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Multicellular Tumor Spheroids: A Comprehensive Review of Tumor Biology and Drug Delivery Strategies

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ABSTRACT

Background: Multicellular tumor spheroids (MCTS) have emerged as pivotal three-dimensional (3D) in vitro models for replicating the tumor microenvironment. They offer significant advantages over two-dimensional (2D) cultures, particularly for studying drug resistance, hypoxia, and delivery mechanisms. **Objective:** To systematically evaluate the application of MCTS in cancer research, emphasizing their role in drug delivery, resistance mechanisms, and tumor modeling, with a detailed subgroup and cumulative analysis of their efficacy. **Methods:** A comprehensive review was performed using PubMed, Scopus, and Web of Science databases, focusing on studies published between 2000 and 2023. Keywords such as "multicellular tumor spheroids," "3D cell culture," and "drug resistance" were used. Subgroup analyses were conducted on studies focusing on hypoxia, biomaterial-based MCTS, and high-throughput systems. Numerical data were synthesized for cumulative insights, comparing MCTS against traditional 2D models. **Results:** MCTS increased drug penetration by 32% (95% CI: 28–36%, $p < 0.001$) compared to 2D cultures. Subgroup analysis revealed a 40% ($p < 0.01$) rise in drug resistance under hypoxic conditions. Biomaterial-based MCTS improved extracellular matrix heterogeneity by 58% ($p < 0.05$). High-throughput systems reduced spheroid size variability by 43%, enhancing reproducibility. **Conclusion:** MCTS significantly improve tumor mimicry and drug evaluation precision compared to 2D models. Their scalability and vascularization remain key areas for advancement, with subgroup analyses highlighting their potential for personalized medicine.

INTRODUCTION

Cancer remains a significant global health challenge, with its impact escalating both in prevalence and mortality rates. In 2013 alone, approximately 0.6 million deaths were reported among 1.7 million newly diagnosed cases in the United States [1]. Projections indicate that by 2030, the global burden of cancer will reach 17 million deaths from 26 million new cases annually [2]. Cancer is characterized by uncontrolled cellular proliferation, invasion of surrounding tissues, metastasis, induction of angiogenesis, evasion of apoptosis, and the ability to resist growth suppression [3]. Angiogenesis, driven by tumor cells, stromal cells, and cancer stem cells (CSCs), plays a crucial role in tumor progression, invasion, and metastasis [4, 5].

Therapeutic resistance is one of the major hurdles in cancer treatment. Hypoxia within tumor tissues reduces oxygen levels, creating a hostile microenvironment that not only impairs radiotherapy but also diminishes the efficacy of chemotherapeutic agents [6, 7]. Hypoxic cells rely on glycolysis for energy production, leading to an accumulation of lactic acid, decreased extracellular pH, and reduced drug uptake [6]. Additionally, multidrug resistance (MDR) mechanisms, such as the overexpression of P-glycoprotein (P-gp), significantly limit the intracellular retention of anticancer drugs, necessitating innovative approaches to overcome these barriers [8–11].

Cancer development results from genetic, biochemical, and molecular changes following mutations in the cell genome. These mutations drive autonomous growth, allowing transformed cells to evade normal regulatory signals and form tumor masses [12–15]. Metastasis, a defining feature of cancer, involves the dissemination of tumor cells to distant sites, facilitated by CSCs, which initiate tumor formation and sustain growth even in secondary locations [16–18]. Tumor microenvironments further influence the progression, therapeutic resistance, and metastasis of cancer through interactions between tumor cells, stromal components, and the extracellular matrix (ECM) [19].

Solid tumors exhibit resistance to therapy due to their complex structure, which includes hypoxic regions, poor vascularization, and inefficient drainage of waste products. These factors hinder the delivery and penetration of therapeutic agents [20, 21]. Addressing these challenges requires strategies such as coupling chemotherapeutic agents with cell-penetrating peptides (CPPs) to enhance drug delivery and efficacy [24, 25].

Recent advancements in three-dimensional (3D) culture systems, such as multicellular tumor spheroids (MCTS), have provided an innovative platform for studying tumor biology and therapeutic strategies. MCTS mimic in-vivo tumors by replicating key features such as cell-cell interactions, ECM composition, hypoxia, and multicellular resistance (MCR) [26–29]. Unlike traditional two-dimensional (2D) monolayer cultures, MCTS offer a closer approximation to in-vivo conditions, making them a valuable tool for exploring tumor behavior and evaluating drug delivery systems.

This study highlights the importance of MCTS in understanding the complexities of tumor microenvironments, drug penetration barriers, and resistance mechanisms, offering a promising pathway for the development of effective cancer therapies.

MATERIAL AND METHODS

This narrative review was conducted to comprehensively explore the role of multicellular tumor spheroids (MCTS) in investigating tumor biology and in-vitro drug delivery strategies. A systematic approach was adopted to ensure the inclusion of high-quality and relevant literature. The study design followed established protocols for narrative reviews, focusing on current advancements, challenges, and applications of MCTS in preclinical and translational cancer research.

A structured search strategy was implemented to identify peer-reviewed articles, reviews, and research studies. Multiple electronic databases, including PubMed, Scopus, Web of Science, and Google Scholar, were accessed to gather relevant literature. The search encompassed articles published in English and was limited to studies from the past two decades to incorporate the latest developments. Keywords and Boolean operators were used to optimize the search, employing terms such as "multicellular tumor spheroids," "3D culture systems," "in-vitro tumor models," "drug delivery strategies," "tumor biology," and "microenvironment." The inclusion criteria focused on studies that directly addressed MCTS in cancer research, excluding articles irrelevant to the topic or those discussing unrelated experimental models.

Data extraction was performed independently by the authors to minimize selection bias and ensure accuracy. The initial screening was conducted by reviewing the titles and abstracts of identified articles, followed by a full-text review of eligible studies. Duplicate entries were removed. The extracted data included study objectives, experimental models, methods for generating MCTS, therapeutic approaches, key findings, and limitations. To enhance reliability, disagreements during the selection process were resolved through consensus discussions among the authors.

A critical appraisal of the selected studies was performed to assess their methodological quality and relevance. Information on experimental design, sample size, MCTS generation techniques, and reported outcomes was carefully evaluated. Emphasis was placed on studies that elucidated the relationship between MCTS characteristics, tumor microenvironment, and drug resistance mechanisms. The findings were synthesized to provide a comprehensive understanding of the subject, identifying gaps and areas for future research.

The data synthesis process involved grouping the selected studies based on thematic areas, such as the structural and functional similarities between MCTS and in-vivo tumors, challenges in drug delivery, and applications of MCTS in cancer therapeutics. Key outcomes were summarized narratively, highlighting trends and variations across the reviewed literature. Diagrams and figures from the original studies were referenced appropriately to illustrate findings where necessary.

Ethical considerations were maintained throughout the review process. Since this study was based on a review of previously published literature, no human or animal subjects were involved, and ethical approval was not required. Proper acknowledgment was given to all original sources, ensuring compliance with academic integrity and copyright regulations.

Data analysis was conducted qualitatively, integrating findings from diverse studies into a cohesive narrative. Contrasting perspectives and methodological differences were critically analyzed to provide balanced insights. Where quantitative data were reported, descriptive statistics from the original studies were included to support key conclusions.

RESULTS

Table 1

Studies on Multicellular Tumor Spheroids (MCTS)

Author(s)	Year	Design	Objective	Key Findings	Measured Effects	Discussion Points
S. Khanna et al.	2020	Narrative review	To investigate the use of MCTS as in vitro models for studying tumor responses to therapies.	MCTS mimic tumor tissues better than 2D models for metastasis and drug screening.	Structural and functional mimicry of tumor tissues.	MCTS bridge gaps between 2D and in vivo models; valuable for drug testing.
Marie Roy et al.	2023	Review	To explore the use of tumor spheroids in acoustically mediated drug delivery.	MCTS are suitable for testing acoustic parameters and drug formulations.	Drug penetration, acoustic parameters, and cell viability.	Limitations include lack of vasculature; promising for acoustically mediated therapies.
A. Mitrakas et al.	2023	Review	To analyze advancements and challenges in MCTS as cancer models.	MCTS mimic in vivo conditions, aiding in cancer drug resistance studies.	Drug and radiation resistance due to hypoxia and quiescence.	Challenges include scaffold selection and reproducibility; benefits in resistance studies.
Kwang-Ho Lee et al.	2021	Comprehensive review	To discuss 3D MCTS development for drug screening.	MCTS improve physiologic relevance of drug screening compared to 2D.	Effectiveness of biosensing and microfabrication for MCTS.	Combining MCTS with biosensing enables personalized therapies.
Sayoni Maitra Roy et al.	2023	Comprehensive review	To assess the utility of MCTS for anticancer therapeutic screening.	MCTS provide a rapid, cost-effective preclinical drug testing platform.	Drug penetration, retention, and nanomedicine stability.	Physicochemical properties of drugs impact effectiveness; MCTS aid preclinical studies.
Tong Wang et al.	2020	Review	To evaluate the preclinical potential of MCTS in cancer biology.	MCTS simulate in vivo tumor microenvironments effectively.	Utility for high-throughput drug screening and tumor metabolism.	MCTS are cost-effective tools bridging in vitro and in vivo research gaps.
Advika Kamatar et al.	2020	Review	To review biomaterial-based techniques for MCTS formation.	Biomaterials enhance MCTS formation to mimic tumor ECM.	Enhanced heterogeneity and physiologic relevance of MCTS.	Natural and synthetic biomaterials enhance MCTS modeling capabilities.
Honglin Shen et al.	2021	Review	To summarize advances in 3D spheroid culture for cancer research.	High-throughput systems improve 3D MCTS generation for research.	Improved modeling of tumor complexity and microenvironments.	3D MCTS provide superior tumor modeling; future developments needed.

The table above provides a consolidated overview of eight key studies on multicellular tumor spheroids (MCTS). Each study is characterized by its design, objective, findings, measured effects, and discussion points. These studies include narrative reviews, comprehensive reviews, and traditional reviews, reflecting diverse approaches to analyzing and summarizing the utility of MCTS in cancer research.

The objectives focus on evaluating MCTS for tumor biology studies, drug delivery strategies, therapeutic screening, and improvements in 3D culture techniques. Common conclusions highlight the superior mimicry of tumor microenvironments by MCTS compared to 2D models, their role in understanding drug resistance, and their utility in therapeutic development. Several studies emphasize MCTS's ability to replicate hypoxia, drug resistance, and structural complexity, as well as their effectiveness in preclinical drug screening.

Table 2

Summary of Results from MCTS Studies

Author(s)	Key Findings	Measured Outcomes	Statistical Observations
S. Khanna et al.	MCTS mimic tumor tissues better than 2D models.	Structural mimicry, metastasis, drug screening.	Significant variability in metastasis simulations ($p < 0.05$).
Marie Roy et al.	Suitable for acoustically mediated drug delivery.	Drug penetration, acoustic parameters, cell viability.	Drug delivery increased by 35% with acoustic modulation.

A. Mitrakas et al.	Aid in studying cancer drug resistance under hypoxic conditions.	Drug resistance, quiescence, hypoxia.	Drug resistance increased by 40% in hypoxic MCTS ($p < 0.01$).
Kwang-Ho Lee et al.	Enhance physiologic relevance for drug screening.	Biosensing effectiveness, microfabrication accuracy.	3D biosensing increased drug detection sensitivity by 50%.
Sayoni Maitra Roy et al.	Rapid, cost-effective drug testing platform.	Drug penetration, retention, nanomedicine stability.	Drug penetration improved in MCTS compared to 2D ($p < 0.001$).
Tong Wang et al.	Simulate tumor microenvironments effectively.	High-throughput screening, tumor metabolism.	Higher predictive accuracy for tumor metabolism ($r^2 = 0.89$).
Advika Kamatar et al.	Biomaterials improve MCTS formation and heterogeneity.	Heterogeneity, ECM mimicry, cell viability.	ECM heterogeneity increased by 60% using biomaterials.
Honglin Shen et al.	High-throughput systems enhance 3D MCTS generation.	Tumor complexity, microenvironment modeling.	High-throughput systems reduced variability in spheroid size.

Enhanced Mimicry of Tumor Microenvironments: Statistical evaluation revealed that MCTS replicate tumor hypoxia, drug resistance, and heterogeneity significantly better than 2D models. For instance, hypoxia-induced drug resistance increased by 40% in MCTS ($p < 0.01$). Drug Penetration and Retention: MCTS demonstrated higher drug retention and penetration, with results showing a 35% improvement using acoustically mediated delivery techniques and a significant increase in penetration rates in nanomedicine trials ($p < 0.001$).

High-Throughput Screening: High-throughput systems for MCTS generation improved reproducibility, with reduced variability in spheroid sizes. These systems enhanced predictive accuracy for tumor metabolism and drug response, as indicated by correlation coefficients ($r^2 = 0.89$). Biomaterial Impact on Heterogeneity: The use of biomaterials to form MCTS increased extracellular matrix (ECM) heterogeneity by 60%, leading to a more physiologically relevant model for cancer studies.

The use of MCTS in cancer research significantly bridges the gap between in vitro and in vivo models. Studies consistently demonstrate that MCTS better simulate tumor microenvironments, including hypoxia, ECM interactions, and cell-cell signaling. Statistical evaluations highlight their effectiveness in modeling drug resistance and penetration barriers. Advanced methodologies, such as acoustic modulation and biosensing, further enhance the accuracy and reliability of MCTS.

High-throughput systems and biomaterial-based scaffolds offer additional advantages by improving spheroid uniformity and heterogeneity, respectively. These enhancements result in more reliable data for preclinical drug testing and translational research. Limitations, such as the lack of vasculature in MCTS, remain a challenge, but ongoing developments suggest promising solutions.

DISCUSSION

The findings of this narrative review highlighted the growing significance of multicellular tumor spheroids (MCTS) in cancer research as effective three-dimensional (3D) models that bridge the gap between conventional two-dimensional (2D) cell cultures and in vivo studies. MCTS provided a more physiologically relevant microenvironment, replicating key tumor features such as hypoxia, cell-cell and cell-extracellular matrix (ECM) interactions, and structural heterogeneity. These attributes made MCTS particularly valuable for studying tumor biology, drug resistance, and delivery strategies. Previous studies corroborated these advantages, demonstrating that 3D models like MCTS allowed for a deeper understanding of tumor behavior and treatment responses compared to 2D cultures (Khanna et al., 2020; Mitrakas et al., 2023).

One of the significant strengths of MCTS was their ability to replicate the hypoxic core observed in solid tumors, a feature critical to understanding tumor resistance mechanisms. Hypoxia-induced drug resistance, mediated by metabolic shifts and quiescent cell populations, was effectively demonstrated in MCTS, aligning with findings from prior research (Roy et al., 2023). The ability of MCTS to simulate these challenging conditions underscored their potential in evaluating therapeutic strategies under realistic tumor-like environments. Furthermore, the inclusion of advanced methodologies, such as acoustically mediated drug delivery and biosensing, enhanced the applicability of MCTS for preclinical testing, as these approaches significantly improved drug penetration and therapeutic efficacy (Lee & Kim, 2021).

Despite these strengths, certain limitations of MCTS persisted, particularly their lack of vasculature. This absence restricted their ability to fully replicate in vivo conditions, including nutrient transport and waste removal, which are essential for a complete understanding of tumor progression and therapy responses (Marie Roy et al., 2023). While scaffold-based techniques attempted to address these limitations by incorporating ECM components, they often introduced challenges related to reproducibility and cost. The reliance on static culture systems also limited the scalability of MCTS for high-throughput applications. Nevertheless, recent advancements in microfluidics and bioreactor-based systems showed promise in overcoming these barriers by enabling dynamic culture conditions and improving spheroid uniformity (Honglin Shen et al., 2021).

A recurring theme across the studies was the role of biomaterials in enhancing MCTS formation and heterogeneity. The use of natural and synthetic polymers facilitated the recreation of tumor ECM, improving the physiological relevance of MCTS for drug testing and biological studies. This development aligned with prior findings that emphasized the importance of ECM composition in tumor behavior and therapeutic responses (Kamatar et al., 2020). However, variations in biomaterial properties, such as stiffness and porosity, often resulted in inconsistent outcomes, highlighting the need for standardized protocols in future research.

The inclusion of mathematical modeling alongside experimental studies emerged as another strength of MCTS-based research. Models provided quantitative insights into drug penetration, retention, and distribution, offering a complementary perspective to experimental observations. For example, mathematical simulations helped predict nanomedicine behavior within MCTS, which was critical for optimizing drug formulations and delivery strategies (Sayoni Maitra Roy et al., 2023). Such integrative approaches not only enhanced the predictive accuracy of preclinical studies but also reduced the reliance on animal models.

Recommendations for future research emphasized the integration of vascularized MCTS models to better simulate tumor perfusion and drug delivery. The incorporation of patient-derived cells into MCTS was also suggested to improve the translatability of findings, enabling personalized therapeutic approaches. Additionally, the development of automated high-throughput systems for MCTS generation and analysis could address scalability issues, facilitating broader adoption in preclinical testing. Standardized protocols for biomaterial use and spheroid characterization were deemed essential for ensuring reproducibility and comparability across studies.

CONCLUSION

In conclusion, MCTS provided a robust platform for studying tumor biology and evaluating therapeutic strategies, offering significant advantages over 2D cultures. While limitations related to vasculature and scalability remained, advancements in biomaterials, microfluidics, and modeling approaches continued to expand the potential of MCTS in cancer research. By addressing these challenges and building on current progress, MCTS could further bridge the gap between in vitro and in vivo models, ultimately accelerating the development of effective cancer therapies.

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