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REVIEW ARTICLE

3D Cell Culture and Organoids: Emerging Analytical Strategies for *In Vitro* Drug Testing

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ABSTRACT

The shift from traditional two-dimensional (2D) cell cultures to three-dimensional (3D) models such as spheroids and organoids has transformed in vitro drug testing by offering more physiologically relevant platforms that better mimic human tissue architecture and function. These models enable more accurate assessment of drug efficacy, toxicity, metabolism, and pharmacokinetics, thereby enhancing the predictive power of preclinical studies. However, the complex structural and biochemical nature of 3D systems presents significant analytical challenges, necessitating the development of advanced and adaptable techniques. This review highlights the current analytical approaches used in the characterization and evaluation of 3D cultures and organoids, including high-content imaging, mass spectrometry-based metabolomics, and high-throughput screening platforms. We also discuss the integration of microfluidic technologies, artificial intelligence in image analysis, and the standardization needs for regulatory compliance. By addressing these emerging strategies, the review emphasizes the pivotal role of analytical science in bridging innovation and translational success in modern drug discovery pipelines.

Keywords*: 3D cell culture, organoids, in vitro drug testing, analytical techniques, high-content imaging, mass spectrometry*

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INTRODUCTION

The accurate evaluation of drug efficacy, toxicity, and pharmacokinetics is a fundamental requirement in the drug development pipeline. Traditionally, two-dimensional (2D) cell cultures have been widely used as in vitro models to assess these parameters due to their simplicity, low cost, and ease of handling. However, 2D cultures fail to recapitulate the complex architecture, cell–cell interactions, and microenvironment of living tissues, often resulting in poor predictive power for in vivo and clinical outcomes [1].

To overcome these limitations, the scientific community has witnessed a paradigm shift toward three-dimensional (3D) cell culture systems, including spheroids, organoids, and scaffold-based models. These systems offer enhanced physiological relevance by better mimicking in vivo tissue morphology, mechanical properties, oxygen/nutrient gradients, and multicellular organization [2]. 3D cultures have shown improved predictability in drug screening and have become instrumental in studying disease mechanisms, tumor heterogeneity, and host–pathogen interactions [3].

Among the various 3D models, organoids—self-organizing, stem cell-derived structures that resemble miniature organs—have gained particular attention due to their capacity to mimic organ-level functions and pathology. Organoids derived from patient tissues provide an opportunity to develop personalized drug testing platforms, accelerating progress in precision medicine [4].

Despite the biological advantages of 3D systems, their analytical interrogation presents considerable challenges. The complex spatial organization, increased matrix density, and non-uniform drug penetration require the adoption of advanced analytical tools for accurate characterization and quantification. Traditional assays, which work effectively in 2D models, often fail to produce reliable results in 3D systems due to interference from the extracellular matrix and the inability to access inner cell layers [5].

Consequently, there is a growing need for innovative analytical techniques such as high-content imaging, spatially resolved mass spectrometry, microfluidic sampling, and AI-assisted data interpretation. These approaches enable a deeper understanding of the pharmacodynamic and pharmacokinetic behavior of drugs within 3D systems and are crucial for the successful translation of in vitro findings into clinical applications [6].

This review aims to explore the analytical landscape supporting 3D cell culture and organoid-based drug testing, with a focus on imaging methods, mass spectrometry, high-throughput screening (HTS), and emerging tools such as bioprinting and lab-on-chip systems. Additionally, we discuss regulatory considerations, standardization efforts, and future directions to bridge the gap between analytical innovation and practical implementation in pharmaceutical and biomedical research. Figure 1 provides a comparative illustration of traditional 2D monolayer cultures versus advanced 3D cell culture systems, highlighting differences in morphology, nutrient diffusion, and cellular interactions.

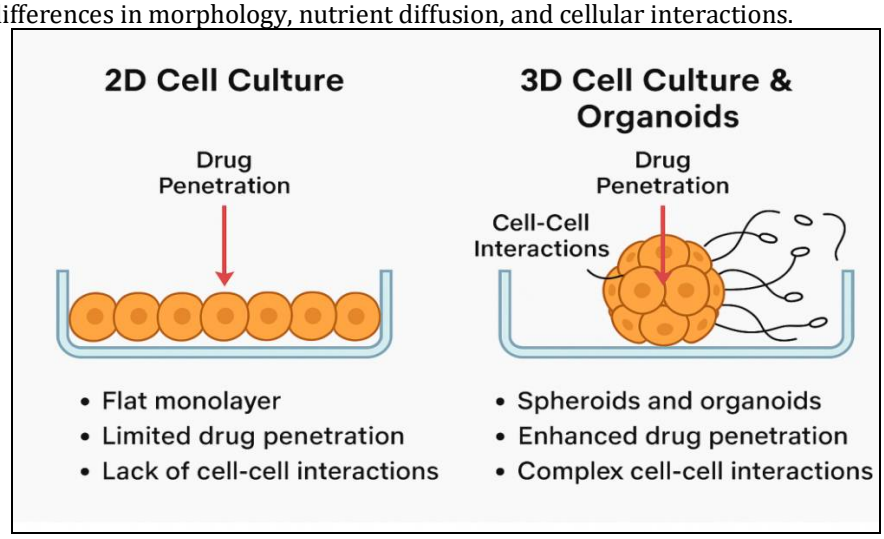

Figure 1. Comparative illustration of 2D vs. 3D cell culture systems.

TYPES OF 3D MODELS USED IN DRUG TESTING

In modern drug discovery and biomedical research, three-dimensional (3D) cell culture systems have gained prominence due to their ability to more closely replicate the physiological and architectural characteristics of native tissues compared to traditional two-dimensional (2D) cultures. These 3D models preserve key cell–cell and cell–extracellular matrix (ECM) interactions, provide more accurate oxygen and nutrient gradients, and allow for realistic tissue mechanics, thereby enhancing the relevance of preclinical testing. The most commonly used 3D models include spheroids, organoids, scaffold-based systems, and 3D bioprinted constructs, each with distinct characteristics, applications, and limitations.

SPHEROIDS

Spheroids are multicellular aggregates that self-assemble under non-adherent conditions without the need for scaffolds. These structures are typically formed using techniques such as hanging drop, ultra-low

attachment (ULA) plates, or spinner flasks. Due to their ability to form gradients of oxygen, nutrients, and waste, spheroids simulate avascular tumor regions and are widely used in cancer research, cytotoxicity screening, and drug penetration studies [7]. Despite their simplicity, spheroids can develop a necrotic core and demonstrate drug resistance patterns similar to those observed in vivo.

ORGANOIDS

Organoids are miniaturized, 3D representations of whole organs derived from adult stem cells (ASCs), embryonic stem cells (ESCs), or induced pluripotent stem cells (iPSCs). Unlike spheroids, organoids self-organize into tissue-like structures with defined cell lineage, polarity, and functionality. They have been established for several organs, including intestine, liver, brain, kidney, and pancreas, and are considered ideal for modeling organ development, disease mechanisms, and patient-specific drug responses [8]. Organoids are increasingly used in precision oncology to predict therapeutic outcomes in individual patients.

SCAFFOLD-BASED 3D SYSTEMS

These models rely on natural or synthetic biomaterials (e.g., collagen, Matrigel, alginate, or PLGA) to support the spatial organization of cells into 3D structures. The scaffold provides mechanical strength and ECM-like features, allowing cells to adhere, proliferate, and differentiate within a controlled architecture. Scaffold-based models are widely used in tissue engineering, regenerative medicine, and toxicity testing due to their ability to mimic the mechanical and biochemical cues of native tissues [9].

BIOPRINTED TISSUE MODELS

3D bioprinting is an advanced technique that uses computer-aided design (CAD) to deposit bioinks composed of cells and hydrogels layer by layer, forming complex tissue structures. These models allow for precise spatial distribution of multiple cell types, vascular-like channels, and control over the geometry of the construct. Bioprinted models are emerging as customizable platforms for high-throughput drug screening, disease modeling, and personalized medicine, though standardization and scalability remain challenges [10]. The major types of 3D culture systems—including spheroids, organoids, scaffold-based constructs, and bioprinted tissues—differ significantly in origin, complexity, and application scope, as summarized in Table 1.

Table 1. Classification and Features of 3D Culture Models

Model Type	Source	Structural Complexity	Major Applications
Spheroids	Tumor or normal cell lines	Moderate	Cancer drug screening, penetration studies, apoptosis research
Organoids	iPSCs, ESCs, ASCs	High	Disease modeling, personalized therapy, developmental biology
Scaffold-based systems	Natural or synthetic biomaterials	Variable	Tissue engineering, toxicity testing, regenerative medicine
Bioprinted constructs	CAD + bioinks (cells + hydrogels)	Very high	Custom drug testing, complex tissue modeling, vascularized structures

ANALYTICAL CHALLENGES IN 3D SYSTEMS

Although three-dimensional (3D) cell culture models offer superior physiological relevance over traditional 2D monolayer cultures, they also introduce unique analytical challenges that hinder their widespread implementation in drug screening, toxicity testing, and disease modeling. These limitations span various domains, including drug diffusion, experimental reproducibility, and biological complexity.

DRUG PENETRATION AND DIFFUSION LIMITATIONS

One of the most prominent challenges in 3D cell models is the limited penetration and uneven distribution of drugs within the scaffold or spheroid. Unlike monolayer cultures where all cells are equally exposed to the drug, 3D systems feature multiple layers of cells embedded within extracellular matrix (ECM)-like structures, creating diffusion barriers [11].

This spatial organization leads to drug concentration gradients—with cells at the periphery receiving higher drug exposure than those at the core—resulting in heterogeneous drug responses [12]. For instance, large tumor spheroids often develop hypoxic or necrotic centers due to restricted oxygen and nutrient diffusion, mimicking in vivo solid tumors but complicating dose–response analyses [13].

Furthermore, the physicochemical properties of drugs, such as molecular size, polarity, and lipophilicity, can significantly influence their ability to penetrate deeply into 3D tissues. Hydrophilic and larger molecules, including many biological therapeutics, often fail to reach inner cell layers, thereby underestimating their therapeutic potential during screening [14].

SAMPLE HANDLING AND REPRODUCIBILITY ISSUES

Handling 3D cultures requires more intricate protocols compared to 2D systems. Techniques such as spheroid formation, organoid culture, or bioprinting often yield non-uniform constructs varying in size, cell density, and morphology. This heterogeneity poses a challenge to standardization and reproducibility, especially in high-throughput settings [15].

Moreover, many analytical assays optimized for 2D cultures (e.g., Western blotting, qPCR, ELISA, or viability assays) require either tissue dissociation or penetration of assay reagents into dense 3D matrices. These processes may result in cell loss, altered gene/protein expression, or inconsistent signal readouts, compromising data quality [16]. Even common viability assays such as MTT or resazurin reduction may yield skewed results due to inadequate penetration or interference from ECM components [17].

The requirement for specialized tools and platforms, such as 3D-optimized imaging systems (e.g., confocal microscopy or optical clearing), also raises the cost and technical barrier for laboratories unfamiliar with 3D methodologies [18].

COMPLEXITY OF CELL–CELL AND CELL–MATRIX INTERACTIONS

A key strength—but also a major analytical challenge—of 3D culture systems is their incorporation of physiologically relevant cell–cell and cell–ECM interactions. These dynamic interactions influence cellular behavior in a context-dependent manner, affecting cell differentiation, proliferation, migration, and drug responsiveness [19].

However, due to this complexity, dissecting cause-effect relationships in response to experimental manipulation (e.g., drug treatment) becomes difficult. For instance, a particular phenotypic change may be driven by the interplay of mechanical cues (matrix stiffness), biochemical gradients (growth factors), and intercellular signaling—not just the drug itself [20].

Quantifying such effects often requires advanced technologies, including spatial transcriptomics, live-cell imaging, and 3D single-cell RNA-sequencing, which are not yet widely accessible or standardized [21]. Furthermore, batch-to-batch variability in ECM components (e.g., Matrigel) and sensitivity to culture conditions add layers of unpredictability to experimental outcomes [22].

INTEGRATION AND INTERPRETATION OF COMPLEX DATA

The voluminous and multidimensional data generated from 3D cultures—especially when using omics platforms or high-resolution imaging—pose computational challenges. Unlike 2D cultures, where outputs are relatively uniform and easy to quantify, data from 3D models require complex image analysis, spatial mapping, and mathematical modeling for accurate interpretation [23]. To date, there is no universal pipeline for data analysis in 3D drug screening, making cross-study comparisons difficult and impeding regulatory acceptance for routine use in drug development [24].

IMAGING TECHNIQUES FOR 3D CULTURES

Three-dimensional (3D) cell cultures offer significant advantages over two-dimensional systems, including improved structural complexity and cellular behavior closer to in vivo tissues. However, their increased thickness and heterogeneity introduce challenges in visualization and analysis. Advanced imaging techniques have therefore been developed and adapted to address these issues, enabling precise monitoring of cell viability, morphology, differentiation, and drug response within 3D environments.

CONFOCAL AND MULTIPHOTON MICROSCOPY

Confocal laser scanning microscopy (CLSM) has become a staple in 3D culture imaging due to its optical sectioning ability, which enables acquisition of high-resolution images at different depths within a specimen. However, CLSM has limited penetration depth (typically up to 100–200 μm) and can cause photobleaching and phototoxicity during prolonged imaging sessions. Multiphoton microscopy, on the other hand, uses near-infrared excitation to overcome these limitations, allowing for deeper tissue imaging (up to 500–800 μm) with reduced photodamage. These techniques have been effectively used to study cell migration, proliferation, and morphological changes within spheroids and organoids [25,26].

LIGHT SHEET FLUORESCENCE MICROSCOPY (LSFM)

Light Sheet Fluorescence Microscopy (LSFM) represents a cutting-edge advancement that enables fast, high-resolution 3D imaging with minimal phototoxicity. By illuminating the sample with a thin sheet of light from the side, LSFM allows optical sectioning without scanning the entire volume, significantly reducing photodamage. LSFM is particularly suitable for long-term imaging of live organoids and embryonic development models. It also supports multi-view acquisition, improving resolution and data reconstruction across large 3D samples [27,28].

LABEL-FREE IMAGING TECHNIQUES

To avoid the need for fluorescent dyes, which can interfere with biological processes or be toxic over time, label-free imaging methods such as phase contrast microscopy, coherent anti-Stokes Raman scattering (CARS), and Raman spectroscopy have been increasingly adopted. These techniques exploit differences in refractive index or molecular vibrations to generate contrast, enabling visualization of 3D cultures without external staining. Raman microscopy, in particular, offers molecular-level insights into drug distribution and metabolic activity within spheroids and scaffold-based systems [29,30].

IMAGE ANALYSIS SOFTWARE AND AI-BASED QUANTIFICATION

Due to the complexity and volume of data generated from 3D cultures, robust image analysis tools are essential for quantitative interpretation. Software such as ImageJ (with 3D plugins), Imaris, and Arivis Vision4D are commonly used for 3D reconstruction, cell segmentation, and volumetric analysis. Moreover, artificial intelligence (AI) and machine learning-based tools are now being integrated for automated pattern recognition, phenotype classification, and high-throughput analysis. Deep learning algorithms can enhance contrast, identify subcellular structures, and classify drug response patterns in large 3D datasets with high accuracy and reproducibility [31–33]. A range of imaging techniques, including confocal microscopy, light sheet fluorescence microscopy (LSFM), and label-free modalities, are employed to analyze 3D cultures, each offering distinct advantages and limitations as outlined in Table 2.

Table 2: Comparative Overview of Imaging Methods for 3D Cultures

Imaging Method	Key Features	Advantages	Limitations	Applications
Confocal & Multiphoton Microscopy	Optical sectioning, fluorescence-based	High resolution, depth imaging (~100–500 µm)	Limited penetration depth, phototoxicity	Imaging spheroids, tissue sections
Light Sheet Fluorescence Microscopy (LSFM)	Plane illumination, low photodamage	Fast acquisition, ideal for live imaging of large samples	Requires transparent samples, complex setup	Organoid imaging, real-time development studies
Label-free Imaging (e.g., Raman, Phase Contrast)	No staining required, molecular fingerprinting	Non-invasive, real-time monitoring	Lower resolution, limited molecular specificity	Metabolic imaging, drug uptake monitoring
AI-based Image Analysis Software	Automated segmentation, tracking, quantification	High-throughput, objective analysis	Needs large training datasets, computational resources	Phenotypic screening, drug response quantification

MASS SPECTROMETRY AND OMICS-BASED APPROACHES

The integration of mass spectrometry (MS) and omics technologies has significantly enhanced the analytical depth and resolution of studies involving three-dimensional (3D) cell culture systems. These advanced methodologies allow for the precise detection of drug uptake, metabolism, and cellular responses within complex biological matrices.

LC-MS/MS FOR DRUG UPTAKE AND METABOLISM

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a cornerstone analytical tool used to investigate drug penetration and biotransformation in 3D models. Compared to traditional two-dimensional cultures, 3D models mimic the in vivo tumor microenvironment more closely, which can influence drug absorption and enzymatic activity. LC-MS/MS provides high sensitivity and specificity for detecting parent drugs and their metabolites within cell lysates or conditioned media [34]. When applied to tumor spheroids or organoids, this technique helps map the pharmacokinetic behavior of compounds across multiple time points and tissue depths, aiding in the optimization of therapeutic regimens [35].

MALDI-TOF IMAGING FOR SPATIAL MAPPING

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) imaging mass spectrometry has emerged as a powerful tool for spatially resolved analysis of molecular distributions. It enables label-free visualization of drugs, lipids, and endogenous metabolites directly within intact 3D tissue sections [36]. Unlike conventional MS that requires homogenization, MALDI-TOF imaging preserves spatial context, making it particularly useful for mapping drug diffusion gradients or metabolic heterogeneity across spheroid cores and peripheries [37]. This technique contributes significantly to understanding intra-tumoral variability in drug response and nutrient availability.

METABOLOMICS AND PROTEOMICS FOR RESPONSE PROFILING

Omics-based strategies such as metabolomics and proteomics are extensively used to decode the biochemical and molecular shifts induced by drug treatments or environmental stresses in 3D cultures.

Metabolomics, via techniques like GC-MS and LC-MS, offers snapshots of intermediary metabolism, redox states, and energy fluxes, thereby revealing stress adaptation or cytotoxic effects [38]. Meanwhile, quantitative proteomics using tandem mass tag (TMT) labeling or stable isotope labeling by amino acids in cell culture (SILAC) identifies differentially expressed proteins involved in pathways such as apoptosis, proliferation, or epithelial-mesenchymal transition (EMT) [39]. These profiles enable researchers to pinpoint mechanistic biomarkers of treatment efficacy or resistance in physiologically relevant models.

MICRO-SAMPLING TECHNIQUES AND SPATIAL RESOLUTION

To achieve higher spatial resolution in omics analysis, micro-sampling approaches such as laser capture microdissection (LCM) and microprobe extraction have been developed. These allow selective sampling of core versus peripheral regions of 3D cultures, enhancing the resolution of localized metabolic or proteomic data [40]. When integrated with spatial MS or single-cell omics, such techniques provide detailed maps of biochemical activity within distinct niches of the 3D structure, aiding in precision modeling of tumor microenvironments and cellular heterogeneity.

Various mass spectrometry-based approaches such as LC-MS/MS, MALDI imaging, and GC-MS have been utilized to study drug distribution and metabolism in 3D models, with their respective applications and strengths summarized in Table 3.

Table 3: Summary of MS-Based Analytical Tools for 3D Systems

Technique	Target Analyte	Sample Type	Strengths
LC-MS/MS	Drugs and metabolites	Extracted spheroids/organoids	High sensitivity, quantification, broad applicability
MALDI Imaging	Drugs, lipids, metabolites	Intact 3D models	Spatial resolution, label-free detection, visualization of gradients
GC-MS	Volatile metabolites	Media or lysate	High specificity, ideal for metabolic fingerprinting
Proteomics (LC-MS)	Proteins and peptides	Whole model or layers	Expression profiling, pathway elucidation
Micro-sampling + MS	Region-specific analytes	Core/periphery sampling	Heterogeneity mapping, integration with spatial omics

HIGH-CONTENT AND HIGH-THROUGHPUT SCREENING (HCS/HTS)

MINIATURIZED ASSAYS FOR 3D CULTURES

The transition from 2D to 3D cell cultures in drug screening has necessitated the development of miniaturized assay platforms compatible with high-throughput workflows. Miniaturization of 3D assays into 96-, 384-, and 1536-well formats has become possible through advancements in automated liquid handling, robotic pipetting, and microfluidic technologies. These platforms enable parallel testing of multiple compounds on spheroids, organoids, or scaffold-based cultures, significantly improving screening throughput while reducing reagent consumption and assay cost [41,42]. For example, 384-well ULA (ultra-low attachment) plates have been optimized for uniform spheroid formation and drug exposure, supporting reproducible toxicity assessments in cancer models [43].

AUTOMATED IMAGING AND ANALYSIS PIPELINES

Automated imaging systems coupled with robotics and machine learning algorithms have greatly improved the capacity for high-content screening (HCS) in 3D systems. These platforms utilize automated confocal or spinning disk microscopy to collect z-stacks and analyze parameters such as cell proliferation, apoptosis, and morphological changes at cellular resolution [44]. AI-based image analysis tools like CellProfiler, ImageJ with plugins, and commercial platforms such as Harmony® or MetaXpress® now support 3D segmentation and quantification of volumetric data, enabling multi-parametric assessment of complex phenotypes [45,46]. Additionally, time-lapse imaging allows dynamic tracking of treatment responses in real-time, which is particularly valuable for long-term toxicity or efficacy studies [47].

MULTIPARAMETRIC READOUTS AND DATA INTEGRATION

Multiparametric screening allows simultaneous assessment of multiple endpoints, such as cytotoxicity, cell viability, mitochondrial function, and caspase activation, within the same 3D culture well. This multiplexing capability reduces variability and enhances the biological relevance of the findings. In 3D cultures, the use of fluorescent and luminescent reporters, combined with readouts from flow cytometry or microplate readers, has improved the sensitivity of detecting subtle changes in cell health and drug efficacy [48,49]. Integration of imaging data with omics outputs (e.g., transcriptomics or metabolomics) further enhances screening precision and supports better lead candidate selection [50].

ADVANCEMENTS IN ROBOTICS AND AI IN SCREENING

Recent innovations in robotics, artificial intelligence, and cloud computing are revolutionizing the scalability and analysis speed of HCS/HTS platforms. AI-enhanced systems enable rapid pattern recognition and predictive modeling of compound responses, reducing the time from screening to hit identification. Integration of cloud-based systems also allows remote access to data, facilitating collaborative and large-scale multi-site screening campaigns [51,52]. The general workflow for high-throughput screening in 3D organoid models—from organoid seeding to data analysis—is illustrated in Table 4.

Table 4: Workflow for High-Throughput Screening in 3D Organoid Models

Step	Description
Organoid Plating	Dispensing of uniform-sized organoids into multi-well plates (e.g., 96/384-well).
Compound Treatment	Exposure to various drug concentrations using automated liquid handlers.
Incubation	Allowing time for drug-organoid interaction, typically 24–72 hours.
High-Content Imaging	Use of confocal or automated microscopy to capture morphological and viability changes.
Image/Data Analysis	Quantitative analysis using AI-based or software-assisted segmentation and scoring.
Hit Identification	Identification of compounds with significant biological effects across metrics like apoptosis, proliferation, or viability.

EMERGING TECHNOLOGIES

ORGANS-ON-CHIPS AND MICROFLUIDICS INTEGRATION

Organs-on-chips are microengineered biomimetic systems that simulate key physiological functions of tissues and organs. These microfluidic platforms allow for continuous perfusion, enabling dynamic cell culture conditions that better replicate in vivo environments than static 3D cultures. When integrated with 3D cell models, these chips offer fine control over mechanical cues such as shear stress and interstitial flow, which are essential for mimicking tissue-specific functions like lung breathing or gut peristalsis [53,54]. Notably, they enable co-culture of multiple cell types in spatially defined compartments, facilitating studies of drug transport, toxicity, and immune interactions in a highly controlled setting [55].

3D BIOPRINTING FOR TISSUE MIMICRY

3D bioprinting has emerged as a transformative tool for fabricating architecturally complex tissue constructs with high spatial resolution. Using layer-by-layer deposition of bioinks—composed of cells, growth factors, and matrix materials—researchers can recreate tissue-like microenvironments that preserve cellular heterogeneity and functionality. This technology enables the generation of reproducible and patient-specific models for drug testing and disease modeling [56,57]. Bioprinted constructs can also be integrated with perfusable vasculature and mechanical cues, thereby enhancing their physiological relevance.

ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING FOR ASSAY INTERPRETATION

Artificial Intelligence (AI) and Machine Learning (ML) are playing an increasingly vital role in the analysis and interpretation of data from 3D cultures. High-content imaging and omics-based approaches generate large and complex datasets that require advanced computational tools for meaningful insights. AI-driven algorithms are being employed to identify subtle phenotypic changes, predict therapeutic responses, and classify compounds based on their mechanism of action [58,59]. Additionally, ML models trained on annotated datasets can automate segmentation, pattern recognition, and anomaly detection, thereby improving the throughput and reproducibility of screening campaigns.

STANDARDIZATION, VALIDATION, AND REGULATORY PERSPECTIVES

STANDARDIZATION AND HARMONIZATION OF 3D ASSAYS

One of the major barriers to the widespread adoption of three-dimensional (3D) cell culture systems in pharmaceutical research is the lack of standardization in experimental design, reporting, and validation. Unlike two-dimensional (2D) systems, 3D cultures introduce variability due to differences in scaffold materials, cell sources, and culture conditions. To address this, various international organizations, including the Organisation for Economic Co-operation and Development (OECD) and the International Organization for Standardization (ISO), have proposed guidelines for quality control in in vitro models, though specific standards for 3D assays are still under development [60,61].

Efforts such as the BioAssay Ontology (BAO) and initiatives like the European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) aim to harmonize data reporting and method documentation to ensure consistency and reproducibility in high-content 3D assays [62,63]. The adoption of Minimum Information About a 3D Cell Culture Experiment (MIACCE) has also been recommended to enhance transparency and reproducibility across laboratories [64].

VALIDATION OF 3D ASSAYS: RELEVANCE TO ICH GUIDELINES

Validation of analytical assays, including those used for drug screening in 3D models, must align with regulatory expectations such as those outlined in the International Council for Harmonisation (ICH) guidelines, particularly ICH Q2(R1), which defines criteria for analytical validation parameters like accuracy, precision, specificity, linearity, and robustness [60,65]. However, translating these criteria to complex 3D systems remains challenging due to assay variability and dynamic cellular behavior within these cultures. Inter-laboratory reproducibility studies are crucial to confirm the robustness of 3D models and qualify them as valid tools for regulatory submissions [66].

REGULATORY ACCEPTANCE AND THE 3RS PRINCIPLE

The development and validation of 3D assays are increasingly driven by the need to reduce reliance on animal testing, aligning with the 3Rs principle (Replacement, Reduction, Refinement). The U.S. Food and Drug Administration (FDA), European Medicines Agency (EMA), and other global agencies have expressed interest in the use of physiologically relevant models like organoids, spheroids, and organ-on-chip platforms for safety pharmacology and toxicology assessments [67,68].

Validated 3D models can provide more predictive insights into human physiology and drug responses, potentially improving the translational relevance of preclinical studies and reducing the number of animals used in experimental research [69]. Regulatory authorities are actively funding initiatives such as SEURAT-1 and Tox21 to accelerate the integration of 3D models into regulatory science [70].

FUTURE DIRECTIONS AND RESEARCH GAPS

PERSONALIZED DRUG TESTING USING PATIENT-DERIVED ORGANOIDS

The evolution of 3D cell culture technologies, particularly patient-derived organoids (PDOs), holds immense potential in the realm of personalized medicine. These organoids, cultivated directly from patient tissues, replicate the genetic and histological features of the original tumors or tissues, making them highly predictive for drug response studies. By employing PDOs, clinicians and researchers can evaluate drug efficacy and resistance mechanisms in a patient-specific context before initiating therapy, significantly improving treatment outcomes [71,72]. Furthermore, biobanks of PDOs representing various cancer types and genetic backgrounds are enabling large-scale screening to inform patient-specific therapeutic regimens and stratification [73].

INTEGRATION OF MULTI-OMICS AND COMPUTATIONAL MODELING

A critical future direction involves the convergence of multi-omics (genomics, transcriptomics, proteomics, and metabolomics) with advanced computational modeling to generate a systems-level understanding of drug responses in 3D models. Integration of these datasets allows identification of novel biomarkers, resistance pathways, and therapeutic vulnerabilities that are often masked in 2D cultures [74]. Tools such as machine learning and artificial intelligence are increasingly being applied to analyze omics data from 3D systems, revealing complex interactions and enabling predictive modeling for treatment outcomes [75,76]. Such integrative approaches will be vital for uncovering the molecular underpinnings of tissue-specific responses to pharmacological agents.

REGULATORY FRAMEWORK FOR 3D MODEL APPROVAL IN PRECLINICAL PIPELINES

Despite the promising nature of 3D cell culture systems, their adoption into mainstream drug development pipelines remains limited due to a lack of standardized validation criteria and regulatory guidelines. Regulatory bodies, including the FDA and EMA, have started recognizing the potential of 3D models and organ-on-chip platforms in toxicity and efficacy testing. However, there is still a need for harmonized protocols that ensure reproducibility, scalability, and translatability of findings derived from these systems [77]. The implementation of Good Cell Culture Practice (GCCP) guidelines, as well as standard operating procedures for assay validation, will be pivotal in achieving regulatory acceptance [78]. Moreover, collaboration between industry, academia, and regulatory agencies is essential to establish benchmarks that support the inclusion of 3D models in Investigational New Drug (IND) applications.

CONCLUSION

The integration of advanced analytical tools in the study of 3D cell culture systems represents a transformative shift in the landscape of drug discovery and development. Technologies such as high-

resolution imaging, mass spectrometry, and omics platforms have enabled researchers to gain deeper insights into the structural, functional, and molecular dynamics of complex three-dimensional models. These analytical advancements address many of the limitations posed by traditional two-dimensional cultures and animal models by offering more physiologically relevant data.

Despite substantial progress, several challenges remain. Standardization and validation of protocols across laboratories are still evolving, and there is a critical need for harmonized guidelines to ensure reproducibility and regulatory compliance. Moreover, while many cutting-edge tools are being developed and validated, their widespread adoption is often hindered by cost, technical complexity, and data integration challenges.

The future of 3D systems in drug discovery lies in fostering interdisciplinary collaboration among biologists, chemists, engineers, data scientists, and regulatory bodies. Such partnerships will be key to integrating diverse platforms—ranging from patient-derived organoids and multi-omics profiling to machine learning algorithms and organ-on-chip systems—into cohesive, robust pipelines. With the increasing regulatory interest in alternatives to animal testing and the emphasis on personalized medicine, 3D culture systems are poised to become central to the mainstream drug development workflow. Their continued refinement, coupled with advances in computational modeling and high-throughput platforms, will pave the way for more predictive and ethically sound pharmaceutical research.

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