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Review article

The importance of 3D cell culture in drug discovery and development

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Abstract

Three-dimensional (3D) cell culture techniques represent a transformative advancement in biomedical research, particularly in drug discovery and development. By more closely replicating the physiological and microenvironmental conditions of *in vivo* tissues, 3D cell cultures enable more accurate assessments of drug efficacy, toxicity, and therapeutic potential compared to traditional two-dimensional (2D) cultures. These systems not only provide a more realistic model for preclinical testing but also allow for the study of complex cell-cell and cell-matrix interactions, which are often overlooked in 2D systems. This review provides a comprehensive examination of studies utilizing spheroids and organoids in 3D culture systems for drug screening and development. Furthermore, it highlights the critical role of these models in uncovering novel therapeutic targets, understanding disease mechanisms, and optimizing drug delivery strategies. Key challenges, such as scalability, standardization, and integration with high-throughput screening platforms, are also discussed. In conclusion, 3D cell culture techniques hold immense promise for revolutionizing the drug discovery pipeline, offering a more predictive and ethical approach to preclinical research while bridging the gap between laboratory findings and clinical outcomes.

Keywords: *3D cell culture; drug discovery; organoids; spheroid*

1. Introduction

The process of drug discovery and development is notoriously challenging, characterized by low success rates in clinical trials. This is largely due to the slow progression of the process, the high costs involved, and the complexities of translating preclinical results into successful treatments. In addition to these challenges, there is a significant gap in the availability of effective and safe treatment options for a variety of diseases, particularly complex conditions like cancer, neurodegenerative disorders, and cardiovascular diseases (Arrowsmith and Miller, 2013, Jordan et al., 2024). Furthermore, safety concerns arising from the inability to accurately predict human responses to drugs in preclinical models exacerbate the situation. As a result, there is a critical need to find innovative technologies that can improve the predictability of drug efficacy and safety in clinical trials. This would, in turn, enhance the success rates of drug discovery and development, providing new hope for the treatment of currently unmet medical needs.

One of the most promising areas for improving drug discovery outcomes is the advancement of 3D cell culture technology. 2D cell cultures have been widely used in drug discovery for decades; however, these cultures fail to adequately replicate the complex *in vivo* environment. Unlike 2D cultures, 3D cell cultures offer a more realistic representation of human tissues by allowing cells to grow and interact in a three-dimensional space, similar to how they would behave in the body. This approach has the potential to significantly improve the accuracy of drug testing and allow for better prediction of drug responses in humans (Biju et al., 2023). The initial studies that demonstrated the potential of 3D cell cultures to mimic the

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fundamental factors of tissues and replicate the extracellular matrix (ECM) in cell behavior were conducted in the 1980s by Mina Bissell and her team (Ravi et al., 2015; Langhans, 2018). These early studies laid the foundation for the development of 3D cell culture models that could more effectively simulate the natural environment of human tissues, which is essential for understanding cellular responses to drugs. Since then, advances in 3D culture technologies have led to the creation of more sophisticated models that enable the study of various diseases, including cancer, and the testing of new drugs in a more relevant setting. Despite the rapid advancements in 3D cell culture technologies, traditional single-layer 2D cell cultures are still commonly used in drug studies due to their simplicity, lower cost, and long-standing familiarity. However, as the limitations of 2D cultures in predicting drug responses become more apparent, the advantages of 3D cell cultures are becoming increasingly evident. In particular, 3D cell cultures are now being integrated into high-throughput screening (HTS) platforms, where they provide more accurate and reliable results in drug discovery applications (Wang and Jeon, 2022). This shift towards 3D culture-based screening has resulted in the identification of new drug candidates with better efficacy and fewer side effects, ultimately improving the chances of success in clinical trials (Sittampalam et al., 2015; Langhans, 2018).

In this review, we will explore the growing importance of 3D cell culture technologies in enhancing drug utilization and discovery. We will focus on the various types of 3D culture systems currently being employed, including spheroids, organoids, and bio-printed tissues, and their respective applications in drug testing. Additionally, we will discuss the key advantages of using 3D cell cultures in preclinical research, including their ability to mimic complex tissue structures, better represent disease models, and predict drug responses more accurately. However, despite their potential, the adoption of 3D cell cultures still faces challenges, such as the scalability of these systems, their integration into existing drug screening pipelines, and the need for standardized protocols. These challenges must be addressed to fully realize the potential of 3D cell culture technology in revolutionizing the drug discovery process. Ultimately, 3D cell culture systems hold the promise of improving the drug discovery pipeline by providing more predictive, accurate, and ethical alternatives to traditional preclinical models. The adoption of these advanced technologies could bridge the gap between laboratory findings and clinical outcomes, leading to the development of more effective and safer drugs for patients in the near future.

2. 3D cell culture

3D cell culture is a technique that simulates the environment where cells grow, mimicking cell-cell and cell-matrix interactions similar to those found in natural tissues. The aim of this technique is to model cellular hierarchy progression, investigate cell-cell interactions, observe cellular behavior, and develop treatment strategies. Using this technique, 3D models such as spheroids and organoids are created (Ajjarapu et al., 2023).

Spheroids are spherical or round-shaped cell clusters formed by the self-organization of cells. Organoids, on the other hand, are 3D models that resemble human tissues and organs in structure and function, developed from stem cells or progenitor cells with differentiation potential (Ravi et al., 2015; Simian and Bissell, 2017; Temple et al., 2022; Ajjarapu et al., 2023). Spheroids establish an architecture involving cell-cell and cell-matrix (ECM) interactions. Cell-surface integrins and cadherins play a critical role in activating signaling pathways that regulate several biological processes, including adhesion, organization, and maintaining structural integrity among cells (Białkowska et al., 2020; Sara Biju et al., 2023). Some of these techniques are illustrated in Fig. 1.

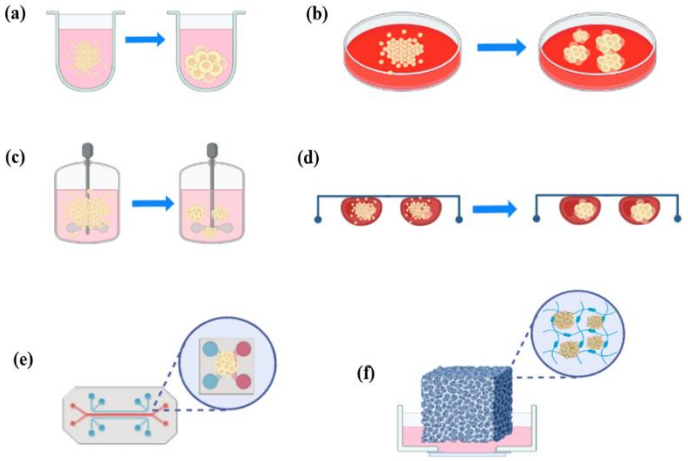

Fig. 1. The techniques used to create spheroids include: (a) Pellet culture, (b) surface coating, (c) bioreactor, (d) hanging drop method, (e) microfluidics, and (f) embedding onto or into a matrix (Demirel, 2023).

All these techniques enable the production of 3D cellular aggregates, spheroids, in a rapid and reproducible manner. However, spheroids produced using these techniques often display a low percentage of some ECM components, alongside cell-ECM interactions. Therefore, the dynamics of ECM components are crucial for mechanisms regulating cancer cell metabolism and responses to therapeutic molecules (Lu et al., 2012).

The pellet culture technique involves cells concentrating at the bottom of a tube under the influence of centrifugal force, leading to the formation of spheroids (Fig. 1-a). The adhesion between cells at the bottom of the tube plays a crucial role in forming spheroid cultures. To culture the formed cell aggregates, supernatants are removed, and the spheroids formed in the supernatant are resuspended in the cell culture medium. However, a disadvantage of this technique is that changing the environment in this way can damage spheroids, potentially disrupting their structure (Achilli et al., 2012; Maritan et al., 2017).

Spheroid formation using the surface coating technique (Fig. 1-b) is a widely used and straightforward method. Among spheroid formation techniques, the simplest approach involves seeding cells onto a surface where they cannot adhere. In this method, the surface is coated with materials such as agarose, poly-HEMA, polyethylene glycol (PEG), galactose, or polyvinyl alcohol (PVA) to create a low-adhesion surface (Liu et al., 2021).

The disadvantages of this technique include the inability to adequately support spheroids, its chemically sensitive structure, and weaker cell-cell interactions compared to other methods (Raghavan et al., 2016). For creating a low-adhesion surface, agar or agarose gel is commonly used. Agarose is a preferred material for adhesion inhibition and demonstrates lower adhesion properties compared to agar. On this low-adhesion surface, cells stimulate cell-cell adhesive molecules, leading to the formation of spheroids (Costa et al., 2018). However,

agarose struggles to interact with tumor cells and cannot activate signaling pathways (Carvalho et al., 2016). Recently, hyaluronic acid has emerged as the most suitable biomaterial to overcome these disadvantages of agarose (Demirel et al., 2024). This is due to hyaluronic acid's ability to interact with surface receptors of cancer cells, which facilitates the transmission of cellular signals related to proliferation, angiogenesis, survival, and differentiation, as well as increasing resistance to therapeutics (Carvalho et al., 2017).

In the technique of spheroid formation through a bioreactor, as depicted in Fig. 1-c, the cultured cells are subjected to a continuous rotating force that prevents them from statically adhering to a surface. Under these conditions, cells tend to cluster and form spheroids (Tostões et al., 2012). The bioreactor technique is suitable for long-term cultures and can be used to produce a larger quantity of spheroids. However, it produces heterogeneous spheroids, and it is impossible to perform simultaneous monitoring and tracking. Moreover, transferring the resulting spheroids to another culture environment is necessary for their examination (Kahn-Krell et al., 2021).

The hanging drop technique relies on cell sedimentation, or in other words, the settling of cells, to form cell clusters (Fig. 1-d). In this technique, cells are cultured in droplet form suspended on the lid of a petri dish. The foundation of the commonly encountered hanging drop technique lies in the simultaneous effect of surface tension and gravity, leading to the formation of droplets. At the apex of these droplets, 3D spheroids form (Achilli et al., 2012). In addition, due to the small volumes used, implementing this technique can be challenging, and spheroids can be easily lost. Furthermore, changing the culture medium in such a technique is not easy (Timmins and Nielsen, 2007). Unlike other techniques, the microfluidic technique, different from non-microfluidic methods, plays a significant role in spheroid formation in 3D cell culture. However, it has some disadvantages. The limitations in other techniques can include variations in spheroid diameters, low throughput, or difficulty of use, as well as issues like the reduction of oxygen and nutrients and the increase in osmolality and metabolite levels (Whitesides, 2006).

In Fig. 1-e, the microfluidic technique involves the creation of controlled concentration gradients, lower reagent consumption, and the application of pressure on cells and a regular perfusion system, which are among its most significant advantages (Moshksayan et al., 2018). Additionally, microfluidic chips provide a dynamic environment to better mimic the *in vivo* conditions. Spheroids cultured in a continuously dynamic environment within a microwell plate exhibit higher resistance to drugs compared to other techniques (Ruppen et al., 2014). Among the disadvantages of this technique are the occurrence of clogging problems in microchannels, the relatively low permeability and transparency of silicone and PDMS (polydimethylsiloxane), which can pose challenges during microscopy, and the possibility of the system being multi-channeled, leading to the emergence of a microenvironment that could cause contamination (Chueh et al., 2010; Ruppen et al., 2014).

The final spheroid formation technique is the 3D cell culture method created by embedding cells onto or into a matrix (Fig. 1-f). Hydrogels are used in this technique for spheroid formation. Tissue scaffolds created with hydrogels not only support the 3D structure of cells but also provide a microenvironment that supports cell-cell and cell-matrix interactions, influencing tumor cell functions (Nath and Devi, 2016; Li and Kumacheva, 2018; Ozkan and Ozturk, 2024). 3D culture systems commonly utilize tissue scaffolds, which include ECM-based natural hydrogels, synthetic hydrogels, and commercial hydrogels that attempt to mimic the natural ECM (Li and Kumacheva, 2018).

Organoids, also known as mini-organs, are complex clusters of cells that develop from stem cells or organ progenitors, capable of self-renewal and exhibiting organ-specific properties (Fang and Eglen, 2017; Lee et al., 2023). The source of organoid formation can be embryonic stem cells, induced pluripotent stem cells, or adult stem cells. The most significant difference from spheroids is that due to their stem cell potential and ability to differentiate in a complex manner similar to *in vivo* organogenesis, organoids can exhibit organ-like properties (Spence et al., 2010; McCauley and Wells, 2017). Organoids summarize the *in vitro* development of organs due to their *in vivo*-like architecture; therefore, they are highly useful tools in organogenesis, genetics, and pathology studies (Takebe et al., 2013; Dutta et al., 2017). They are typically used for modeling and investigating the functionality of a specific organ, while spheroids are employed for studying more general cellular behaviors and interactions (Table 1). Especially in cancer research, the complex architecture of organoids can better reflect the histological and genetic characteristics of cancerous tissue (Sato et al., 2009; Baillargeon et al., 2019; Lee et al., 2023).

Table 1
Representation of different characteristic features of spheroids and organoids.

Feature	Spheroids	Organoids
Definition	Three-dimensional structures formed by cells coming together in a spherical or round shape	Three-dimensional structures where cells mimic the architecture and functionality of a specific organ
Production Process	Typically involve simple production processes	Production process is generally more complex
Applications	Studying cell-cell interactions, understanding general cellular behaviors	Modeling specific organs, investigating organ-specific functionality, creating disease models, drug discovery, and development of treatment strategies
Cell Behavior	Do not mimic the complex architecture of a specific organ	Mimic the complex architecture of specific organs
Advantages	- Simple production process - Suitable for studying cell-cell interactions - Suitable for understanding general cellular behaviors	- Investigation of organ-specific functionality - Creation of disease models - Drug discovery and development of treatment strategies
Disadvantages	- Do not mimic the complex architecture of a specific organ - Do not reflect organ-specific functionality - Not suitable for modeling specific organs	- Production process is generally more complex - May require more resources and time - May require a long time for tissue maturation

3. The importance of spheroids and organoids in efficient drug discovery

The beneficial use of spheroids as a 3D model was first explored in 1970 to understand the phenotype of *in vitro* tumors and their responses to chemotherapy during radiotherapy applications (Sutherland et al., 1970). Since then, spheroids have been widely used in various cell types (Fig. 2).

3D cell cultures have emerged as a promising alternative in drug discovery and development, providing more accurate and faster results compared to *in vivo* models. These innovative cell culture models significantly contribute to the advancement of drug research and personalized medicine. It has been reported that mathematical models have been exemplified for the use of Leedale et al. (2020) in simulating drug transport and activity in hepatic spheroids. Through this study, it is emphasized that 3D spheroids can guide researchers on how dosage and culture conditions can be regulated to optimize drug distribution. Using 3D tumor spheroids, drug delivery system associated with Aluminum chloride phthalocyanine, for early-stage diagnosis and treatment of breast cancer, has been employed (Jayme et al., 2022). This system demonstrates the compatibility of DNA polymeric films with cells and increases cell death through visible light photoactivation, targeting breast cancer cells with photodynamic therapy. In a study conducted on PDAC, 3D spheroids and organoids were utilized (Shah et al., 2022).

Pisheh et al. (2024) conducted comparative experiments with 2D and 3D cell cultures to demonstrate that antibody-drug conjugates (ADCs) yield more accurate responses in 3D cell culture. This study aimed to directly destroy tumor cells through a monoclonal antibody conjugated with a cytotoxic agent. It was reported that ADCs targeting the epidermal growth factor receptor (EGFR) were combined with aminobisphosphonates, which bind to drugs like cetuximab administered to colorectal cancer patients. The results showed that the outcomes obtained in 3D cell culture closely resemble those observed *in vivo*. Nakazawa (2024) discovered an ADC through organoids, named EBET, which induces protein cleavage. This ADC consists of bromodomain and extra-terminal (BET) proteins. The cytotoxic effects of EBET on various PDAC organoids demonstrated its potential efficacy when delivered via EBET. A comparative study was conducted between 3D and 2D cell cultures to demonstrate that more accurate results can be obtained using 3D cell cultures. The study proposed that liposomes could be used as a new drug delivery system for photodynamic therapy (PDT) (De Leo et al., 2024). It was noted that the therapeutic efficacy of second-generation photosensitizers (PS) is enhanced by loading them onto nanocarriers, with methylene blue (MB) dye being considered a potential PS. MB, which is thought to exhibit photodynamic activity, was loaded onto liposomes and demonstrated higher photodynamic potency in 3D cell cultures. These findings suggest that MB-based PDT delivered via liposomes could be an effective drug delivery system, as demonstrated through the *in vivo* similarity of 3D cell cultures. However, organoid production is quite challenging with automated microfluidics and is not feasible. An important study in the field of personalized therapy also utilized 3D cell culture. Schuster et al. (2020) study an automatic microfluidic platform was developed to facilitate organoid formation and enable rapid and combinatorial drug screening. To validate this system, drug screenings were conducted on human-derived pancreatic cancer

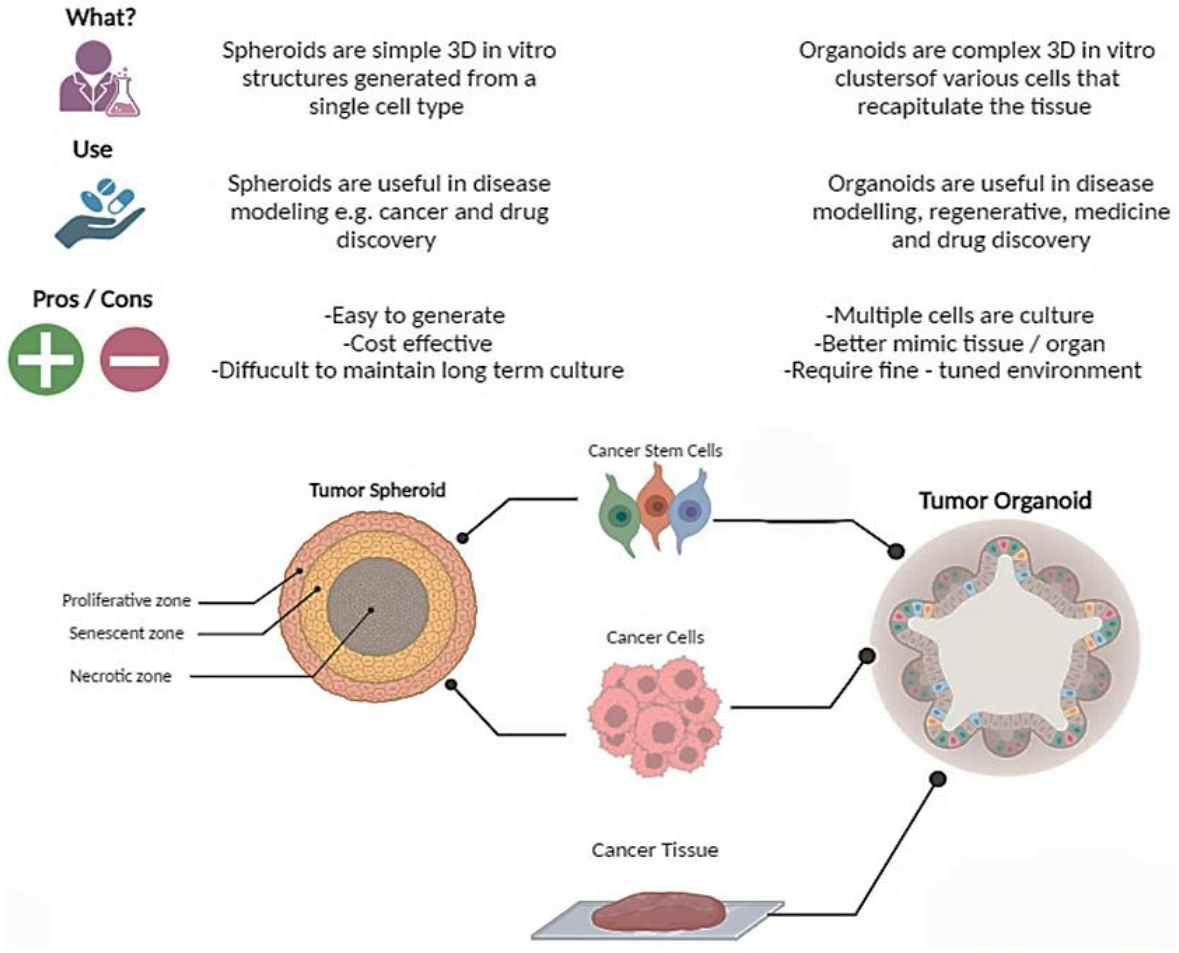

Fig. 2. Comparison and schematic representation between spheroids and organoids. (Created with Biorender).

organoids. Significant differences were observed in the responses of individual patient-based organoids to drug treatments, and it was reported that this represents an important advancement in personalized therapy for decision-making in treatment strategies for patients.

In a study conducted by a research team that hypothesized that organoids could show drug sensitivity and resistance similar to an *in vivo* environment, ovarian cancer organoids were specifically studied (Nanki et al., 2020). Within three weeks, ovarian cancer cells formed organoids, with each subtype showing different responses. For example, an organoid harboring a pathogenic variant in BRCA1 showed increased sensitivity to the PARP inhibitor olaparib, while an organoid derived from clear-cell ovarian cancer exhibited resistance to conventional drugs. According to the results, patient-derived organoids could serve as suitable *ex vivo* models for screening effective personalized ovarian cancer treatments.

Olijnik et al. (2024) developed bone marrow organoids derived from human induced pluripotent stem cells (hiPSCs). These organoids provide a powerful model for modeling disease phenotypes and evaluating the efficacy of pharmacological inhibitors by reflecting the natural architecture and functions of hematopoietic and stromal microenvironments. This approach preserves the characteristics of hematological cancer cells, which are difficult to maintain in 2D cell cultures, thereby reducing failure rates by more accurately predicting drug efficacy during the clinical transition process. One of the key advantages of 3D models is their ability to replicate the vascularization system. Since tumors *in vivo* are nourished through blood vessels, mimicking this system is highly beneficial for evaluating drug efficacy. For instance, Ascheid et al. (2024) developed vascularized tumor spheroids (VTS). These spheroids can be used to assess drug efficacy, such as in anti-angiogenic treatments, and enable a more accurate selection of drug candidates.

4. Challenges in 3D cell culture

3D cell culture models have gained significant attention as an important technique in drug efficacy evaluations. However, there are some limitations to the success of this technique. One of the most crucial factors is the determination and optimization of the culture medium components (Langhans, 2018). The components of the culture medium and substrate play a vital role in sustaining the culture. The limiting factor in this technique is the effect of the components in the culture medium on cellular growth and function (Lin and Chang, 2008). Moreover, the cells' ability to attach to the substrate, morphology, proliferation, and differentiation are affected by this. However, the balance and stability of the substrate interaction determine the success rate of the culture. Therefore, regulating and optimizing this balance is critical (Verma et al., 2020). The management of these components and the resulting cellular waste is a crucial detail. It has been emphasized that the management of components and waste is a limiting factor for the sustainability of this culture technique (Chaicharoenaudomrung et al., 2019). The bioactivity and potential toxicity of the materials used in 3D cell cultures are decisive factors for success. While the effect of each material may not be equally important, the combined effect of these components on the cells in the culture medium is expected to be positive (Moroni et al., 2008). One limiting factor in this technique is the presence of nutrients and oxygen in the inner parts of the cells, which negatively affects cell development. To address this issue, methods to increase the circulation of the culture medium are crucial (Demirel, 2021). The circular diffusion rate of the environment changes the interaction and organization level between cells (Lancaster and Knoblich, 2014). Cellular conditions vary with chemical and biomechanical signals. Therefore, the culture medium must be adjusted according to biomechanical signal limitations (Fennema et al., 2013). The formation of organoids and spheroids takes longer than in 2D cell cultures (Anton et al., 2015). The short lifespan of the obtained tissues and their inability to maintain viability after cell implantation require the engineering of biological materials (Murphy and Atala, 2014). One of the limitations of this technique is productivity and cost. The complexity and cost of long-term production processes are barriers to industrial-scale production (Lin and Chang, 2008). Standardization and reproducibility of this technique are essential for its reliability and safety (Handschel et al., 2007).

In 3D cell culture applications, the cell source and donor diversity are significant limitations. Genetic and phenotypic differences between different cell sources and donor diversity affect the results. Therefore, more studies are required to eliminate the lack of analysis and modeling (Knowlton et al., 2015; Gu et al., 2018). This lack of data validation and interpretation limits the applicability and validity of findings in clinical settings. Further research and validation are necessary for the application of clinical findings in studies (Tanner and Gottesman, 2015). 3D cell cultures are slightly more costly than 2D cell cultures, with the main reason being the biomaterials used to create the 3D structure. These biomaterials, which are a crucial parameter for mimicking the *in vivo* environment, can be commercially obtained or derived from natural polymers. However, researchers often have limited knowledge and expertise in using this technique (Ingber et al., 2006). There are ethical restrictions when using primary cells directly taken from patients, but these processes are crucial for testing drug efficacy for personalized treatments (Cvetkovic et al., 2014).

5. Conclusion

Significant progress has been made in drug discovery and development through the use of 3D cell culture models. These models, which reflect both the genomic and phenotypic characteristics of cancer and other diseases, provide highly reliable results. This system allows us to observe drug interactions in conditions similar to the *vivo* environment, offering a more accurate representation compared to traditional 2D models. Furthermore, 3D models play a critical role in the advancement of personalized treatments. The unique cellular characteristics of each patient enable the creation of accurate and effective personalized treatment protocols, allowing for more efficient application of the treatment process. However, challenges such as standardization and automation remain for the widespread adoption of 3D models in drug development. Despite these hurdles, the continuous advancements in drug efficacy testing through 3D cell cultures are expected to significantly contribute to the future of personalized medicine and drug discovery, ultimately improving treatment strategies.

Conflict of interest: The authors declare that they have no conflict of interests.

Informed consent: The authors declare that this manuscript did not involve human or animal participants and informed consent

was not collected.

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