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Review

Navigating Cancer Treatment: A Journey from 2D to 3D Cancer Models and Nanoscale Therapies

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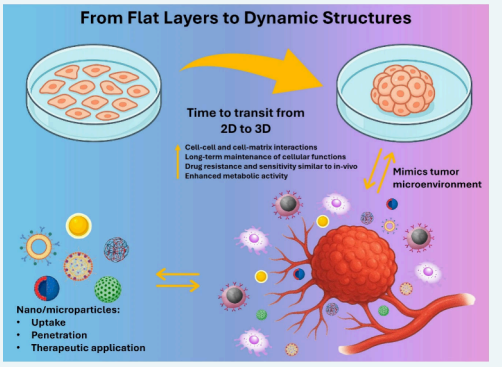
ABSTRACT: Cancer remains one of the most significant global health challenges, characterized by an increasing incidence and mortality rate worldwide. *In vitro* models play a significant role in the initial investigations of cancer biology, drug screening, and therapeutic development. However, although widely used, conventional 2D cell cultures fail to replicate the complex tumor microenvironment, leading to discrepancies in drug response and therapeutic efficacy. This perspective explores the transition from 2D to 3D culture models, highlighting their advantages, limitations, and impact on cancer research. Various 3D culture approaches, including scaffold-based systems, hydrogels, 3D-printed models, microfluidics, and organ-on-a-chip technologies, are discussed in terms of their relevance to cancer modeling and drug testing. Additionally, the review also highlights the integration of theranostic nano- and microparticles in cancer treatment, focusing on their application in drug delivery and interactions with 3D tumor spheroids and organoids. A comparative analysis of uptake mechanisms and interactions between particles and 3D models is presented along with advanced techniques for probing nanoparticle behavior and drug screening in these models. By bridging the gap between *in vitro* assays and clinical outcomes, 3D culture systems integrated with nanotechnology offer promising tools for improving cancer therapeutics.



KEYWORDS: *3D cell culture, nanotechnology, microparticles, organoids, 3D bioprinting, nanoparticles uptake*

INTRODUCTION

Cancer is the second leading cause of death worldwide.[1] It is characterized by the uncontrolled growth and development of cells caused by aberrant DNA expression. These genetic changes can occur because of random errors during DNA replication, exposure to carcinogens, or inherited from one of our parents.[2] The genetic alterations impact three primary categories of genes including proto-oncogenes, tumor suppressor genes, and DNA repair genes, which play a crucial role in the initiation and progression of the disease. Abnormal growth and division of cells result in the formation of a cluster of cancer cells known as tumors. Tumors can be benign or metastatic. Benign tumors are confined to one place, do not invade nearby tissues, and can be easily removed through surgery.[2] However, metastatic or cancerous tumors are life-threatening as they can spread to nearby tissues and other body parts, resulting in the formation of new tumors. There are more than 200 types of cancer, and they have been classified into different groups based on the type of cells from which they originated, including carcinoma, sarcoma, leukemia, lymphoma, and myeloma.[3] Traditional cancer therapies, such as surgery, chemotherapy, radiation therapy, immunotherapy, hormonal therapy, and laser therapy, have been employed in the treatment of cancer. However, these therapies may lead to incomplete tumor removal and side effects, potentially causing adverse effects on other organs in the body.[4]

Micro- and nanooncology is an emerging field with the potential to enhance cancer therapy while minimizing the side effects associated with traditional therapies. It involves the use of nano- and microsized particles (∼100−1000 nm) loaded with anticancer drugs for targeted delivery and controlled release. Nanoparticles (NPs) are mostly used owing to their small size, high surface-to-area ratio, and surface functionalization allowing them to easily enter the tumor environment and be retained for a long period of time. There are diverse types of NPs, including polymeric, liposomal, metallic, magnetic, quantum dots, graphene, carbon nanotubes, and dendrimers, which are used in treatment and cancer diagnosis. Cancer-specific antibodies, peptides, and targeting ligands can be

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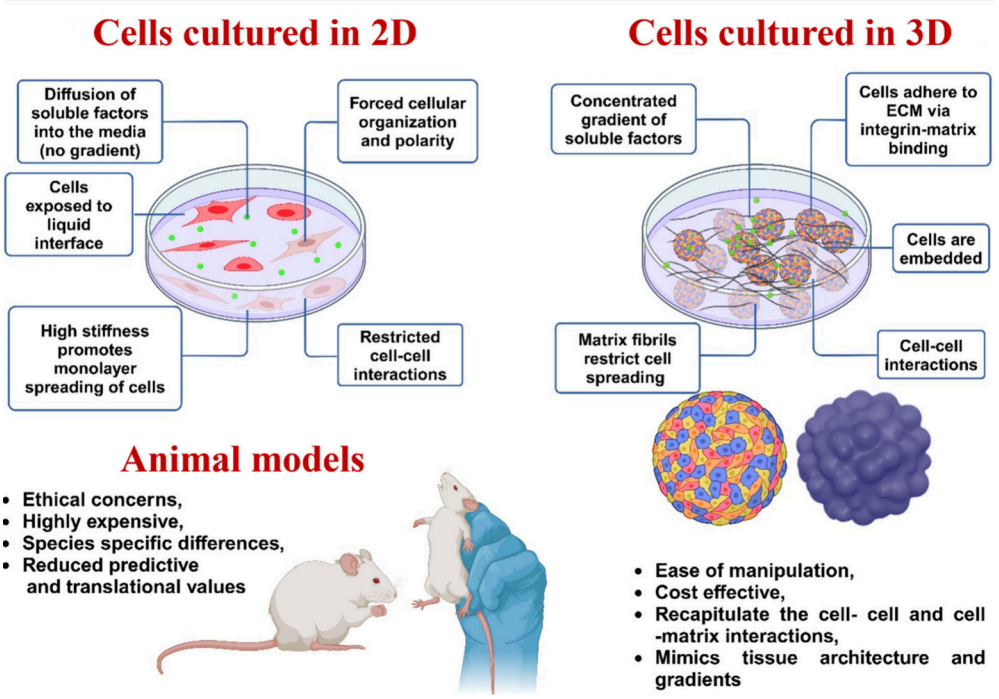

Figure 1. Schematic representation illustrates the key differences between 2D, 3D, and *in vivo* cancer models. Created with biorender.com.

conjugated with NPs for targeted delivery and efficient cancer diagnosis. Furthermore, microparticles (MPs) have recently become a popular area of research. Microparticles are also easily taken up by cells; however, the size of microparticles affects uptake among different cell lines. Thus, microparticles are selectively taken up more by cancerous cells compared to healthy cells, in contrast to nanoparticles.[5] This, in turn, can increase drug loading, reducing the frequency of drug administration due to their sustained release over an extended period.

For decades, micro/nanoparticles have been tested for cytotoxicity, biocompatibility, and uptake against *in vitro* bidimensional models (2D) of cancer cells, where cells can grow on a flat surface. The simplicity and cost-effectiveness of a 2D culture make it an accessible approach for exploring basic cellular responses. However, 2D culture platforms have significant limitations in matching native tumor biology, as tumors are not flat in the human body. They exist in a three-dimensional (3D) space with intricate cell−cell and cell-extracellular matrix (ECM) interactions along with a complex and dynamic microenvironment. Furthermore, while animal models are considered the gold standard for uptake and drug screening investigations, *in vivo* preclinical models are expensive, challenging to work with, and present ethical challenges (Figure 1). In addition, animal models are challenging to analyze for human-specific effects. Although patient-derived xenografts (PDXs) and genetically engineered mouse models offer promising platforms for personalized cancer medicine, they can be costly, time-consuming, and exhibit a low performance rate (3%) concerning the clinical success of anticancer therapeutics.[6,7] To address these limitations, there has been a notable shift toward 3D tissue culture in cancer research. In 3D cancer models, cell growth occurs at a more natural and sustained rate, and cells can have an extended lifespan compared to 2D models, where cell proliferation often happens at an abnormally fast pace. 3D cell culture models provide unique advantages in mimicking the microenvironment for tumors over *in vivo* models, effectively addressing the interspecies disparities present in PDX models. 3D cell culture models mimic the inherent architecture of tumors, featuring an outer proliferating zone, an inner quiescent region with restricted oxygen, nutrient, and growth factor diffusion, and a central core characterized by necrosis and hypoxia. There have been numerous approaches utilized to produce 3D cancer models, including spheroids,[8,9] organoids,[10] cell-seeded scaffolds,[11,12] 3D printed models,[13,14] microfluidics,[15,16] and organ on chip.[17,18] Moreover, distinct responses are observed in cells cultured in 2D and 3D environments when exposed to NPs and MPs. These responses are influenced by the cellular phenotype, functions, and physiological properties, including size, charge, and surface functionalities of particles.[19] In 3D cultured cells, there is distinct particle toxicity, limited penetration depth, and uneven particle distribution, resulting in lower uptake compared to 2D cultures.[20,21] Here, in particular, the extracellular matrix plays a crucial role in creating penetration barriers in the *in vivo* environment.

In this review, we examine the existing gaps in *in vitro* cancer culture models for recapitulating *in vivo* behavior, highlighting the superiority of 3D culture techniques and exploring various approaches to 3D cancer models. Additionally, we investigate the uptake, penetration, and interactions of nano- and microparticles within 3D tumor models. We examine different parameters and techniques that can enhance model *in vitro* systems, offering a closer representation of native human tumor physiology, which can aid in limiting the need for small animal models, and providing improved drug delivery systems with enhanced therapeutic efficacy.

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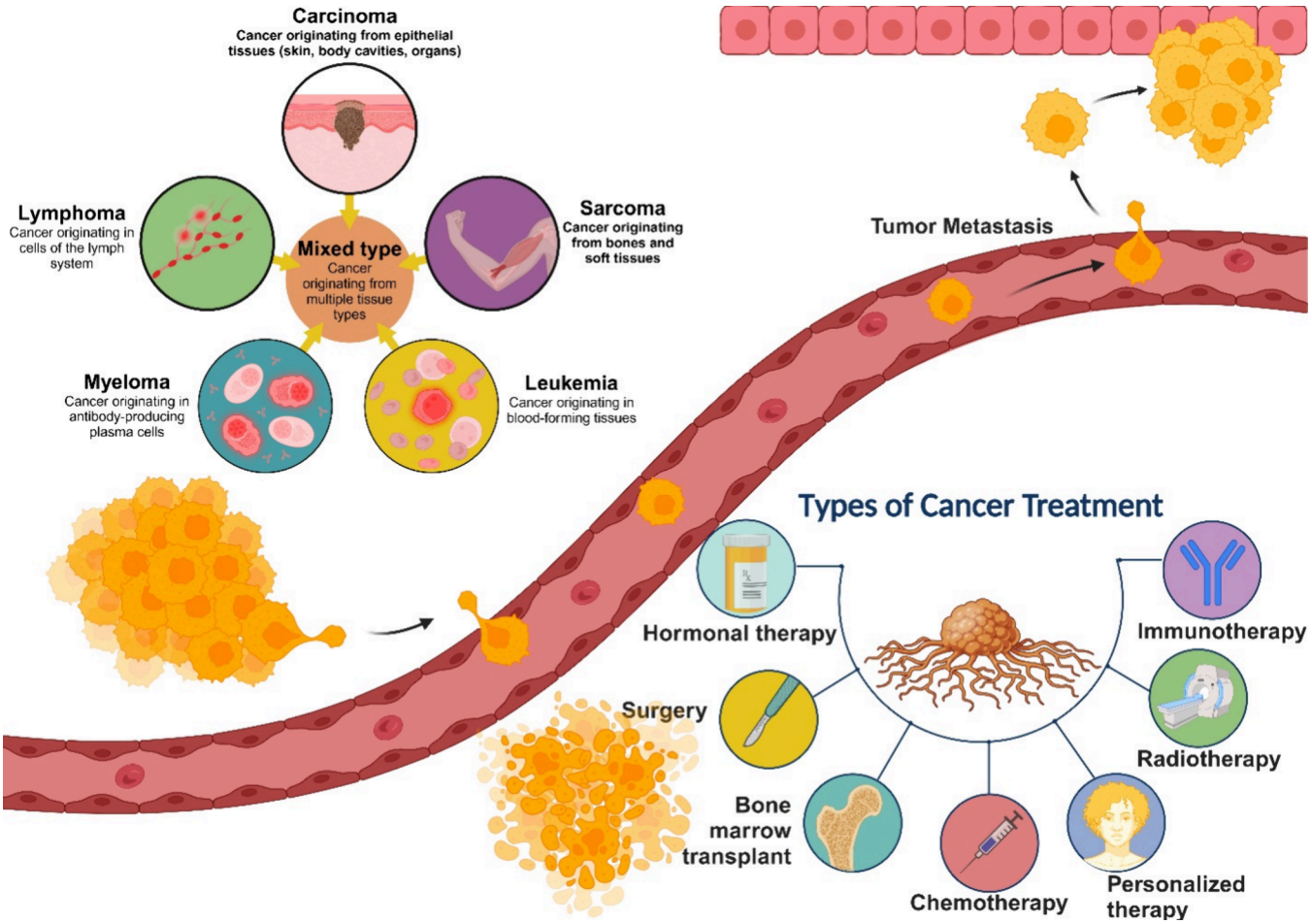

Figure 2. Schematic representation of cancer types and metastatic progression. Created with biorender.com.

CANCER: TYPES, TREATMENT CHALLENGES, AND THE GAP IN RESEARCH

Cancer is a complex group of diseases characterized by the uncontrolled growth and spread of abnormal cells. There are various types of cancer, each with distinct characteristics, origins, and treatment approaches (Figure 2). Uncontrolled and aberrant cell growth, a hallmark of cancer, is driven by various risk factors including age, lifestyle factors (alcohol, cancer-causing substances, diet, obesity, and sun exposure), and precancerous or genetic conditions. 90% of cancers are associated with alteration of genes. Major genes involved in cancer include oncogenes, tumor suppressor genes, and mismatch-repair genes, which can affect cell growth and are altered in some types of cancer. Genetic damage that leads to cancer occurs in oncogenes, tumor suppressor genes, and DNA repair genes. Oncogenes, such as HER2/ERBB2 and KRAS, are mutated proto-oncogenes that become permanently active, causing uncontrolled cell growth. Tumor suppressor genes, including TP53, BRCA1/2, and APC, normally slow cell division, repair DNA, and trigger apoptosis; when mutated, they fail to control cell proliferation, allowing cells with DNA damage to survive and divide.[22] DNA repair genes such as MLH1, MSH2, MSH6, and PMS2 normally correct replication errors, but mutations in these genes lead to accumulation of additional harmful mutations in oncogenes and tumor suppressor genes. These mutations disrupt the normal regulation of cell growth, division, and death. For example, TP53 mutations prevent damaged cells from undergoing apoptosis, while BCR-ABL fusion genes produce abnormal enzymes that drive blood cell proliferation. Mutated oncogenes and inactivated tumor suppressors together allow cells to grow uncontrollably, evade apoptosis, accumulate further genetic errors, and ultimately form tumors.[23,24]

Moreover, cancer cells exhibit remarkable heterogeneity within the primary tumor and during metastatic spread, resulting in difficulty in creating effective treatments. Also, the tumor microenvironment, including factors such as the ECM and immune cells, plays a crucial role in shaping the behavior of cancer cells, influencing their response to therapies. The tumor microenvironment plays a central role in regulating cancer cell behavior from the earliest stages of tumor development. Early neoplastic lesions are initially detected by immune cells, including T cells and dendritic cells. However, as tumors progress, the TME shifts toward an immunosuppressive state, characterized by reduced cytotoxic CD8+ T cells and NK cells, expansion of regulatory T cells (Tregs), and impaired dendritic cell function. This immune suppression allows cancer cells to survive and proliferate despite the presence of the adaptive immune system.[25] Chronic inflammation and recruitment of myeloid cells, such as tumor-associated macrophages and neutrophils, further shape the TME. These cells secrete reactive oxygen species and cytokines such as EGF, HGF, TGF-β, and IL-6, which promote cancer cell proliferation, migration, stemness, and DNA damage. They also remodel the extracellular matrix and stimulate angio-

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genesis, ensuring that tumors receive sufficient oxygen and nutrients to support their growth.[26] Metabolic changes within the TME also support tumor progression. Myeloid cells and other stromal components consume critical nutrients, alter glycolytic and lipid metabolism, and produce immunosuppressive mediators, which limit T cell activation and antitumor responses.[27] Together, these interactions create a tumor-supportive niche, enabling cancer cells to evade immunity, thrive under stress, and progress toward more aggressive and therapy-resistant states.

Understanding and targeting these diverse traits of cancer cells would help in developing innovative and personalized approaches to cancer diagnosis and treatment. The different types of cancers are categorized based on the tissue where they originate or their location.[28] The most common type of cancer is carcinoma, which originates from epithelial cells that line the body's internal and external surfaces. It typically develops in organs such as the skin, lungs, breast, colon, and prostate. The common types of carcinomas include basal cell carcinoma, squamous cell carcinoma, adenocarcinoma, and ductal carcinoma. Treatment approaches for carcinoma often include surgery, chemotherapy, radiation therapy, targeted therapy, and immunotherapy depending on the specific type and stage of the cancer. Leukemia affects the blood and bone marrow, resulting in the overproduction of abnormal white blood cells. It originates from hematopoietic stem cells in bone marrow. Leukemia is mainly classified into four types: acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, respectively. Common treatments for leukemia typically involve chemotherapy, targeted therapy, radiation therapy, stem cell transplantation, or a combination of these approaches. Sarcoma is a rare type of cancer that develops in the body's connective tissues, including bones, muscles, cartilage, tendons, and blood vessels. It can be further categorized into two main types: soft tissue sarcoma and bone sarcoma. Common types of sarcomas include osteosarcoma, chondrosarcoma, liposarcoma, and angiosarcoma. Treatment for sarcoma often involves surgery to remove the tumor followed by chemotherapy, radiation therapy, targeted therapy, or a combination of these treatments. Lymphoma is cancer of the lymphatic system, which includes lymph nodes, lymphatic vessels, and lymphoid organs such as spleen and thymus. It originates from lymphocytes, a type of white blood cell involved in the body's immune response. Lymphoma is categorized into two main types: Hodgkin lymphoma and non-Hodgkin lymphoma. Common subtypes of non-Hodgkin lymphoma include diffuse large B-cell lymphoma, follicular lymphoma, and mantle-cell lymphoma. Treatment for lymphoma may include chemotherapy, radiation therapy, immunotherapy, targeted therapy, or stem cell transplantation, depending on the subtype and stage of the disease. Myelomas, also known as multiple myeloma, are cancers that develop in plasma cells, which are a type of white blood cell responsible for producing antibodies. They typically originate in the bone marrow and can cause bone damage, anemia, and impaired immune function.[28] Treatment for myeloma may include chemotherapy, targeted therapy, immunotherapy, stem cell transplantation, or a combination of these approaches.

Brain and spinal cord cancer, also known as central nervous system (CNS) tumors, originates from abnormal cells in the brain or spinal cord. They can be primary tumors, which arise in the brain or spinal cord tissue, or secondary tumors, which metastasize from cancer elsewhere in the body. Common types of brain and spinal cord tumors include gliomas, meningiomas, and medulloblastomas. Treatment for these tumors depends on the tumor type, location, size, and grade. It may involve surgery, radiation therapy, chemotherapy, targeted therapy, or a combination of these treatments.

CHALLENGES IN CANCER TREATMENT

Cancer treatment today is a complex and diverse process to eradicate or control cancer, while minimizing damage to healthy tissues. One of the primary treatment strategies for cancer involves **surgical resection of tumors**, which is most effective for localized disease and can be curative in many cases. Surgery offers the advantage of immediate tumor removal and provides tissue for pathological evaluation; however, it is not suitable for patients with advanced or metastatic cancer, and access to specialized surgical facilities remains limited in many low- and middle-income countries.[29,30] **Radiation therapy** is another common treatment that utilizes high-energy radiation to destroy malignant cells. Recent innovations, including intensity-modulated radiation therapy, proton beam therapy, and stereotactic radiosurgery, have allowed for more precise tumor targeting, thereby sparing surrounding healthy tissues.[31] The major advantages of radiotherapy are its noninvasive nature and applicability to both curative and palliative applications.[32] Nonetheless, radiation can still cause collateral damage, such as mucositis, fibrosis, and secondary malignancies, and advanced radiotherapy technologies remain largely available only in high-income regions due to high infrastructure cost.[32]

Chemotherapy. Chemotherapy remains a cornerstone of systemic cancer therapy, particularly for cancers that have been disseminated. Chemotherapeutic agents work by targeting rapidly dividing cells, thereby reducing tumor burden.[33] While effective, their lack of specificity often results in damage to healthy proliferating cells, causing side effects such as nausea, alopecia, bone marrow suppression, and organ toxicity.[34] Furthermore, the development of drug resistance is a significant limitation. Despite these challenges, chemotherapy remains more globally accessible compared to advanced therapies.

Targeted Therapies. These represent a more refined approach, designed to interfere with specific molecular drivers of cancer progression, such as tyrosine kinases, growth factor receptors, or mutated oncogenes.[35] These therapies generally offer improved efficacy and reduced toxicity compared with chemotherapy, but resistance mechanisms can limit long-term effectiveness. Furthermore, in environments with limited resources, targeted medications are less accessible due to their high cost and need on advanced molecular diagnostics.[36]

Immunotherapy. This has revolutionized cancer treatment in the past decade by utilizing the patient's own immune system. Approaches such as immune checkpoint inhibitors (e.g., PD-1/PD-L1 and CTLA-4 inhibitors), CAR T-cell therapies, and therapeutic cancer vaccines have shown durable responses in subsets of patients, especially in hematological malignancies and some solid tumors.[37] However, immunotherapy is associated with unique toxicities, including severe autoimmune reactions, and is not universally effective.[38] Additionally, the production and delivery of advanced immunotherapies such as CAR T-cell therapy are highly complex and costly, limiting their availability to specialized centers in developed countries.[39] **Hormone therapy** remains

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essential for cancers that are driven by hormonal signaling, such as breast and prostate cancers.[40] By blocking hormone production or interfering with receptor-mediated signaling, hormone therapy can significantly prolong survival and improve quality of life.[41] Despite its efficacy, hormone therapy may lead to resistance over time and can cause metabolic side effects, such as osteoporosis and cardiovascular complications. Nonetheless, these treatments are relatively inexpensive and widely available compared to newer targeted or immune-based therapies.

Despite these advancements, the treatment of cancer remains a significant challenge. Malignant cells can adapt and develop resistance to therapies, which reduces their effectiveness over time. Moreover, side effects from treatments such as chemotherapy and radiation can be severe, affecting a patient's functional organs (liver, kidney, spleen, etc.) along with prolonged fatigue, nausea, immune suppression, and neuropathy. Late-stage diagnosis is another significant challenge, as many cancers are not detected until they are in advanced stages, when treatment options are less effective and chances of survival are lower. Moreover, the metabolic heterogeneity of cancer, a genetically unique process, can vary significantly even within the same tumor site, making it difficult to develop highly effective treatment approaches.[42]

Cancer research faces several critical gaps, including the need for effective, noninvasive early stage detection methods and affordable screening programs, particularly for cancers often diagnosed at advanced stages, like pancreatic and ovarian cancers.[43,44] Metastasis, the leading cause of most cancer-related deaths, requires a deep understanding of its mechanisms to develop therapies targeting the metastatic process.[42] Existing immunotherapies need to improve, as they have great potential to overcome resistance and enhance efficacy. Another major gap in cancer research exists between the findings from traditional 2D cell cultures and *in vivo* assays. Cultures in 2D, where cells are grown on flat, rigid surfaces, do not accurately mimic the 3D structure, cellular heterogeneity, and complex microenvironment of tumors in the human body. Thus, it often results in drug failure in later stages of development. Despite advances in drug discovery, the success rate for new drugs reaching FDA approval has decreased, with most failing in early clinical trial phases (approval rate of 5.3% between 2011 and 2020).[45] Many drugs that pass trials may still face severe side effects, leading to market withdrawal, highlighting the need for a shift in how drugs are prioritized for testing.[46] However, while *in vivo* trials are more physiologically relevant than *in vitro* 2D models, they have their own set of limitations. Animal models do not always accurately represent human cancer due to species-specific differences. Ethical considerations, high costs, and the complexity of isolating specific cellular interactions complicate *in vivo* research. Animal testing, especially for pharmaceuticals, often fails to predict human responses due to poorly designed experiments and significant species differences. While there are many examples (e.g., penicillin) where there is a close alignment between the effects of small molecules on mice and humans, there are many more examples where there are very different outcomes observed between humans and mice, such as the differing reactions to corticosteroids, troglitazone, and many more.[47] Systematic reviews of animal screening test are rare, and their methodological limitations, such as small sample sizes and inadequate randomization, hinder their reliability for human predictions.[48] Improving experimental quality could help bridge the gap between animal research and human clinical outcomes. Thus, there is a need for potential systems to bridge the gap between 2D cultures and *in vivo* systems. Addressing these gaps could significantly enhance our ability to prevent, detect, and treat cancer, ultimately improving patient outcomes and the quality of life.

TRANSITION FROM 2D TO 3D CELL CULTURE MODELS

Culturing cells in 3D has emerged as a promising method to create cancer tissues that replicate the *in vivo* microenvironment, presenting a bridge between the gap of 2D cultures and *in vivo* models.[49] Although still in its early stages of development, 3D cell culturing has already demonstrated considerable advantages over 2D cultures. Current research continues to investigate the comparative benefits and drawbacks of 2D versus 3D cell cultures, highlighting the potential of 3D models to provide more physiologically relevant data for preclinical trials, allowing for patient-specific therapies. Cell proliferation and gene expression: One of the key advantages of 3D models is the prolonged cell viability and proliferation. Work using colorectal cancer cell lines and patient FFPE samples found that 3D cultures showed significant differences in cell proliferation, gene expression, and drug responsiveness compared to 2D cultures. Additionally, 3D models and FFPE samples shared similar epigenetic profiles, while 2D cultures exhibited altered methylation and microRNA patterns, highlighting the limitations of 2D models in cancer research.[50] It was also found that the cells in 3D showed a similar gene profile to that found *in vivo*. Two novel fully synthetic 3D scaffolds made from polyhydroxybutyrate, solvent casting particle leaching membrane, and an electrospun membrane were developed for culturing B16 F10 melanoma and 4T1 breast cancer cells. These models were compared with traditional 2D cultures. Cells in all 3D models formed spheroids, showed increased chemotherapy resistance, and had gene expression profiles more similar to *in vivo* tumors.[51] Later, they compared the transcriptomic profiles of SiHa cervical cancer cells in 3D versus 2D cultures, revealing upregulation of immune activation, angiogenesis, and tissue remodeling pathways in 3D cultures. Higher HPV16 gene expression in 3D cervical cancer cultures indicates that the system better replicates the tumor microenvironment because these viral oncogenes are tightly linked to tumor-relevant processes, such as proliferation, apoptosis evasion, and genomic instability. The 3D structure provides physiological cues, such as cell−cell interactions and ECM signaling, that enhance viral transcription and activate pathways involved in immune evasion, angiogenesis, and tissue remodeling. In contrast, 2D monolayer cultures lack these complex interactions, resulting in lower HPV16 gene expression and a less physiologically relevant model.[52] Further, a meta-analysis reviewed prior work to compare gene expressions in 3D and 2D OvCa cultures. The analysis found significant differences in gene regulation based on the scaffold used for 3D cultures, including genes such as C3, CXCL1, CXCL2, CXCL8, IL1B, SLP1, FN1, IL6, DDIT4, PI3, LAMC2, CCL20, MMP1, IFI27, CFB, and ANGPTL4, which were differentially expressed in 3D versus 2D cultures.[53] Genes are essential in cancer research because they represent hallmarks of tumor biology that become more evident in 3D cultures compared with 2D. These genes regulate inflammation, immune signaling, angiogenesis, extracellular matrix remodeling, hypoxia response, and

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stress adaptation processes that drive tumor progression, invasion, and therapy resistance. Their consistent enrichment across multiple scaffolds highlights their importance in capturing the complexity of the tumor microenvironment and underscores the value of 3D models for probing physiologically relevant cancer biology.

Cell migration: Cancer cell migration work has revealed significant differences between 2D and 3D culture models. In 3D cultures, cancer cells generally exhibited slower migration speeds but more realistic patterns compared to 2D cultures due to the increased physical constraints and complex extracellular matrix architecture. ECM plays a significant role in cell movement and is essential for coordinated 3D movement. This investigation showed that collective cell migration through collagen matrices occurred via a dynamic pulling mechanism, with tensile forces increasing at the front to both propel movement and regulate cell-matrix interactions.[54] Migration mechanisms vary, with 3D environments requiring more proteolytic ECM degradation and cell deformation. 3D models also allow for more directional and persistent migration, better capture collective cell movement, and provide a more physiologically relevant microenvironment, including ECM composition, oxygen and nutrient gradients, and cell−cell interactions. These factors significantly influence migration strategies and cellular responses. 3D cultures also affect the migration of cells. This work explored the impact of synthetic hydrogels, specifically PEG-based matrices, on the behavior of primary human fibroblasts in 3D culture. The research revealed that fibroblast proliferation differed significantly between 2D and 3D environments, with matrix stiffness (elastic modulus >1200 Pa) acting as a barrier to cell growth. By comparing biomimetic PEG-based and collagen gels, these results highlighted how variations in gel viscoelasticity and network structure influence cell-mediated matrix remodeling, offering valuable insights into cell-matrix interactions for regenerative medicine and cancer research.[55] Another article showed a 3D cell culture system using inorganic nanoscale scaffolding via nanoimprinting technology. By reducing cell-to-substrate contact, the system promoted spontaneous tumor cell migration, intercellular adhesion, and the formation of 3D spheroids with hypoxic cores, while maintaining cellular proliferation and viability.[56] Moreover, porosity of the 3D matrix affects the movement of cells. Dynamic hydrogels with tunable microporosity were developed for tissue engineering by using stimuli-responsive porogens made from gelatin, alginate, and hyaluronic acid. The hydrogels formed sequential pores in response to specific stimuli (temperature, chelation, and enzymatic digestion), allowing for controlled macroporosity during cell culture. Bovine chondrocytes encapsulated in the hydrogels showed enhanced viability, proliferation, and collagen production, demonstrating the potential of these scaffolds for tissue regeneration.[57,58]

The mechanical responses of cells differ significantly between the 2D and 3D culture environments. These differences arise from altered cellular behaviors and interactions. In 2D cultures, cells are exposed to high stiffness surfaces that provide supraphysiological mechanical signals.[59] This flat, continuous surface allows for adhesion, spreading, and migration. The stiffness of glass or plastic used in 2D cultures is multiple orders of magnitude higher than that seen in soft tissues, directly affecting cell adhesion, spreading, migration, and differentiation. In contrast, 3D cultures offer a tunable, relatively low-stiffness environment that more closely resembles tissues.[60] The 3D extracellular matrix features lower stiffnesses, local structures like collagen fibers, and pores that help in migration and invasion.[61] This environment leads to reduced proliferation, altered migration patterns, smaller and more distributed focal adhesions, and directional cell elongation along fibers. 2D cultures constrain cells to a single layer, limiting their ability to form complex 3D structures.[62] Particle-tracking microrheology was used to investigate molecular motor function and stiffness on metastatic breast cancer cells embedded in 3D collagen gels. They showed that increasing collagen concentration in 3D environments led to elevated intracellular stiffness and motor activity, with fluctuations influenced by ECM concentration even in the presence of various chemotherapeutic and anti-MMP drugs, highlighting the critical role of ECM density in drug screening.[63] In other work, alginate hydrogels with varying stiffnesses were used to understand how substrate elasticity affects breast cancer cell activity. The hydrogel stiffness (150−4000 kPa) was measured using atomic force microscopy. Breast cancer MCF-7 cells cultured in 3D gels formed spheroid clusters, similar to *in vivo* conditions, unlike the flat shape seen in 2D cultures. They observed that cell viability decreased as hydrogel stiffness increased, with the highest cell growth occurring in the softest gels (150−200 kPa). This highlights the importance of using physiologically relevant 3D models for cancer research.[64]

APPROACHES TO 3D CANCER CULTURE MODELS

In recent years, there has been a significant shift toward utilizing 3D fabrication techniques to create *in vitro* tissue models that better mimic the intricate cellular environments seen *in vivo*.[65−67] Thus, with growing interest in using 3D fabricated systems for *in vitro* investigations, various 3D systems have been developed utilizing different materials, procedures, and applications.[66,67] One prevalent approach for 3D cell culture is using structural scaffold models. These models are physical structures that support and structure cell growth in an environment that more closely mimics physiological conditions.[68] Various scaffold models have been employed in a wide range of cancer investigations ranging from breast to lung, colon, or prostate cancer.[67,68]

3D scaffold models have enhanced our understanding of the factors influencing cancer cell proliferation and response to anticancer therapeutics. Scaffolds are designed to mimic tissue structure, facilitate cell−cell and cell-matrix interactions, and allow oxygen and nutrient diffusion. These design criteria are essential for cell growth and behavior regulation.[69] Thus, scaffolds must satisfy the fundamental criteria of being biocompatible, immunologically inert, and biodegradable.[69] Moreover, carefully choosing scaffold material is a crucial step in the fabrication process and should be guided by a given material's tunability and inherent properties. This importance is underscored by the collective influence of 3D topography, surface attributes, and scaffolds' biophysical and biochemical characteristics on cellular processes, such as adhesion, proliferation, migration, and differentiation.[70] Hence, scaffold models are derived from various materials ranging from natural to synthetic, including biodegradable polymers and fabrication techniques such as freeze-drying or particle leaching. Natural scaffolds used as scaffolding materials are derived from biological systems such as plants, microorganisms, or algae.[71] Naturally derived scaffolds are widely used because they are biocompatible and biodegradable, thus allowing for the culture

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of diverse cell types. The most used natural-based scaffolds are composed of polysaccharides, proteins, nucleic acids, or polyhydroxyalkanoates.[71,72] The most frequently used polysaccharides are alginate, gelatin, collagen, chitosan, cellulose, amylose, or hyaluronic acids.[73−76] Most of these widely used naturally derived polymers have structural properties comparable to those found *in vivo*. For instance, chitosan is derived from crustacean shells with a similar chemical structure to glycosaminoglycan, and it is highly used because of its properties such as hydrophilicity and biodegradability.[73,75]

Various reports have highlighted the utility of these polymers as 3D scaffolding materials for investigating cancer behaviors.[75] One of the limitations of using natural polymers for scaffolding material is limited mechanical stability and suitability for cell growth; therefore, most natural-based scaffolds are formulated using polymer composites to enhance their mechanical properties. For instance, Xu et al. investigated the effects of matrix stiffness on prostate cancer cell response using chitosan-alginate (CA) scaffolds with different stiffness levels. Their work utilized a lyophilization technique to fabricate CA scaffolds of varying stiffness, simulating different stages of prostate cancer tumors. In other works, Zhou et al. introduced a 3D porous scaffold-based high-throughput platform for cancer drug screening utilizing a chitosan-hyaluronic acid (CHA) composite scaffold. The CHA scaffold, fabricated through freeze-drying, offered tunable pore sizes (60, 120, and 180 μm) and demonstrated significant potential in assessing drug responses and tumor behavior in glioblastoma multiforme cell lines.[74]

Another limitation is insufficient attachment sites, thus affecting cell adhesion, which can be overcome via conjugation to other polymers or proteins, such as Arg-Gly-Asp (RGD) peptides, for enhancing cell adhesion. For instance, alginate generally lacks cell-adhesion sites and thus can be used with polydopamine (PDA) to enhance its properties. Thus, in the work of Luo et al., the alginate-PDA scaffold was used as a platform for breast cancer therapy and breast tissue repair. This scaffold demonstrated mechanical properties akin to human breast tissues, promoting the adhesion and proliferation of normal breast epithelial cells. The findings suggest promising applications for this scaffold in postsurgery photothermal therapy of breast cancer and tissue repair, further enriching the landscape of natural scaffold materials in 3D culturing platforms for anticancer therapeutics.[77]

Synthetic scaffolds are also commonly used. Synthetic polymers offer tunable properties and enhance mechanical stability compared with their natural counterparts. Some commonly used synthetic polymers for 3D cancer research include polycaprolactone (PCL), polyacrylamide (PAA), poly(lactic acid) (PLA), poly(lactide-*co*-glycolic acid) (PLGA), or polyurethane.[78,79] Rabionet et al. fabricated two polycaprolactone materials with different fiber concentrations through electrospinning for 3D breast cancer culture.[80] Their results show enhanced cell proliferation, elongation, and aldehyde dehydrogenase activity on a 15% PCL scaffold compared to cells cultured on lower concentrations of PCL and cells grown on traditional tissue culture plates. They also showed that the mammosphere-forming index was significantly higher for the 15% PCL scaffold than for 2D culture plates. These results highlighted the utility of using electrospinning as a fabrication technique to design porous PCL scaffolds for 3D cancer investigations. Additionally, Rabionet et al. highlighted that PCL can be used as a noncytotoxic polymer capable of facilitating and persevering cellular functions for future cancer applications.

In other work, Sun et al. used waterborne biodegradable polyurethane (WBPU) scaffolds as an *in vitro* platform for investigating lung cancer.[79] In this investigation, a series of WBPU scaffolds ranging from 1 to 10 kPa were fabricated in 96-well plates by using the lyophilization technique. Their results showed that L929 cells seeded onto the PU-based scaffold developed multilayered structures after 7 days of incubation. However, the stiffness of the scaffolds contributed to the degree of cell elongation and aggregation behaviors similar to those observed on culture plates. However, lung cancer cells in PU4 scaffolds (1.63 kPa) displayed morphologies like the clinical morphology in lung cancer. Controlling the scaffold stiffness to match the native stiffness environment on the tissue allowed for physiologically relevant circumstances. Their results also showed higher expression of cancer stem cell markers, cell proliferation and invasion-related proteins, and drug resistance proteins for lung cancer WBPU scaffolds and xenograph models, suggesting that PU-based lung cancer models are more physically relevant to native lung cancer tissue models.

While some have demonstrated the feasibility of using some synthetic-based scaffolds without any additional modification,[81] synthetically derived scaffolds often require additional functionalization to support cell adhesion, viability, and cell proliferation.[82−85] For example, Pal et al. developed a hybrid scaffold composed of gelatin methacrylate (GelMA) and poly(lactide-*co*-glycolic acid) (PLGA) to understand the epithelia-mesenchymal transition in cancer progression.[83] In this work, the hybrid scaffold was composed of electrospun PLGA fibers embedded in GelMA hydrogels, and their findings show that it emulates the fibrous complexity of the native ECM microenvironment. Compared to cells cultured independently on GelMA and PLGA scaffolds, the epithelial-mesenchymal transition was more prevalent in hybrid scaffolds with higher expression of N-cadherin and vimentin in hybrid scaffolds for both breast cancer (MDA-MB231) and gastric cancer (MKN74) cell lines. They also demonstrated that a hybrid scaffold promotes multicell aggregation. Their results suggest that epithelial-mesenchymal transition is facilitated by the hybrid scaffold's topographical and biochemical cues.

FABRICATION METHODS: 3D BIOPRINTING

3D bioprinting, a subset of additive manufacturing (AM), is one of the most promising fabrication techniques for producing 3D scaffolds[67] (Figure 3). Unlike traditional AM, 3D bioprinting uses living cells encapsulated in biomaterials, collectively termed *bioinks*, which are deposited layer-by-layer to generate constructs with complex geometries and high spatial resolution.[86] This allows for controlled cell distribution within scaffolds and enables the fabrication of biologically functional tissue-like structures.[87,88] Several distinct 3D bioprinting methods are employed for scaffold fabrication, each with unique strengths and limitations.[68−70] Inkjet, or droplet-based, bioprinting is one of the earliest 3D printing methods adapted from 2D inkjet desktop printers. This technique uses either a continuous inkjet or a "drop-on-demand" approach to deposit precise droplets of low-viscosity bioinks.[89] It is cost-effective and well-suited for high-throughput applications, such as drug screening. However, its reliance on low-viscosity materials limits scaffold stiffness, and low initial cell seeding densities can restrict tissue matura-

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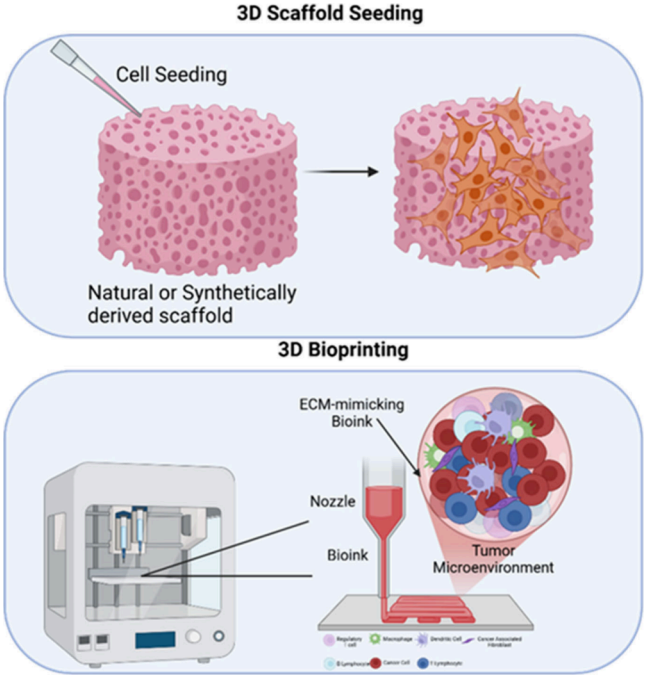

Figure 3. 3D scaffold-based cell seeding and 3D extrusion-based bioprinting. Created with biorender.com.

tion.[90] Extrusion-based bioprinting is the most widely used method in tissue engineering, which involves the continuous deposition of bioinks through a nozzle to produce 3D filaments and build scaffolds. It is compatible with a broad range of natural and synthetic polymer blends, enabling tunable viscoelastic properties that mimic those of native cell microenvironments. Despite being scalable and cost-effective, this method often suffers from lower resolution and shear-induced cell damage. Another method is laser-based bioprinting, which is a high-precision and high-resolution method that can achieve microscale features (~10−100 μm), making it useful for applications requiring fine architectural detail. Nevertheless, its use is constrained by the limited range of compatible biomaterials and risks of cell cytotoxicity from laser exposure and curing agents.[67] Lastly, vat-polymerization-based bioprinting (e.g., stereolithography) relies on light-based curing of photosensitive bioinks. It enables rapid fabrication of complex geometries but is similarly limited by material compatibility and potential cytotoxicity from photoinitiators.[68−70] Regardless of the selected bioprinting method one common critical factor, and often limiting factor, considered is the initial cell seeding density achievable with each printing method to generate scaffolds with desired functionalities for downstream applications such as drug screening and tissue regeneration.[91,92]

Collectively, these techniques provide versatile options for tailoring scaffold architecture, mechanical properties, and biological functions to suit diverse biomedical applications. Bioprinted scaffolds have been used *in vitro* to create models of functional human organs (e.g., heart, kidney, skin, liver) and to support applications in tissue regeneration, disease modeling, drug testing, and personalized medicine.[68,69] In cancer research, 3D bioprinting offers a significant advantage over traditional 2D culture by providing the means to replicate the tumor microenvironment (TME) with higher physiological relevance. By incorporating multiple cell types, such as immune cells, fibroblasts, and endothelial cells, into bioprinted scaffolds, researchers can recreate tumor-stromal interactions, angiogenesis, metastasis, and drug resistance.[71] For example, Shi et al. used electrohydrodynamic jet (E-jet) bioprinting to fabricate drug-loaded scaffolds for breast cancer treatment, demonstrating both inhibition of recurrence and promotion of wound healing. Similarly, Sun et al. applied extrusion-based bioprinting with patient-derived colorectal cancer cells to develop personalized chemotherapy models, providing a reliable platform for individualized treatment.[72]

Other work has leveraged stereolithography bioprinting to integrate osteogenic cells or mesenchymal stem cells within GelMA hydrogels and hydroxyapatite composites, creating bone-like matrices cocultured with breast cancer cells to model sternal metastasis.[73] Such approaches highlight the ability of 3D bioprinting to generate physiologically relevant cancer models for investigating tumor progression, drug screening, radiation therapy, immunotherapy, and biomarker discovery.[93,94] By combining different bioprinting methods with advanced bioinks, researchers can recreate complex tissue microenvironments and accelerate the translation of cancer models into personalized therapeutic strategies

Exploring Drug Toxicity in 2D and 3D Models. 2D cell culture models have been most utilized in drug treatment research and experimentation. However, they lack physiological relevance, as cells lose their natural architecture and fail to mimic *in vivo* interactions, leading to inaccurate predictions of drug toxicity.[95] As a result, when the cancer drugs progressed to later phases of clinical trials and proved ineffective, it raised questions, as earlier tests in 2D cultures had demonstrated its effectiveness.[96] Only a small percentage of new drugs successfully pass clinical trials, often due to poor translation from preclinical models. Current experimental models, such as 2D cell cultures, fail to replicate the complexity of tumors, particularly lacking cell−matrix interactions and apicobasal polarity. While 3D models such as spheroids better mimic tumor architecture, they still lack essential components like vasculature. Animal models also have limitations, such as species differences and incomplete immune systems, and they raise ethical concerns. In response, advanced technologies, including 3D bioprinted tumor models and organ-on-a-chip systems, are increasingly used to create physiologically relevant in vitro platforms that closely mimic the tumor microenvironment. 3D bioprinting allows precise spatial organization of cancer cells, stromal cells, and extracellular matrix components, enabling the fabrication of tissue-like architectures with controlled porosity, stiffness, and nutrient gradients. These features facilitate the realistic evaluation of nanoparticle penetration, cellular uptake, and drug release kinetics. Organ-on-a-chip systems integrate microfluidic channels to simulate blood flow, interstitial fluid dynamics, and tissue−tissue interfaces, providing dynamic mechanical and biochemical cues that influence nanoparticle distribution, immune interactions, and tumor response. Both platforms allow for incorporation of patient-derived cells, supporting personalized assessment of nanoparticle-based therapies. Collectively, these models offer superior predictive power compared to traditional 2D cultures, enabling systematic testing of nanoparticle efficacy, toxicity, and delivery mechanisms in a manner that bridges the gap between preclinical trials and clinical translations.[97] Here, drug sensitivities were compared between 2D and 3D cultures in triple-negative breast cancer cell lines, and it was found that 3D cultures were generally more resistant

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to epirubicin, cisplatin, and docetaxel. The IC50 values were higher in 3D cultures, with significant correlation for cisplatin and epirubicin but not for docetaxel. These findings suggest that a 2D culture may still be useful for testing DNA-damaging agents like cisplatin, while a 3D culture offers a more accurate model for overall drug resistance.[98] In another investigation, 65 drugs were screened for their efficacy against patient-derived glioma stem cells in 2D cultures and 3D glioblastoma organoids. The screening revealed that different drugs showed selective efficacy across patient-derived models, with costunolide, a TERT inhibitor, proving effective in reducing cell viability in both primary and therapy-pretreated tumor models.[99] Further, the report compared the effects of doxorubicin and cisplatin on 3D multicellular structures and 2D monolayers of endometrial cancer cells. It was found that 3D structures formed distinct morphologies and exhibited greater resistance to the drugs compared to 2D cultures, with cancer cell responses varying by cell line. Doxorubicin induced less apoptosis and had weaker effects on cell proliferation and Erk1/2 phosphorylation in the 3D structures. Also, both drugs paradoxically stimulated VEGF secretion, and 3D cultures showed higher SOD-1 levels and sustained Akt phosphorylation, indicating the importance of the microenvironment in drug responses. These results suggest that 3D models offer a more accurate platform for drug screening in endometrial carcinoma.[100]

Microwell platforms for the high-throughput generation of 3D prostate cancer microaggregates have been developed for providing a more accurate model for drug testing than traditional 2D cultures. In one report, the response of these aggregates to docetaxel was more consistent with *in vivo* results than 2D cultures, suggesting that 3D prostate cancer microaggregates are a better model for drug testing and may improve the predictability of clinical outcomes.[100] Even the type of 3D matrix affects drug toxicity. For instance, how different matrices affect the behavior of prostate cancer cells in 3D culture has been explored, comparing biologically derived matrices (Matrigel, Cultrex BME) and a synthetic matrix (Alvetex scaffold). It was found that the matrix type influenced cell proliferation, drug responses, and protein expression related to drug sensitivity and resistance. For example, in DU145 cells, β-III tubulin expression correlated with docetaxel resistance, while EGFR expression linked to rapamycin sensitivity. In LNCaP cells, p53 dimer expression was associated with docetaxel resistance. These findings underscore the importance of matrix selection for 3D culture models in drug screening and cell biology research.[101]

Vesa et al. developed a high-throughput three-dimensional drug screening method to compare drug responses in JIMT1 breast cancer cells grown in 2D, poly(2-hydroxyethyl methacrylate)-induced 3D, and Matrigel-based 3D cultures. After screening 102 compounds, results showed significant variations in drug responses between the models. Cells cultured on Matrigel were generally more sensitive to drugs than those grown in 2D, while poly(2-hydroxyethyl methacrylate)-cultured cells resembled 2D cultures. Gene expression profiles of matrigel-cultured cells closely matched those of xenograft tumors, suggesting that 3D cultures offer a more *in vivo*-like environment for drug screening and can be used for high-throughput testing of compound libraries.[102]

CHALLENGES IN 3D CULTURES

Three-dimensional cell culture has emerged as a powerful tool for more accurately modeling physiological conditions *in vitro*, but it also presents several challenges that need to be addressed. Challenges including uniform cell distribution, hypoxia, high costs, difficulties in replicating cell microenvironments, culture analysis, and the complexity of the matrices hinder widespread acceptance. Microscopy imaging is one of the primary difficulties in 3D cell culture. The increased size and complexity of 3D structures can make traditional imaging and analysis techniques less effective. 3D imaging poses challenges due to sample thickness exceeding the focus depth of conventional systems and light scattering, leading to poor image quality. Techniques like confocal laser scanning microscopy, optical sectioning structured illumination microscopy, and light sheet fluorescence microscopy are preferred for optical sectioning, although they face limitations such as phototoxicity and photobleaching, complicating data acquisition and analysis. Researchers often struggle with obtaining clear images throughout the entire depth of a 3D culture, and quantitative measurements may be hindered by the inability to easily dissociate cells or access the inner regions of spheroids. This can lead to inaccurate results or difficulties in interpreting data.[103]

Another significant hurdle is the formation of nutrients and oxygen gradients within 3D cultures, especially as they grow larger. Factors including cell seeding density, media height, cellular oxygen consumption rate, and aggregate size need to be carefully considered for reproducible and relevant results. The role of oxygen in 3D cultures, especially for cells with high metabolic demands such as primary islets of Langerhans and stem cell-derived SC-β cells, was discussed. Oxygen distribution within 3D aggregates can lead to hypoxia and anoxia in core regions, particularly with increased media height and oxygen consumption. Static gas-permeable systems help alleviate this issue, and dynamic systems such as rotational culture offer a better oxygen supply but require careful management to prevent mechanical stress.[104]

Reproducibility is another concern in a 3D cell culture. The lack of standardization in materials and protocols can lead to significant variability between experiments and between different laboratories. Even commercial products like Matrigel can suffer from batch-to-batch variations, potentially affecting experimental outcomes.[105−107] Moreover, adapting existing assays and analytical methods to 3D cultures presents ongoing challenges. Many established techniques for cell viability, drug screening, and molecular analysis were developed for 2D systems and are sometimes difficult to translate directly to 3D models. Researchers must often modify or develop new protocols to account for the unique properties of 3D cultures such as incomplete cell lysis or reagent penetration issues. Researchers often need to modify or develop new protocols to address challenges unique to 3D models, such as incomplete cell lysis or limited reagent penetration, which can make the transition process time-consuming and technically demanding.

NANO/MICROPARTICLES IN CANCER TREATMENT

Cancer can originate in almost any tissue or organ and has the potential to metastasize to distant sites.[108] Approaches to cancer therapy include surgery, radiation, chemotherapy, immunotherapy, and targeted therapies, often used in combination to achieve significant outcomes.[109] However,

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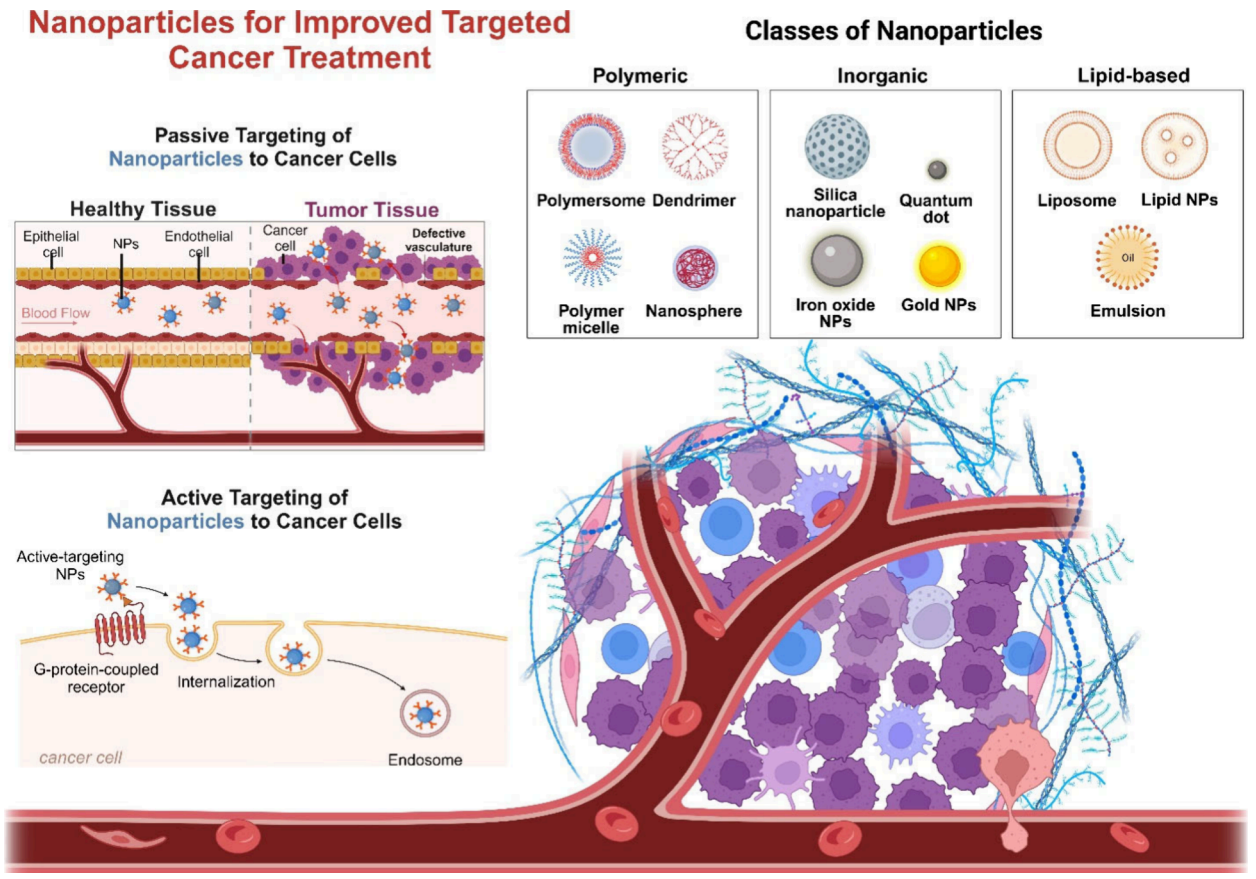

Figure 4. Different classes of nanoparticles and their targeting mechanisms to cancer cells. Created with biorender.com.

these treatments frequently result in severe side effects, lack precise targeting, and may lead to resistance over time.[110] Nanoparticles offer a promising alternative by improving drug delivery to tumor sites, reducing systemic toxicity, and minimizing damage to healthy tissues.[111] Nanoparticles are uniquely equipped to overcome the intricate biological barriers associated with tumors, such as the dense extracellular matrix and irregular vasculature (Figure 4).[112] Their ability to achieve intracellular delivery and exploit the enhanced permeability and retention effect allows for passive targeting of tumor tissues while minimizing off-target effects.[113] Microparticles, ranging from 1−1000 μm, offer advantages including enhanced drug loading, controlled drug release, and sustained depot systems, which ensure prolonged therapeutic efficacy at the tumor site.[114] Together, these platforms tackle critical issues like poor drug solubility, systemic toxicity, and the need for precision targeting, demonstrating their potential to revolutionize cancer therapeutics.[115,116] Despite these advancements, realizing the full potential of nano- and microparticles requires a deeper understanding of their interactions within biological systems, particularly in the context of preclinical research.[117] Traditional *in vitro* models, often limited to 2D cell cultures, fail to replicate the complex 3D architecture, dynamic microenvironment, and cellular heterogeneity of tumors. These oversimplified models can lead to limited predictive accuracy in evaluating drug delivery systems.[118]

Advanced 3D culture systems, such as tumor spheroids and organoids, have emerged as powerful platforms to address these limitations.[117] These models mimic the tumor microenvironment by mimicking essential features, such as hypoxic gradients, extracellular matrix dynamics, and cellular heterogeneity. 3D systems have the potential to investigate the uptake, penetration, and therapeutic effectiveness of nanoparticles and microparticles. Furthermore, 3D models have the potential to assess particle-tumor interactions in a controlled but physiologically similar environment, offering a way to improve cancer therapies and drug delivery techniques.[119−121]

NANO/MICROPARTICLES IN 3D CELL CULTURES

There are different types of nano- and microparticles that have been extensively used for cancer therapy in 2D cell culture models. While 2D cell cultures have been widely explored for nano/microparticle uptake due to their simplicity and ease of use, they poorly represent the conditions found in the human body, which often results in failure during clinical trials.[117] Owing to these limitations, there has been a shift from 2D research to 3D cell culture models, which better mimic the *in vivo* microenvironment, facilitate cell−cell and cell-matrix interactions, and offer advantages such as enhanced drug penetration, sustained delivery, and more realistic cellular responses.[118] Several nanoparticles, including polymeric nanoparticles,[122−124] lipid-based nanoparticles,[125,126] and organic[127,128] and inorganic nanoparticles,[129−132] have been explored for targeted drug delivery and imaging.

Polymer nanoparticles are solid particles ranging from 10 to 1000 nm, composed of macromolecular polymers. They protect encapsulated drugs from enzymatic degradation, alter tissue distribution, and enhance stability *in vivo*. Drugs can be encapsulated, dissolved, or adsorbed on their surface. Polymer nanoparticles are ideal for drug delivery, owing to their low toxicity, biodegradability, and biocompatibility. Common

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polymers include polyesters (PLA, PLGA, PCL), polyalkylcyanoacrylates (e.g., PBCA, PiBCA), and amphiphilic block copolymers (e.g., PEG−PLA, PEG−PCL). Different preparation methods, including emulsion polymerization, solvent evaporation, and double emulsion, are used to encapsulate hydrophilic and hydrophobic drugs. Research has been published on 2D *in vitro* cell culture and polymeric particles for cancer therapy, including the encapsulation of various anticancer drugs in particles,[133−135] as well as the incorporation of photosensitizers and near-infrared (NIR) dyes.[136,137] This work aimed to develop combined therapeutic approaches utilizing photothermal therapy, photodynamic therapy, and chemotherapy.[138] Researchers have explored the use of polymeric nanocarriers to improve tumor targeting and reduce drug resistance, while also developing photosensitizer-functionalized nanocomposites for light-activated cancer treatments.[139,140] The integration of NIR dyes, such as indocyanine green and its derivatives, into nanocomposites has been investigated for both imaging and combined phototherapy applications.[141] Groups have explored 3D cell cultures for more relevant results. For example, pH-responsive histidine-modified star-shaped PLGA nanoparticles (sPLGA-His NPs) were developed for tumor-targeted delivery of docetaxel and disulfiram. The sPLGA-His NPs demonstrated enhanced tumor penetration, rapid drug release at acidic pH, and superior efficacy in 2D and 3D models compared to unmodified sPLGA NPs, making them a promising carrier for efficient cancer therapy.[142] Another group developed MMP-9-responsive PEGylated nanovesicles using a collagen-mimetic lipopeptide for targeted drug delivery. These nanovesicles encapsulated gemcitabine, releasing it in response to tumor-specific conditions, and demonstrated effective tumor growth reduction in both 3D spheroid models and a xenograft mouse model.[143] Moreover, researchers developed pH- and thermoresponsive NIMPS by integrating doxorubicin-loaded gold-core silica shell nanorods with salicylic acid-loaded PLGA microparticles. Triggered by an NIR laser or acidic conditions, NIMPS showed biocompatibility and efficient uptake in 2D HeLa cells. In 3D models, NIMPS combined with NIR laser irradiation significantly reduced HeLa spheroid size by up to 48%, supporting their potential for cervical cancer combinatorial therapy.[144] The size and surface topology of the nanoparticles significantly influence their uptake by cultured cells and their penetration into tumor models. Using 3D HCT116 colorectal cancer spheroids, this work evaluated nanoparticle penetration and uptake based on physicochemical properties. Smaller NPs showed superior penetration into the spheroid core compared to larger ones with reduced penetration for highly charged surfaces. PEG modification enhanced NP penetration, highlighting the potential of 3D models for optimizing nanomedicine design.[145] Estradiol-conjugated hypoxia-responsive polymersomes were developed for the targeted delivery of doxorubicin to hypoxic niches in ER-positive breast cancer. These polymersomes exhibited hypoxia-specific drug release and enhanced cytotoxicity in 3D hypoxic spheroids, highlighting their potential for targeted cancer therapy.[146] 3D tumor spheroids provide a more accurate model of the *in vivo* tumor microenvironment than 2D cultures, revealing drug resistance mechanisms. This work highlighted the enhanced efficacy of DTX-loaded micelles over Taxotere in spheroid models, supporting their use as a platform for evaluating nanomedicines.[147]

Lipid-based nanoparticles (LNPs) are spherical vesicles composed of lipids, ranging in size from 10 to 1000 nm. LNPs offer several advantages, such as biocompatibility, low immunogenicity, and the ability to enhance the stability and bioavailability of drugs.[148] Several types of lipid-based nanoparticles have been developed, including liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs). Size, surface charge, and morphology of lipid nanoparticles significantly influence their stability, biodistribution, and therapeutic efficacy. Smaller LNPs (<100 nm) enhance cellular uptake, while larger ones offer higher drug-loading capacities.[149] LNPs with a highly positive or negative zeta potential have strong surface charges, promoting stability in suspension by preventing aggregation through electrostatic repulsion.[150,151] This strong surface charge also enhances cellular uptake by facilitating interactions with cell membranes. Surface modifications and formation of protein corona such as PEGylation improve immune evasion and circulation time.[152] Stability, loading efficacy, and entrapment efficiency further optimize LNP performance.[153] LNPs have been extensively investigated for several types of cancer, including breast, ovarian, lung, pancreatic, prostate, glioblastoma, colorectal, leukemias, Kaposi's sarcoma, multiple myeloma, osteosarcoma, and soft tissue sarcoma. Several LNP formulations have been successfully approved for clinical trials and are already in the market for certain cancer therapies.[154] Various liposomal formulations of DOX were developed using cholesterol, phospholipids, and surfactants and modified with DSPE-mPEG2000. It was reported that liposomal DOX exhibited significantly higher cytotoxicity against Saos-2 cells in a 3D alginate hydrogel culture compared to free DOX in 2D cultures, highlighting the superior drug delivery and efficacy of liposomal NPs in mimicking the tumor microenvironment.[155] Solid tumors often suffer from poor blood flow, leading to hypoxic regions in which cancer cells adapt to survive. Hypoxia promotes tumor progression by enhancing angiogenesis and other cellular changes. In another article, lipid nanoparticles with hypoxia-responsive lipids and peptide conjugates were developed, showing improved drug delivery to hypoxic tumor areas in 3D spheroid cultures. The lipid nanoparticles penetrated deep into the spheroids, releasing encapsulated drugs under low oxygen conditions, reducing pancreatic cancer cell viability to 35% in hypoxic environments.[156] Lipid nanoparticles have been widely explored for targeted delivery by attaching various ligands to enhance the specificity and improve therapeutic outcomes. In one article, cationic solid lipid nanoparticles loaded with siRNAs targeting the EphA2 receptor, overexpressed in prostate cancer, were developed to enhance gene silencing. The nanoparticles demonstrated improved cellular uptake and silencing efficiency in both 2D and 3D prostate cancer cell cultures. Co-treatment with histone lysine demethylase inhibitor JIB-04 further enhanced the silencing effect, reducing EphA2 expression and cellular responses. This novel siRNA delivery system shows great potential for targeted therapy in 3D tumor models, offering improved transfection efficiency and therapeutic outcomes.[157]

Extracellular vesicles (EVs)—including exosomes (30−150 nm), microvesicles (100−1000 nm), and other vesicular subsets—are secreted by most cell types and have emerged as natural nanocarriers for therapeutic delivery. Their lipid bilayer membranes encapsulate proteins, RNAs, and metabolites that reflect the parental cell, providing intrinsic homing properties, biocompatibility, and immune evasion capabilities.

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In cancer therapy, EVs have been engineered to deliver chemotherapeutics, siRNAs, miRNAs, and CRISPR-Cas components to tumor cells. Clinical translation has begun; for example, the "iExosomes" Phase I trial (NCT03608631) administers mesenchymal stromal cell-derived exosomes loaded with KrasG12D-siRNA to pancreatic cancer patients, demonstrating feasibility and tolerability.[158−161] In 2D monolayer cultures, EVs demonstrate high apparent potency due to unrestricted contact with cells and the absence of stromal barriers. For example, engineered exosomes delivering siRNA or doxorubicin achieve efficient gene silencing and apoptosis in breast and pancreatic cancer cell lines.[160,161] The plasma membrane proximity and high endocytic activity of proliferating monolayer cells favor EV uptake via endocytosis and membrane fusion pathways. However, these data often overestimate therapeutic potential as 2D models lack the extracellular matrix, hypoxic gradients, and interstitial pressures of tumors. This discrepancy contributes to the translational gap seen when monolayer-validated therapies underperform *in vivo*.[162] Investigations with 3D tumor spheroids show that EVs penetrate spheroids more effectively than many synthetic NPs of similar size, likely due to fusogenic membrane components and active transcytosis. For example, exosomes engineered to carry paclitaxel penetrate deeply into multicellular tumor spheroids, delivering drug to hypoxic cores and achieving higher apoptotic fractions compared with liposomes.[163] Still, dense ECM and elevated interstitial pressures can limit the uniform distribution, requiring strategies such as ECM modulation or exosome surface engineering.

Similarly, hybrid nanoparticles combine synthetic cores (liposomes, polymers, and metallic nanostructures) with biologic membranes (from EVs or whole cells) to merge the tunability of synthetic nanocarriers with the biofunctionality of natural vesicles. Membrane-coated nanocarriers exhibit extended circulation, reduced macrophage clearance, and homotypic binding that enhances tumor retention.[164−166] Although most hybrid platforms remain at preclinical stages, several groups are advancing red blood cell− or cancer cell−membrane-coated nanoparticles as next-generation oncology therapeutics.[167−169] Hybrid NPs also perform strongly in 2D assays. Membrane-coated nanoparticles exhibit enhanced uptake compared to bare synthetic cores. For instance, HeLa cell-derived membrane-coated polymeric NPs show 2−3-fold higher doxorubicin accumulation and cytotoxicity in monolayers relative to uncoated particles. The presence of adhesion molecules (e.g., E-cadherin, integrins) promotes stronger binding and faster uptake in 2D environments.[170] In 3D systems, hybrid NPs, such as cancer cell−membrane-coated liposomes, often accumulate at spheroid peripheries due to homotypic binding but struggle to penetrate into inner zones unless optimized for size (<70−100 nm) or equipped with ECM-degrading enzymes.[171,172] Incorporation of matrix-metalloproteinase-sensitive linkers or collagenase-functionalized coatings can enhance core penetration. While hybrid biomimetic systems leverage biological membranes to improve tumor targeting and penetration, complementary efforts with inorganic nanoparticles have focused on exploiting their unique optical, magnetic, and catalytic properties to further enhance therapeutic efficacy in 3D tumor models.

Inorganic nanoparticles have been explored for 3D-based cell cultures. These nanoparticles, including gold, silver, silica, and iron oxide, provide a range of functionalities that can be exploited in 3D culture systems. Gold nanoparticles (GNPs) were explored to enhance radiosensitivity and deliver drugs, with the aim to reduce toxicity while increasing tumor targeting. It showed that 3D models revealed significantly higher GNP uptake and penetration, particularly when combined with docetaxel, compared to 2D models. 3D models provided more realistic insights into nanoparticle penetration and uptake, especially when combined with drugs like docetaxel, compared to simpler 2D models.[173] Another investigation compared the effects of size and functionalization of gold nanoparticles on cell responses of 2D and 3D cultures to ionizing radiation. The results indicated that AuNPs improved cellular uptake and reactive oxygen species generation in a time- and concentration-dependent manner with stronger effects observed in 3D models that better represented tumor microenvironments. Clonogenic assays revealed decreased cell survival following irradiation, particularly after AuNP pretreatment, highlighting the critical role of PEG chain length in sensitization efficiency.[174] The size of gold nanoparticles significantly affects their uptake by various 3D models including tumor spheroids and tissues. Ultrasmall gold nanoparticles under 10 nm exhibited superior localization and penetration in breast cancer cells, tumor spheroids, and mouse tumors compared with larger nanoparticles. Au@tiopronin nanoparticles, prepared with sizes ranging from 2 to 15 nm, showed size-dependent accumulation, with smaller nanoparticles penetrating deeper into the tumors. Notably, 2 and 6 nm AuNPs were distributed throughout the cytoplasm and nucleus, whereas 15 nm AuNPs remained confined to the cytoplasm.[175] Moreover, ultrasmall gold nanoparticles, which have been explored extensively for their unique properties, easily penetrated intestinal epithelial cells, colorectal cancer cells, 3D spheroids, and primary gut organoids, unlike dissolved dyes. Confocal microscopy revealed their distribution inside spheroids, while nanoparticles conjugated with doxorubicin exhibited significantly higher cytotoxicity toward CT-26 tumor spheroids than dissolved doxorubicin due to better penetration.[176] Besides gold nanoparticles, other metallic nanoparticles have also been extensively investigated using 3D culture systems. Carboxymethylated PST001-coated iron oxide nanoparticles were encapsulated with the chemotherapeutic drug doxorubicin and can be utilized for targeted drug delivery. These biocompatible nanoconjugates demonstrated effective cancer cell internalization and ROS production in both 2D and 3D culture systems, highlighting their potential for reducing cancer spheroids and tumor burden.[177] Another article developed two types of CNS spheroids from human D384 astrocyte-like and SH-SY5Y neuronal-like cells to test the neurotoxic effects of Fe_3O_4 NPs. Short-term exposure induced cytotoxicity at 10 μg/mL for astrocytes and at 25 μg/mL for neurons, while long-term exposure showed concentration- and time-dependent mortality, with neurons exhibiting higher susceptibility. These 3D models offer a more accurate representation of *in vivo* environments, improving toxicity testing predictability.[178] Further, zinc oxide NPs were examined for toxicity in 2D and 3D cultures. It was observed that the toxicity and inflammatory effects of different-sized ZnO NPs on Caco-2 cells cultured in 3D agarose induce size-dependent toxicity and inflammatory responses. The results highlight that 2D models may overestimate ZnO NP toxicity, and 3D models provide a more realistic mechanism for probing nanoparticle induced but raise safety concerns due to potential toxicity. This work integrated 3D tissue-mimetic models to assess the cytotoxicity

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Table 1. Summary of Different Nanocarriers for Drug Delivery and Cancer Treatment in 2D and 3D Cultured Cells

S. No	Key Features	Size	Drug Encapsulated	Synthesis Method	Applications	ref.
1	pH-responsive, star-shaped PLGA with terminal histidine	153 ± 3.2	docetaxel and disulfiram	Nanoprecipitation	Tumor-targeted drug delivery	142
2	MMP-9 responsive PEG cleavable	86 ± 18 nm	Gemcitabine	Conjugation	Pancreatic cancer treatment	143
3	Gold-core silica shell nanorods with salicylic acid loaded poly(lactic-*co*-glycolic acid) based microparticles (NIMPS)	2.5 μm	Doxorubicin and salicylic acid	Solvent diffusion-evaporation	Cancer cell treatment	144
4	Poly(styrene) nanoparticles	30 nm, 50 nm, 100 nm	Doxorubicin	Not specified	*In Vitro* Screening for Early-Stage Nanoparticle Assessment	19
5	Estradiol-conjugated hypoxia-responsive polymeric nanoparticles	52 ± 6, 695 ± 32	Doxorubicin	Not specified	ER+ breast cancer treatment	146
6	Block copolymer micelles	49.2 ± 2.3 nm	Docetaxel	Polymerization	*In Vitro* and *In Vivo* Models for High-Throughput Therapeutic Screening	147
7	Lipid nanoparticles for mRNA delivery	174 to 109 nm	mRNA	NanoAssemblr Ignite microfluidics mixing system	Respiratory tract delivery	153
8	Doxorubicin-loaded nanoliposomes	86.8 nm	Doxorubicin	Thin film method	Osteosarcoma treatment	155
9	Hypoxia-responsive, peptide−lipid conjugate	180 ± 3 nm	Chemotherapeutics	Microwave-assisted, solid-phase peptide synthesizer	Pancreatic cancer treatment	156
10	EphA2 siRNA-loaded lipid nanoparticles	<200 nm	siRNA	Hot microemulsion method	Prostate cancer treatment	157
11	Gold nanoparticles	17 nm and 46 nm	Docetaxel	Citrate reduction method	3D Tumor Spheroids for Accelerating Nanomedicine Translation	173
12	Functionalized gold nanoparticles	10 ± 2 nm	Not specified	The inverse method and the Turkevich technique	Cancer combined treatment with radiotherapy.	174
13	Au@tiopronin nanoparticles	tunable sizes from 2 to 15 nm	Not specified	Citrate reduction method	High levels of accumulation in tumors	175
14	Functional ultrasmall gold nanoparticles	2 nm	cadaverine and doxorubicin	Adapted Brust−Schiffrin synthesis	Localized tumor therapy	176
15	Carboxymethylated PST001 coated iron oxide nanoparticles	10 ± 2 nm	doxorubicin	Chemical coprecipitation methods followed by APTES coating	Tumor management	177
16	Polyvinylpyrrolidone-coated Fe_3O_4 nanoparticles	19.07 ± 5.5 nm	Not specified	Not specified	3D *in vitro* cell-based models in toxicology	178
17	Poly(D,L-lactic acid) nanoparticles	167 ± 50 nm	Coumarin 6	Nanoprecipitation	Cellular internalization vs tissue penetration in hypo-vascularized cervical cancer lesions	180
18	Single-walled carbon nanotubes (SWNTs)	Not specified	Not specified	Purification and oxidation	*In vitro* toxicity screening assays	179
19	Citrate-protected AuNPs	15, 22, and 60 nm	Not specified	Citrate reduction method	Tissue penetration of nanoparticles	181
20	Fructose-based glycopolymers	30 nm	Paclitaxel	RAFT polymerization	Nanocarriers for anticancer drug delivery	182

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of purified (p-) and oxidized (o-) SWNTs, using ultrasound standing wave traps to create 3D cell aggregates. Results showed that p- and o-SWNTs were not toxic in 3D models after 24 h exposure, while 2D cultures exhibited significant toxicity. Elevated cytokine secretion in 2D cultures but not in 3D models suggests that 3D aggregates offer a more accurate, tissue-mimetic approach for nanomaterial toxicity screening.[179]

INTERACTIONS AND UPTAKE OF NANO/MICROPARTICLES WITH 3D SPHEROIDS

Nanoparticles are generally defined as particles with a size range of 1−100 nm, whereas microparticles range from 1−1000 μm. Both have been explored in cancer drug delivery, but their properties and applications differ significantly. Nanoparticles are advantageous because their small size allows for enhanced permeability and retention in tumor tissues, improved cellular uptake, and the possibility of surface modification for targeted therapy. However, they may be rapidly cleared by the immune system, pose challenges related to large-scale production, and, in some cases, contribute to off-target toxicity. Microparticles, in contrast, are capable of carrying larger drug payloads and can provide sustained or controlled release, making them suitable for localized depot delivery. Their larger size, however, limits tissue penetration and reduces direct cellular uptake compared with nanoparticles.[183]

Nanoparticle uptake varies significantly between 2D and 3D cell cultures due to differences in the cellular environments and interactions present in each culture (Table 1). These variations highlight the importance of using 3D models for more accurate predictions of the nanoparticle behavior in biological systems. There are several factors and differences in nanoparticle uptake in both the 2D and 3D cultures. Key differences in nanoparticle uptake include cellular morphology and environment: cells in 3D cultures exhibit different morphologies compared to those in 2D cultures. The presence of complex cell−cell interactions and a structured extracellular matrix in 3D models influences how nanoparticles are taken up. Since 3D cultures more closely resemble *in vivo* conditions, they provide a better representation of nanoparticle interactions within living tissues. Moreover, the movement of nanoparticles in 3D environments is often restricted by extracellular components, such as collagen and other structural proteins. This barrier limits their penetration and slows the rate of uptake. On the other hand, 2D cultures allow nanoparticles direct access to cell surfaces, leading to potentially exaggerated uptake levels compared to what would occur in a living organism. For example, the Netti group examined the influence of a 3D extracellular matrix on cell behavior and nanoparticle diffusion. Using human dermal fibroblasts and fibrosarcoma cells in a 3D collagen gel, the results showed that cell morphology changed significantly in 3D compared to standard 2D cultures, and NP diffusion was hindered by interactions with collagen fibers, especially for larger NPs. These findings emphasized the importance of nanobio interactions in models that better mimicked *in vivo* conditions for more accurate predictions of drug delivery efficacy.[184] Other work explored how adenocarcinoma cells responded to 3D culture environments with different substrates, focusing on changes in cell morphology, cytoskeletal organization, stiffness, roughness, and biochemical markers. Using advanced techniques like confocal laser scanning, He-ion, atomic force microscopy, ELISA, and Western blotting, the research compared 3D cultures on soft matrices (PuraMatrix and Matrigel) to traditional 2D glass cultures.[185]

Moreover, how substrate stiffness influenced the migration of A549 lung adenocarcinoma cells and its potential link to epithelial-to-mesenchymal transition (EMT) was explored. It was found that stiffer substrates slowed migration and increased directionality but did not change the levels of EMT markers, such as E-cadherin and N-cadherin. TGF-β stimulation induced similar EMT levels, regardless of substrate stiffness. Moreover, stiffer substrates led to reduced phosphorylation of focal adhesion proteins, which correlated with slower migration.[186] Another article investigated fibroblast behavior in 2D and 3D tissue cultures, analyzing adhesion and migration in different extracellular matrices. Fibroblasts in 3D were more spindle-shaped with fewer protrusions and reduced actin stress fibers compared to 2D. Migration was fastest in cell-derived matrices and collagen, while minimal in basement membrane extract.[187] All of these differences significantly slowed down the uptake of nanoparticles in 3D cultures compared to 2D monolayers. Furthermore, physicochemical properties of nanoparticles such as size, shape, charge, and surface functionalization also affect the uptake of nanoparticles.

Nanoparticle size and shape play crucial roles in the uptake efficiency and penetration depth in 3D cultures. The size of NPs plays a critical role in both conditions, with smaller NPs generally exhibiting higher uptake due to enhanced diffusion, whereas larger NPs may experience steric hindrance, particularly in 3D environments where dense ECM structures can further impede their penetration.[188] Nanoparticle size significantly influences uptake in a 3D extracellular matrix, with larger (100 nm) NPs exhibiting reduced cellular internalization due to hindered diffusion through collagen fibers compared to smaller (44 nm) NPs. The same work also reveals that cytoskeletal organization, particularly actin microfilaments, plays a crucial role in NP uptake.[184] The size of the nanoparticles affects their uptake mechanism and penetration pathways in tumors. Another article explored the tumor penetration mechanisms of nanoparticles using a multidimensional quantitative approach in an MDA-MB-231 tumor spheroid model. Gold nanoparticles were synthesized in different sizes, and their distribution was analyzed by using spectrometry, microscopy, and synchrotron techniques. While 15 nm nanoparticles penetrated via an energy-independent transcellular route and 60 nm via an energy-dependent transcellular pathway, 22 nm nanoparticles utilized both transcellular and paracellular pathways, achieving the highest penetration.[181] Furthermore, in another article, a tumor spheroid model was used to evaluate nanoparticle uptake, distribution, and radio-enhancement in a label-free, nanometric resolution approach. The 5 nm gold nanoparticles exhibited similar radio-enhancement in 2D and 3D cultures, whereas the 50 nm nanoparticles showed limited penetration and reduced efficacy in 3D tissues.[189]

Another important factor affecting the uptake of nanoparticles is their morphology as the shape of nanoparticles can significantly influence their interaction with cellular membranes and their ability to penetrate barriers. For example, rod-shaped nanoparticles may have higher cellular uptake compared to spherical ones due to their elongated shape, which facilitates better membrane contact and internalization.[190] Additionally, more complex shapes, such as worm-like or disk-shaped particles, can exhibit different rates of endocytosis and toxicity depending on their surface area,

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aspect ratio, and alignment with the target cell. Fructose-based amphiphilic block copolymer nanoparticles (spheres, rods, and vesicles) were synthesized and tested on breast cancer cell lines in 2D and 3D models.

In 2D monolayer cultures, spheres showed significantly higher internalization in both MCF-7 and MDA-MB-231 cells compared to rods and vesicles, while MCF-7 cells had a greater affinity for rod-like structures than MDA-MB-231 cells. The 3D MCF-7 spheroid model exhibited similar trends to 2D models, with higher uptake correlating to better anticancer efficacy.[182] Spherical NPs (AuNS50, AuNS10) exhibited higher uptake than rod-shaped AuNR (−) in both 2D and 3D models, likely due to the higher energy barrier and inefficient membrane wrapping of rods. In spheroids, spherical NPs penetrated deeper, while rod-shaped NPs faced steric hindrance from the dense ECM, limiting diffusion. At lower concentrations, AuNS50 was not detected in the spheroid core due to reduced NP accumulation.[191]

Furthermore, surface chemistry and charge play significant roles in the uptake of nanoparticles. To visualize the distribution of Au NRs in MCTSs, dark field microscopy was employed, revealing that PSS-coated Au NRs penetrated deeper into the spheroids, while PDDAC- and CTAB-coated Au NRs remained in the outer region. The surface charge influenced nonspecific protein adsorption, affecting nanoparticle size and penetration behavior. Consequently, PSS-coated Au NRs demonstrated superior hyperthermia efficacy, disrupting the spheroid structure more effectively. These findings highlight the impact of surface chemistry on Au NR distribution and thermal therapy, offering insights for optimizing nanoparticle design in cancer treatment.[192] A 3D coculture spheroidal array was developed to mimic tumor stroma and assess the penetration of silica and PLGA nanoparticles. Results showed that fibroblast-rich heterospheroids significantly hindered nanoparticle penetration, with a highly negative zeta potential and smaller nanoparticles achieving deeper infiltration. This 3D model provides a valuable platform for probing tumor stroma barriers and optimizing nanoparticle design for improved drug delivery.[193] Another article evaluated poly(lactic-*co*-glycolic) acid NPs modified with cell penetrating peptides (MPG), poly(ethylene glycol) (PEG), MPG/PEG, and vimentin for cellular uptake and tissue penetration in cervical cancer models. Results showed that MPG NPs had the highest internalization in HeLa cells, while PEG NPs penetrated deeper into 3D cultures. Although internalization was lower in 3D than in monolayers, combining MPG and PEG modifications may optimize NP delivery by balancing cellular uptake and tissue penetration in hypovascularized cervical tumors.[180] Fluid flow and perivascular-like channels also influence deposition. High-resolution imaging has shown that EVs undergo rapid peripheral uptake followed by gradual transcytosis into deeper layers, while uncoated synthetic NPs often remain trapped in outer rims.[194]

Uptake Mechanisms. Nanoparticle uptake mechanisms differ significantly between the 2D and 3D cell culture models. Cell membranes are generally impermeable to large particles, allowing only those between 10 and 30 nm to cross. To overcome this barrier, cells use endocytosis, a mechanism exploited by viruses, to enter host cells. Endocytosis is classified into phagocytosis, which involves the uptake of large particles by immune cells such as macrophages and neutrophils, and pinocytosis, which internalizes solutes. Pinocytosis includes macropinocytosis (nonspecific uptake of fluid and solutes sized 100 nm to 5 μm), clathrin-mediated endocytosis (receptor-specific uptake with ∼ 120 nm vesicles), caveolae-mediated endocytosis (using ∼ 80 nm vesicles), and clathrin- and caveolin-independent endocytosis (∼50 nm vesicles). Nonphagocytic cells primarily use pinocytosis for nutrient intake. The maximum size for internalization varies by pathway, but particles >200 nm are generally not internalized through classical endocytosis. Endocytosis involves a complex interplay of particle size, energy changes, and receptor interactions, influencing the efficiency of nanoparticle uptake.[195] Different cell types utilize distinct endocytotic pathways to internalize the same nanoparticles. For example, HeLa, 1321N1, and A549 cells exhibit variations in clathrin- and caveolin-mediated uptake.[196] Caveolae-dependent endocytosis, prevalent in endothelial cells and fibroblasts, bypasses lysosomal degradation, directing NPs to the endoplasmic reticulum or Golgi apparatus. In contrast, macropinocytosis, occurring in most cells, leads to diverse fates such as lysosomal degradation in macrophages or cargo recycling in A431 cells.[197] In 2D cultures, NPs are internalized through various endocytic pathways, including clathrin-dependent and caveolin-dependent endocytosis, macropinocytosis, phagocytosis, and clathrin- and caveolin-independent mechanisms. Clathrin-mediated and caveolae-mediated endocytosis are particularly relevant for smaller nanoparticles, while larger nanoparticles are taken up through macropinocytosis or phagocytosis.[198,199] Gold nanoparticles are internalized by cells primarily through receptor-mediated endocytosis, a process where ligands on the nanoparticle surface bind to cell surface receptors, leading to membrane invagination and internalization. This uptake mechanism is temperature-dependent, as demonstrated by a significant decrease in nanoparticle absorption at 4 °C compared to 37 °C. Additionally, the shape of AuNPs influences their cellular uptake; spherical nanoparticles are internalized more efficiently than rod-shaped ones.[200] Other work has shown that 40 nm polystyrene nanoparticles are internalized by mouse macrophage (J774A.1) and human alveolar epithelial (A549) cells through multiple endocytic pathways. In J774A.1 cells, uptake is primarily mediated by macropinocytosis, phagocytosis, and clathrin-mediated endocytosis, while in A549 cells, both clathrin and caveolin-mediated endocytosis are involved.[201] Due to the uniform exposure of cells to nanoparticles, uptake kinetics in 2D cultures tend to be faster and more predictable.

However, in 3D cultures, the uptake mechanisms become more complex due to the presence of an extracellular matrix and three-dimensional cellular organization. The ECM can hinder NP diffusion, particularly for larger nanoparticles, reducing their interaction with cells. The outer layers of cells in spheroids have greater access to nanoparticles, whereas deeper cells experience limited exposure due to reduced diffusion and intercellular tight junctions.[202] This results in a gradient of the nanoparticle distribution, often leading to heterogeneous uptake. Additionally, 3D cultures induce changes in cell morphology and behavior, which affect how cells interact with and internalize nanoparticles. Cellular uptake in 3D models is often slower than that in 2D due to these physical barriers and the altered cellular microenvironment. Macropinocytosis remains a primary uptake route in 3D cultures, but other endocytic pathways also play a role, depending on the size, shape, and surface properties of the nanoparticles. For instance, in these models, EV uptake is strongly biased toward the proliferative cells in the spheroid periphery, with slower and

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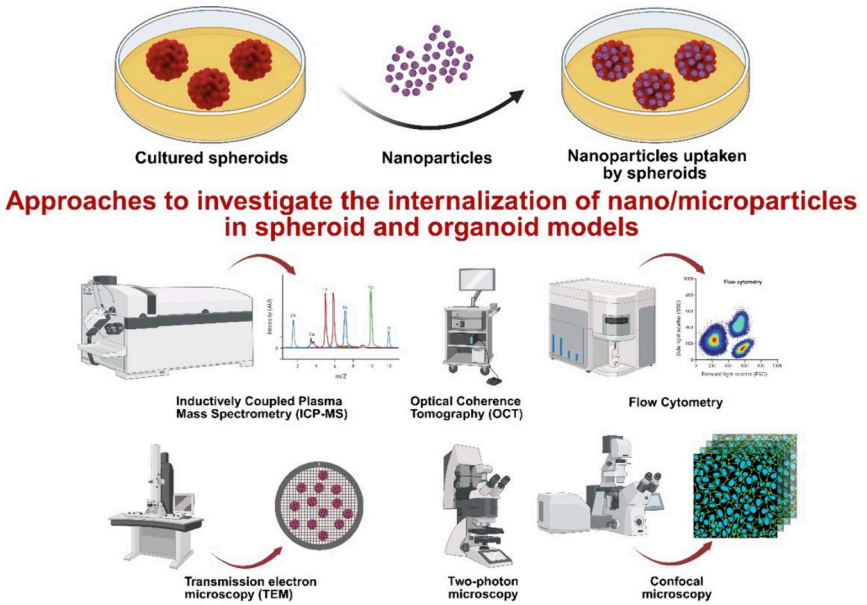

Figure 5. Techniques for investigating the uptake of nano- and microparticles in spheroids and organoids. Created with biorender.com.

less efficient penetration into hypoxic or necrotic cores.[194] Interactions between EVs and ECM components such as collagen and hyaluronan not only delay diffusion but can also serve as temporary retention sites that shape EV biodistribution.[203] Hypoxic conditions in inner spheroid zones appear to further modulate uptake, as hypoxia-inducible factors enhance endocytic and nutrient-scavenging pathways, promoting selective EV internalization in poorly perfused tumor regions.[204] These features explain the superior performance of EVs compared with most synthetic nanocarriers in penetrating tumor-like structures, although their distribution within 3D models remains heterogeneous. Furthermore, smaller NPs generally exhibit higher uptake rates in both models, while the surface charge and coating can modulate their interaction with cells. Overall, while 2D cultures provide insights into basic NP uptake mechanisms, 3D models offer a more physiologically relevant platform for understanding nanobio interactions and predicting therapeutic outcomes.[205]

TECHNIQUES FOR NANOPARTICLE UPTAKE AND DRUG SCREENING

The rising costs and low success rates in drug development highlight the need for better ways to discover new medicines. Many drug candidates fail clinical trials because they do not work well enough or cause unexpected side effects, wasting time and money. Traditional methods, which focus on single molecular targets, often fall short, especially for complex diseases that are not well-understood. As drug failures increase, so do financial challenges, making it crucial to find more effective strategies. New approaches, such as phenotypic screening and 3D cell culture models, provide a more realistic way to determine drug effects early on, helping to improve success rates and lower costs.

Techniques to probe cellular uptake and drug screening of nanoparticles using 2D and 3D models have evolved significantly to investigate nanoparticle behavior and efficacy (Figure 5). Analytical methods such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS) precisely quantify metal-containing nanoparticles but require sample digestion, destroying cellular structures. ICP-MS has become a critical tool in cancer research for quantifying the intracellular and subcellular accumulation of platinum-based chemotherapeutics such as cisplatin and carboplatin, which remain among the most widely used cancer drugs worldwide. Since reduced cisplatin uptake is a common mechanism of therapy resistance and its nonspecific cytotoxicity leads to severe side effects, accurately monitoring its distribution within cells is essential. ICP-MS allows sensitive detection of platinum in DNA, chromatin, and whole cells, helping researchers understand how the drug binds, why resistance develops, and how dosing can be improved. This has advanced cancer research by showing how drug levels inside cells relate to both treatment effectiveness and side effects, and the method can be applied across different cancer models.[206]

Magnetophoresis measures the movement of magnetic nanoparticles in a magnetic field to estimate uptake, while flow cytometry enables single-cell analysis of fluorescently labeled nanoparticles.[207] Imaging techniques, such as confocal microscopy and fluorescence microscopy, allow visualization of fluorescent nanoparticles, whereas brightfield and phase-contrast microscopy are useful for nanoparticles that scatter light. Super-resolution microscopy, including stimulated emission depletion and structured illumination microscopy, helps resolve nanoparticles below the diffraction limit, improving quantification.[208] Automated cell identification tools like cellProfiler and deep-learning models such as cellpose streamline analysis and reduce manual workload.

Another high-throughput approach involves microarray-based cell separation, where cells adhere to predefined patterns that facilitate nanoparticle quantification. These techniques, used individually or in combination, enhance our understanding of nanoparticle-cell interactions for biomedical and toxicological applications.[209] Many techniques for measuring nanoparticle uptake are complex and lack precise quantification. To address this, a plate-reader-based assay was developed for accurately measuring the uptake of fluorescent nanoparticles. The method accounts for potential variations by normalizing the fluorescence intensity to optical density, refining wash steps, and controlling the temperature to minimize errors. This approach was successfully tested on

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Jurkat lymphocytes with 50 nm polystyrene beads and further validated using A549 lung carcinoma and RPMI8226 lymphocyte cells.[210] For 3D models, which better mimic the complex tumor microenvironment, more advanced techniques are required. Spheroids and organoids have become popular 3D culture systems for understanding nanoparticle penetration and accumulation. These models allow researchers to evaluate how nanoparticle properties, such as size, shape, and surface charge, influence their ability to penetrate tumor-like structures. Confocal microscopy with z-stacking, label-free dynamic optical coherence tomography, and two-photon microscopy (TPM) are particularly useful for imaging nanoparticle distribution throughout 3D structures.

TPM has become a valuable tool in translational and clinical cancer research, where it has been applied to visualize human tissue biopsies, monitor structural changes in skin cancer in vivo, and develop microendoscopy techniques for minimally invasive cancer diagnosis. These applications have advanced cancer research by enabling high-resolution, deep-tissue imaging of tumors in their native environment, bridging preclinical findings with clinical translation.[211] A specific example is the use of intravital two-photon microscopy to investigate STA551, a novel anti-CD137 switch antibody, in tumor-bearing human CD137 knock-in mice. TPM imaging demonstrated that STA551 is selectively bound to tumor-infiltrating T cells and NK cells while sparing normal tissues such as spleen. This work highlighted TPM's unique ability to track therapeutic antibody distribution in vivo and provided evidence that STA551 could achieve potent antitumor efficacy with reduced systemic immune activation. Collectively, these examples highlight how TPM advances cancer research by offering real-time, minimally invasive insights into tumor biology, drug distribution, and treatment response.[212] Another article investigated AuNP penetration in 3D colorectal carcinoma spheroids using two-photon microscopy, analyzing the effects of the size, shape, and surface chemistry. Results showed significant differences in uptake between 2D and 3D models with variations in penetration depth and efficacy. Two-photon microscopy proved to be an effective method for large-scale AuNP screening, offering an alternative to labor-intensive electron microscopy.[213]

Flow cytometry has been widely applied in cancer research and clinical practice, particularly for the diagnosis and classification of hematologic malignancies. For example, immunophenotyping by flow cytometry enables differential diagnosis between chronic lymphocytic leukemia and other mature B-cell neoplasms by analyzing the expression of cell surface markers and quantifying cellular subsets. This has advanced cancer research by improving diagnostic accuracy and facilitating timely and precise treatment decisions.[214] Beyond diagnosis, flow cytometry is also used to monitor treatment response and guide personalized immunotherapy, such as by detecting chimeric antigen receptors on engineered T cells and evaluating their functional properties, including proliferation, cytokine secretion, and target specificity. These applications have advanced cancer research by enabling a deeper understanding of tumor heterogeneity, supporting the development of precision therapies, and improving patient prognosis through better treatment monitoring and selection strategies.[215]

A high-resolution transmission X-ray imaging method was developed to quantify nanoparticle uptake in 2D and 3D cells. Tests on gold nanoparticles in EMT and HeLa cells revealed differences in uptake between 2D and 3D cultures, with 3D analysis offering more accurate insights into nanoparticle distribution and clustering. The method effectively measured nanoparticle cluster size and uptake, demonstrating the importance of 3D analysis for better understanding the cellular interactions in more realistic environments.[216] Kou et al., with L-carnitine-conjugated poly(lactic-*co*-glycolic acid) (LC-PLGA) nanoparticles, demonstrated significantly improved uptake and enhanced antitumor efficacy in 3D spheroid models compared to traditional 2D cell cultures. The 3D model allowed for a better assessment of nanoparticle penetration and effectiveness in targeting deep tumor regions. The increased cellular uptake and drug delivery efficiency observed in this model suggest that LC-PLGA NPs could offer greater therapeutic potential in treating colon cancer, reflecting their ability to overcome the challenges of drug delivery in solid tumors.[217]

In terms of drug screening, both 2D and 3D models employ a range of assays to assess the efficacy of nanoparticle-delivered drugs. Viability assays, apoptosis detection, and proliferation measurements are commonly used to evaluate the drug effects. However, 3D models often provide more realistic drug response data due to their tissue-like properties, including the presence of diffusion barriers and hypoxic regions. Several techniques were employed for drug screening, a paper comparing drug sensitivities between 2D and 3D cultures in triple-negative breast cancer cell lines. Cell morphology in 3D cultures was observed by using phase-contrast microscopy, allowing the researchers to examine structural differences between the cultures. Drug sensitivities were assessed through cell viability assays, where the IC50 values for epirubicin, cisplatin, and docetaxel were determined and compared between the 2D and 3D cultures. This work showed that 3D cultures were more resistant to all three drugs than 2D cultures, with the highest correlation for CDDP sensitivity between the two models.[218] Drug efficacy testing in 3D cultures utilized live-cell imaging to quantify the response of patient cells to drugs and drug combinations, enabling the rapid and precise assessment of drug efficacy. Patient samples were cultured in both 2D and 3D formats. After drug exposure, drug sensitivity profiles were generated by measuring cell proliferation and viability. The platform achieved over 90% success in providing results within 6 days, making it a valuable tool for functional precision medicine. Here, 27 drug combinations were evaluated, and the results indicated synergy between drugs like carboplatin and A-1331852, showcasing the platform's ability to rapidly assess both existing and emerging therapies for ovarian cancer.[219]

Immunofluorescence staining was used to analyze cell proliferation and apoptosis. Additionally, the authors compared staining patterns for K_i-67 and caspase-3 in 2D- and 3D-cultured cells derived from patient-derived xenografts and fresh tumors to validate the *in vivo* relevance of the 3D culture system.[220] The APH assay used in this work is a novel and reliable method for determining cell viability in 3D culture models without requiring spheroid dissociation. The assay measures cell viability by detecting the cellular adenosine phosphate levels, which are indicative of active metabolism. This method is advantageous for high-throughput screening as it does not disturb the 3D spheroid structure, allowing for the measurement of viability directly within intact spheroids. Here, the APH assay was validated for use with MIAPaCa-2 and PANC-1 spheroids, showing that it can efficiently quantify cell

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viability even in large spheroids up to 1000 μm in size. The assay's linearity and sensitivity made it suitable for evaluating drug efficacy in 3D cultures, where traditional assays, like those used in 2D cultures, may not be directly applicable due to the complexity of the 3D environment. By employing the APH assay, the researchers demonstrated that spheroid-based cultures were less sensitive to gemcitabine and 5-FU, reflecting the drug resistance often seen in solid tumors. This provides a more accurate platform for assessing drug responses in pancreatic cancer, which could enhance the development of new therapies.[221]

Stock et al. employed several advanced techniques to analyze and compare 2D and 3D culture models for oncology drug discovery. Fluorescence-based growth curves were used to measure tumor cell growth, providing high-throughput data, although they sometimes overlooked specific growth characteristics in complex 3D cultures. Spheroid size analysis was also used to gauge growth and treatment effects, but discrepancies were observed when size did not align with cell viability or treatment response. Shape analysis, aimed at assessing invasiveness, did not provide much additional insight in this work. Molecular imaging with EdU staining was used to track proliferating cells, offering detailed insights into cell division, although it required high-quality imaging and was resource-intensive. Tissue microarrays were used for cross-comparing models and patient tissues, preserving tissue heterogeneity and allowing for in-depth immunohistochemical analysis. Additionally, a 2.5D analysis workflow simplified the analysis of 3D spheroids, combining maximal intensity projection and height mapping to measure proliferation. While labor-intensive, these techniques collectively offered crucial information on treatment responses, stromal contributions, and the complexities of tumor biology in different culture.[222]

The use of label-free dynamic optical coherence tomography (D-OCT) has been used to visualize and quantitatively assess the response of tumor spheroids to three anticancer drugs, including paclitaxel, tamoxifen citrate, and doxorubicin. Abd El-Sadek et al. treated spheroids with varying drug concentrations, and D-OCT imaging revealed distinct patterns in structural changes, volume, and tissue dynamics over time, consistent with fluorescence microscopy results. The findings highlighted D-OCT's potential for evaluating tumor spheroid responses in anticancer drug testing.[223] Moreover, OCT has been used to characterize the 3D morphology and physiology of multicellular tumor spheroids. The technique successfully quantified spheroid volumes and detected necrotic regions, providing a promising tool for high-throughput drug screening without the limitations of conventional imaging methods.[224]

Recent advancements have introduced more sophisticated approaches, such as organ-on-chip systems and bioprinted 3D models, which offer even greater physiological relevance. These platforms allow for the investigation of nanoparticle behavior under dynamic conditions, such as fluid flow, which can significantly impact the uptake and drug delivery. Quantification of nanoparticle uptake has also seen improvements, with techniques such as inductively coupled plasma mass spectrometry and high-performance liquid chromatography providing precise measurements of nanoparticle or drug concentrations in cellular samples. Also, the integration of machine learning and artificial intelligence is enhancing the predictive power of these models, allowing researchers to better understand and optimize nanoparticle design for improved therapeutic outcomes. As the field progresses, the combination of these diverse techniques across both 2D and 3D models is providing a more comprehensive understanding of nanoparticle behavior in biological systems, ultimately contributing to the development of more effective nanoparticle-based cancer therapies.

CLINICAL APPLICATIONS AND RELEVANCE OF NANOPARTICLES IN CANCER THERAPY

Nanoparticles have rapidly transitioned from preclinical exploration to clinical evaluation, driven by their capacity to improve the delivery, specificity, and safety of anticancer therapeutics. Their clinical relevance lies in overcoming pharmacokinetic limitations of conventional drugs, enhancing tumor accumulation through passive and active targeting, and enabling multimodal diagnostic and therapeutic strategies. Among the diverse platforms explored, polymeric, inorganic, and lipid-based nanoparticles, extracellular vesicles, and hybrid nanoparticles are the most advanced, each offering unique translational opportunities.

Polymeric Nanoparticles. These have demonstrated wide clinical applicability, owing to their versatility in drug encapsulation and controlled release. Biodegradable polymers such as poly(lactic-*co*-glycolic acid) and poly(ethylene glycol)-modified carriers are approved or in late-stage clinical testing for cancer therapy.[225] Polymeric micelles, such as Genexol-PM, which encapsulates paclitaxel, have reached clinical use in South Korea and demonstrated reduced systemic toxicity compared to conventional formulations.[226,227] More recently, polymeric nanocarriers are being engineered with tumor-responsive linkers and stimuli-sensitive release mechanisms, improving site-specific drug activation and minimizing off-target effects.[228]

Inorganic Nanoparticles. These have gained clinical relevance through their multifunctional properties, including imaging, photothermal conversion, and radiosensitization. Iron oxide nanoparticles, for instance, are clinically approved as MRI contrast agents and are under investigation for hyperthermia-based cancer therapy.[229] Gold nanoparticles have advanced to clinical trials as radiosensitizers (AuroShell) and drug delivery carriers, capitalizing on their high atomic number and surface modification capacity.[230,231] Silica nanoparticles have also progressed into early phase clinical trials, serving as carriers for doxorubicin and siRNA delivery with controlled release and reduced systemic toxicity.[232] These inorganic systems expand clinical utility beyond drug delivery, enabling therapy and imaging.

Lipid-Based Nanoparticles (LNPs). These represent the most clinically established nanocarrier system, particularly for nucleic acid delivery. LNPs were widely validated through their successful deployment in mRNA COVID-19 vaccines and are now being rapidly translated into oncology for siRNA and mRNA therapeutics.[233] Several LNP-based formulations, such as patisiran for transthyretin amyloidosis, have gained regulatory approval, showcasing their clinical translatability. In cancer therapy, LNPs are being optimized for targeted mRNA-based cancer vaccines, delivery of tumor-suppressor miRNAs, and gene-editing technologies such as CRISPR/Cas9.[234]

Extracellular Vesicles. These have emerged as promising natural delivery vehicles due to their intrinsic biocompatibility, stability in circulation, and ability to cross biological barriers such as the blood–brain barrier. Several EV-based formulations are entering clinical trials for oncology. For example, mesenchymal stem cell-derived EVs loaded with paclitaxel or

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doxorubicin have demonstrated safety and enhanced tumor uptake in phase I trials.[235] EVs are also being explored for delivering immunotherapeutics, such as checkpoint inhibitors or RNA vaccines, given their capacity to modulate the tumor microenvironment and immune responses.[236]

Hybrid Nanoparticles. These, which combine synthetic nanoparticle cores with biological coatings (e.g., cancer cell membranes or EV membranes), represent a frontier in clinical nanomedicine. These biomimetic systems aim to integrate the drug-loading capacity and stability of synthetic platforms with the homotypic targeting and immune-evasive properties of biological membranes. While still largely at the preclinical stage, HNPs show strong translational potential for precision medicine. Cell membrane-coated nanoparticles have been shown to prolong circulation, evade immune clearance, and selectively accumulate in tumors via homotypic binding.[237] With improvements in scalable production and regulatory-compliant manufacturing, HNPs are poised to enter early phase clinical testing in the near future.

Overall, the clinical relevance of nanoparticles lies not only in improving drug pharmacokinetics and tumor delivery but also in expanding the therapeutic toolbox to include genetic, immunologic, and multimodal approaches. Lipidic and polymeric platforms are already in clinical use, and inorganic nanoparticles are advancing in diagnostics and radio sensitization, while EVs and HNPs represent the next generation of biocompatible and personalized nanomedicine. Continued integration of these systems into oncology is expected to improve therapeutic efficacy while reducing systemic toxicity, ultimately reshaping the standard of care for cancer treatment.

■ DISCUSSION AND FUTURE PERSPECTIVES

The shift from traditional 2D cell cultures to 3D culture systems represents a transformative step in cancer research, offering a more accurate representation of the tumor microenvironment. While 2D cultures have been a traditional method for *in vitro* investigations, their inability to replicate the spatial architecture, cell−cell interactions, and extracellular matrix dynamics in *in vivo* tumors has been a limitation. In contrast, 3D models, encompassing scaffold-based systems, hydrogels, 3D-printed constructs, microfluidics, and organ-on-chip technologies, provide a physiologically relevant platform that includes critical tumor features such as hypoxia, nutrient gradients, and drug penetration barriers. This transition not only enhances our understanding of tumor biology but also elevates the accuracy of drug screening and therapeutic development, bridging the gap between laboratory findings and clinical outcomes.

An important development in this field is the use of nano/microparticles in 3D culture models, which has enhanced therapeutic delivery. These particles can precisely deliver and release drugs, minimizing side effects and enhancing treatment effectiveness. Investigations have shown that nanoparticles interact differently in 3D cultures compared to 2D cultures due to the dense ECM and diverse cell structures present in 3D models. Since these factors mimic real tumors, nanoparticles must be specifically designed to improve penetration and drug delivery. Nanoparticles offer benefits, including increased bioavailability, reduced side effects, and controlled drug release, but current systems face challenges with poor drug loading efficiency. The use of microgravity in drug encapsulation introduces an innovative approach. Simulated or space-based microgravity environments can improve nanoparticle self-assembly, enhance drug loading efficiency, and optimize release profiles by reducing aggregation and promoting better dispersion within 3D spheroids. Research has explored microgravity as a novel platform to enhance drug loading, finding a more than 2-fold increase in drug loading efficiency in iron oxide nanoparticles (from 50.4% to 99%) and a 4-fold increase in liposomes (from 14.28% to 97.2%), with sustained drug release. These findings pave the way for innovative drug delivery systems and space-based pharmaceutical advancements.[238] These innovations could lead to formulations that better align *in vitro* results with clinical outcomes, while also providing insights into how gravitational forces affect tumor behavior and treatment responses.

Moreover, self-propelled nano/microparticles with living or bioinspired components further enhanced both cancer screening and therapy.[239] Researchers have engineered self-propelled nano/microscale actuators, catalytic janus particles, magnetically or acoustically guided microrobots,[240] bacteria- and sperm-powered swimmers,[241] and red-blood-cell-derived micromotors[242] that actively navigate interstitial spaces, controlled release of chemotherapeutics, gene editors, or radiosensitizers. These biohybrid devices autonomously probe the TME's biochemical and mechanical cues in 3D systems, offering insights into drug penetration and efficacy that 2D assays fail to capture. Biohybrid particles can mimic tumor microenvironments, enabling high-throughput screening of therapeutic agents with enhanced predictive accuracy for clinical outcomes. Their ability to navigate physiological barriers, respond to stimuli such as pH or temperature, and deliver payloads directly to tumor sites in *in vivo* experiments holds promise for personalized medicine. For instance, researchers engineered a polydopamine-coated core−shell nanomotor carrying indocyanine green (ICG). After peritumoral injection, the first NIR-I laser pulse heated the PDA shell, generating thermophoretic propulsion that moved the motor through dense subcutaneous tissue. Simultaneously, ICG emitted bright NIR-II fluorescence, enabling real-time imaging to guide the motor toward the tumor. This targeted movement enhanced the accumulation of photothermal agents at the tumor site, improving the effectiveness of superficial photothermal therapy.[243] A urease-powered nanomotor carrying an STING agonist showed effective bladder wall penetration and retention, significantly enhancing immune activation and tumor inhibition in a bladder cancer mouse model. This approach showed standard Bacille Calmette-Guerin therapy and pembrolizumab, offering a promising new platform for bladder cancer immunotherapy.[244]

Furthermore, the potential of microgravity in tissue engineering promotes the self-assembly of cells into spheroids and organoids with applications in both space exploration and earth-based research. However, challenges such as optimizing microgravity platforms, acclimatizing tissues to Earth's gravity, and addressing cellular response variability remain. Future research should focus on refining these techniques for reliable, scalable tissue engineering.[245,246] However, these advancements have significant challenges. Standardization remains an ongoing issue, as differences in scaffold materials, culture conditions, and spheroid characteristics lead to inconsistent results across reports. Reproducibility and scalability also fall short compared with 2D systems, limiting high-throughput applications and clinical translation. While integrating nano/microparticles shows promise, their behavior in different 3D

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models needs further investigation using advanced imaging, computational modeling, and real-time tracking. These challenges highlight the need for interdisciplinary investigations to refine protocols, improve nanoparticle designs, and establish standardized benchmarks for 3D culture systems. Moreover, integration of artificial intelligence and machine learning could process intricate data sets including nanoparticle dynamics, drug response profiles, and TME interactions to predict therapeutic outcomes with accuracy. Future research should focus on standardizing 3D culture models to ensure consistency across laboratories and make them scalable for drug testing. Advanced imaging and AI-driven analysis can help track how nanoparticles behave in 3D spheroids. Investigation of microgravity's effects on tumors and drug delivery could offer new treatment approaches. Patient-derived 3D models could help to develop personalized treatments using nanoparticles.

Ultimately, the synergy of 3D cultures and nanotechnology holds the potential to redefine cancer treatments. These advancements could reduce clinical trial failures and provide targeted, effective therapies to patients by more accurately mimicking the tumor microenvironment, improving drug delivery, and using advanced technology.

CONCLUSION

The transition from 2D to 3D culture models marks a significant advancement in cancer research, offering a more physiologically relevant platform for investigating tumor biology and drug responses. While 3D models mimic the *in vivo* tumor microenvironment better, challenges such as standardization, reproducibility, and scalability remain. The integration of nano/microparticles into these models has further enhanced our understanding of drug delivery, cellular uptake, and therapeutic interactions, paving the way for more effective and targeted cancer treatments. As research continues to refine 3D culture technologies and nanoparticle-based approaches, these advancements hold great promise for bridging the gap between *in vitro* laboratory assays and personalized clinical applications.

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Notes

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■ ABBREVIATIONS

AM	Additive manufacturing
APH	Adenosine phosphate high
CA	Chitosan-alginate
CHA	Chitosan-hyaluronic acid
CNS	Central nervous system
D-OCT	Dynamic optical coherence tomography
ECM	Extracellular matrix
EMT	Epithelial-to-mesenchymal transition
EVs	Extracellular vesicles
GNPs	Gold nanoparticles
ICP-MS	Inductively coupled plasma mass spectrometry
LNPs	Lipid-based nanoparticles
MPs	Microparticles
NLCs	Nanostructured lipid carriers
NIR	Near-infrared
NPs	Nanoparticles
PCL	Polycaprolactone
PDXs	Patient-derived xenografts
PDA	Polydopamine
SLNs	Solid lipid nanoparticles
TME	Tumor microenvironment
TPM	Two-photon microscopy
WBPU	Waterborne biodegradable polyurethane

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