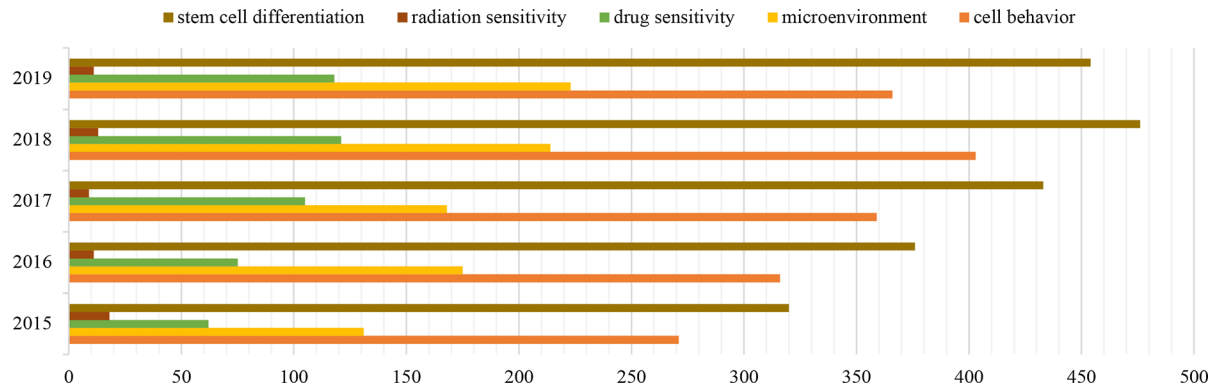

Fig. 3 The histogram of three-dimensional cell culture clinical studies published on Pubmed in the past five years. The number of researches on stem cells is the largest, followed by research on cell behavior related to cell migration and tumor metastasis, which reached a peak in 2018 and has gradually declined since then

formation can be recorded by a specific imaging system, which is conducive to long-term dynamic observation and tracking of tumor invasion and metastasis. Morales et al. (Morales and Alpaugh 2009) used in vitro 3D model to simulate the process of inflammatory breast cancer metastasis in vivo. The results showed that the effect of the collagen matrix on cell morphology and intercellular signal transduction in the 3D culture model was similar to that in vivo microenvironment, which affected the migration behavior of tumor cells. The researchers studied the interactions between colorectal cancer cells, fibroblasts, and endothelial cells in a three-dimensional cell culture environment, and conducted in vivo validation experiments in a mouse model. The results showed that the combined injection of endothelial cells significantly inhibited the growth of the primary tumor, while the combined injection of fibroblasts promoted the growth of lung metastasis of the tumor and reversed the effects of chemotherapy drugs. The invasion and growth of cancer cells in vitro culture can predict in vivo effects(Sarah et al. 2018). Researchers cultivated cervical cancer cells in a three-dimensional cell culture environment to track extracellular vesicles, which can carry and transmit mRNA and miRNA to promote tumor growth. They found that the secretion of extracellular vesicles increased, which indicated that previous two-dimensional related studies may have underestimated the metastasis risk (Thippabhotla et al. 2019).

Research on microenvironment

3D culture technology is suitable for analyzing the effect of microenvironment on cells, revealing some phenomena that cannot be found under traditional experimental methods. Su et al. (Su et al. 2015) Found that in the hCG rich microenvironment pretreated, the number of vascular network structures formed by hCG receptor-positive ovarian cancer cell lines (OVCAR-3 cells) and the expression levels of CD31, VEGF and factor VIII significantly increased. Park et al. (Park et al. 2017) determined the effect of 3D culture on the maintenance of undifferentiated porcine spermatogonial stem cells by analyzing the formation and morphology of cell colonies, transcription and translation regulation of genes related to self-renewal. Stem cells have stronger self-renewal ability in the 3D culture microenvironment than in the 2D culture microenvironment. Zhang et al. (Zhang et al. 2014) cell revealed that contraction collagen hydrogel could promote the growth of cells from G0/G1 phase to S phase, and promote DNA synthesis and cell proliferation. To some extent, these studies indicate that some experimental results in traditional two-dimensional cell culture may have an essential deviation.

Research on drug sensitivity

Monolayer cultured cells in vitro cannot reflect the pathophysiological structure and state of tumor cells in vivo. Therefore, it is a trend to study the drug sensitivity, dose, and efficacy in 3D culture. Perche et al. (Perche and Torchilin 2012) evaluated the

efficacy of chemotherapy drugs by constructing 3D globules of cancer cells. In the model, adriamycin was used as a single drug or in combination with other anti-tumor drugs. In the 3D structure of cancer cells, the drug permeability was limited to the outer cell layer, indicating that globules have higher drug resistance than monolayer cells. Jung et al. constructed a 3D lung cancer model and studied the effects of Cisplatin and etoposide in standard chemotherapy regimens, providing important information to guide therapeutic approaches (Jung et al. 2019). The microfluidic chip is a new technology involving many disciplines. It integrates the basic operation units such as sample preparation, reaction, separation and detection into a micro-scale chip, automatically completes the whole process of sample analysisfor high-throughput screening of tumor drugs (Sabhachandani et al. 2015).

Research on radiation sensitivity

The radio-sensitivity and DNA damage repair ability of two-dimensional growth cells with the same genetic background are different from that of 3D growth cells. The experiment of radio-sensitivity in the 3D environment can better simulate the effect of radiotherapy on the tumor in vivo. Chan et al. utilized a 3D model to elucidate the multifactorial nature of radiation sensitivity in the culture of non-small cell lung cancer cell line A549 (Chan et al. 2016)0.3D cell radiation experiment is more suitable for clinical application. Gomez et al. (Gomez-Roman et al. 2016) cultured glioblastoma based on polystyrene scaffold, the 3D model reliably predicted the clinical efficacy, with potential value in pre-clinical evaluation. Sowa et al. (Sowa et al. 2010) carried out low let ionizing radiation on human mammary epithelial tissue in 2D and 3D culture environment to observed the effect of radiation-induced cytotoxicity. It is found that 3D cell culture has a protective effect on cell survival after irradiation, but a long-term culture in the 3D environment can significantly reduce the cytotoxicity at a given dose.

Research on stem cell differentiation

Stem cell differentiation is a multi-factor regulatory process, and also a hot research direction. Mesenchymal stem cells (MSCs) are a kind of adult pluripotent stem cells with multi-directional differentiation potential, which are derived from mesoderm and neuroectoderm, and do not express hematopoiesis related markers (Wang et al. 2012). According to the source, it can be divided into bone marrow mesenchymal stem cells, umbilical cord mesenchymal stem cells, synovial mesenchymal stem cells, and so on. The differentiation rate of MSCs in 2D culture environment was low. However, 3D culture technology can simulate the physiological environment of cells in the body, which is conducive to gene expression and signal transduction. Chaudhuri et al. (Chaudhuri et al. 2016) regulate the stress relaxation properties of hydrogels. It is found that different elasticity will affect the direction of differentiation of mesenchymal stem cells. Different elasticity can make stem cells differentiate into adipocytes, differentiate into osteoblasts, or do not differentiate. Goulart et al. used 3D bioprinting technology combined with autologous induced pluripotent stem cell (iPS)-derived transplantation technology to explore the treatment of patients with advanced liver disease and revealed the epithelial-mesenchymal transition resulting in rapid loss of hepatocyte phenotype in liver cancer (Goulart et al. 2019). The application of three-dimensional cell culture technology in the above fields has been summarized in the table for quick reading (Table 3).

Conclusion

3D cell culture has created a new experimental system and experimental design ideas. It can well simulate the internal environment, making the experimental results more convincing, and has been widely used in many frontier research fields. Compared with traditional experimental methods, 3D cell culture has many advantages, but there are still differences with the real human body, which provides the required skeleton for cell 3D growth, the simulation of related growth factors and the composition of tissue fluid in vivo environment is not enough, how to improve the culture conditions to simulate the conditions in vivo as much as possible is a direction for further research. And the price of 3D culture is relatively expensive, the process of standardization is not perfect, so there is some resistance in its promotion. With the progress and improvement of technology, 3D cell culture will become a fully used technology.

Table 3 Typical three-dimensional cell culture clinical studies

Categories	Diseases	Related Cells	Way to achieve 3D culture	Result	Country	Reference
Cell behavior	Inflammatory breast cancer	MCF-7 (human breast cancer cells)	3D spheroid assay	The metastatic efficiency might be linked to the formation and maintenance of these architectural features	USA	Morales and Alpaugh (2009)
Cell behavior	Colorectal cancer	SW620, HCT116 (human colorectal carcinoma cell lines) and EA.hy296, HEK-293T (endothelial cells)	3D spheroid assay	The combined injection of endothelial cells significantly inhibited the growth of the primary tumor	Switzerland	Sarah et al. (2018)
Cell behavior	Cervical cancer	Hela cell (cervical cancer cells)	Hydrogel	The secretion of extracellular vesicles increased, which indicated that previous two-dimensional related studies may have underestimated the metastasis risk	USA	Thippabhotla et al. (2019)
Microenvironment	Ovarian cancer	Human umbilical vein endothelial cells (HUVECs) and HTR-8 (trophoblast cells)	Hydrogel	HCG may synergistically enhance hypoxia-induced vascular markers and HIF-1α expression	China	Su et al. (2015)
Microenvironment	N/A	spermatogonial stem cells (SSCs)	Hydrogel	Self-renewal of porcine SSCs can be maintained more effectively in a 3D than in a 2D culture	Korea	Park et al. (2017)
Microenvironment	N/A	L929 (fibroblasts)	Hydrogel	Hydrogel may promote cell cycle from G0/G1 phase to S phase, and DNA synthesis and cell proliferation were enhanced	China	Zhang et al. (2014)
Drug sensitivity	Ovarian adenocarcinoma	NCI-ADR-RES cells (Ovarian adenocarcinoma cells)	3D spheroid assay	In the 3D structure of cancer cells, the drug permeability was limited to the outer cell layer, indicating that globules have higher drug resistance than monolayer cells	USA	Perche and Torchilin (2012)

Table 3 continued

Categories	Diseases	Related Cells	Way to achieve 3D culture	Result	Country	Reference
Drug sensitivity	Lung cancer	Primary culture of small cell lung cancer cells	hydrogel	Constructed a 3D lung cancer model and studied the effects of Cisplatin and etoposide in standard chemotherapy regimens	Korea	Jung et al. (2019)
Drug sensitivity	Breast cancer	MCF-7 (breast cancer cells) and HS-5 (fibroblasts)	3D spheroid assay	Provides a simple and novel, in vitro platform to generate, image and analyze uniform, 3D monodisperse alginate hydrogel tumors	USA	Sabhachandani et al. (2015)
Radiation sensitivity	Non-small cell lung cancer (NSCLC)	A549 (human NSCLC epithelial cell line)	3D spheroid assay	Utilized a 3D model to elucidate the multifactorial nature of radiation sensitivity in NSCLC	USA	Chan et al. (2016)
Radiation sensitivity	Glioblastoma (GBM)	E2, R10, and G7 (GBM cell line)	Polystyrene scaffold	3D model reliably predicted clinical efficacy in preclinical evaluation of drug-radiation combinations for GBM	UK	Gomez-Roman et al. (2016)
Stem cell differentiation	Breast cancer	184A1 (human mammary epithelial cell line)	Hydrogel	The amount of apoptosis following radiation exposure is significantly decreased in 3D culture relative to the 2D monolayer after the same dose	USA	Sowa et al. (2010)
Stem cell differentiation	N/A	Mesenchymal stem cell	Hydrogel	Different elasticity will affect the direction of differentiation of mesenchymal stem cells	Germany	Chaudhuri et al. (2016)
Stem cell differentiation	Liver cancer	Induced pluripotent stem cells	3D print	Indicated the advantage of using spheroid-based bioprinting, built liver physiology and disease modeling	Brazil	Goulart et al. (2019)

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Compliance with ethical standards

Conflict of interest All the authors declare that there is no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

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