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Review

Precision Nanomedicine: A Necessary Convergence of Nanodrug Development, Organotypic Models and Microphysiological Systems

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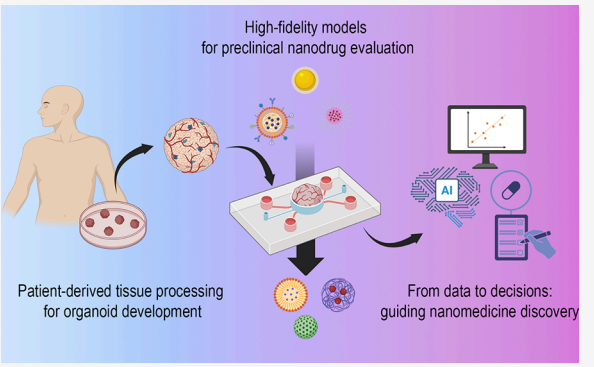
ABSTRACT: Nanoparticle-based therapeutics have been poised to revolutionize medicine for decades. However, despite numerous success stories, translation to the clinic remains hindered by persistent challenges, including toxicity, limited bioavailability, and poor targeting accuracy, which contribute to high failure rates during clinical trials. These limitations stem from the complexity of biological barriers, off-target effects, and patient-to-patient variability in response. Addressing these issues requires more predictive preclinical models that can accurately capture human physiology and disease heterogeneity. In this review we highlight the convergence of nanodrug development with the advent of advanced preclinical testing pipelines, including 3D cell culture with spheroids, organoids and frontier microphysiological systems. We review nanomedicine design criteria with a focus on high efficacy and low toxicity and outline the main barriers to effective disease targeting. Next, we highlight the evolution of 2D cell culture to 3D systems, where advances in nano- and microfabrication have facilitated a rich new ecosystem of biomimetic microphysiological platforms that are accelerating the pace of drug discovery and development. Considering the current stance of regulatory agencies regarding the replacement of animals with human-relevant tissue systems, nanomedicine is at an exciting crossroads with enormous scope to increase the rate of translating nanosolutions from the bench to the bedside.



KEYWORDS: *nanodrug development, micro-physiological systems, organotypic models, nanomedicine*

1. INTRODUCTION

Advances in biology and medicine have spurred enormous growth in our understanding of biological processes, both in normal physiology and in disease, with exquisite detail of the molecular landscape underlying biological processes.[5] Coupled with the ongoing revolution in computational science and artificial intelligence,[7] these developments offer significant potential for deciphering molecular recognition events in biosystems and using these advanced tools to rapidly accelerate structure-based drug design.[7,9,10] Nevertheless, even with a perfectly designed inhibitor, numerous barriers remain that impede desirable therapeutic outcomes including appropriate administration, bioavailability, pharmacokinetics and maintaining concentration within a therapeutic window.[11,12]

A promising solution to the hurdles associated with small molecule-based therapeutics is the use of nanoscale delivery vehicles. The optimal size of these particles aligns well with nature's design principles, many biological entities, from proteins to vesicles, are themselves nanoparticles. Nanoscale objects can be designed to avoid clearance, penetrate and remain in tissue, show organ specificity, and deliver a wide variety of molecules with tunable release rates.[13] First proposed in the 1990s, the idea of nanomedicine has attracted enormous attention in recent decades, with several blockbuster therapeutics clinically available,[14,15] which has improved human health and saved lives. However, as with small molecule drugs, there are concerns with toxicity, nonspecific delivery, variable efficacy, and patient-to-patient variability in response.

Every individual exists in a unique environmental niche, with unique biology that is underpinned by unique molecular networks that govern a vast array of biological processes. The idea of individualized medicine, often termed "personalized" or "precision" medicine, holds that deciphering the unique biology of the individual will facilitate the development of tailored therapies, thereby resulting in more precise disease prevention, diagnosis and treatment.[3] Over the last several decades, the idea of precision nanomedicine has taken root,[18−20] offering a future where nanoparticle-based drugs may be tailored for efficacy based on the individuals' unique circumstances. Considering the

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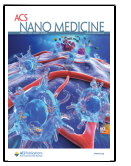

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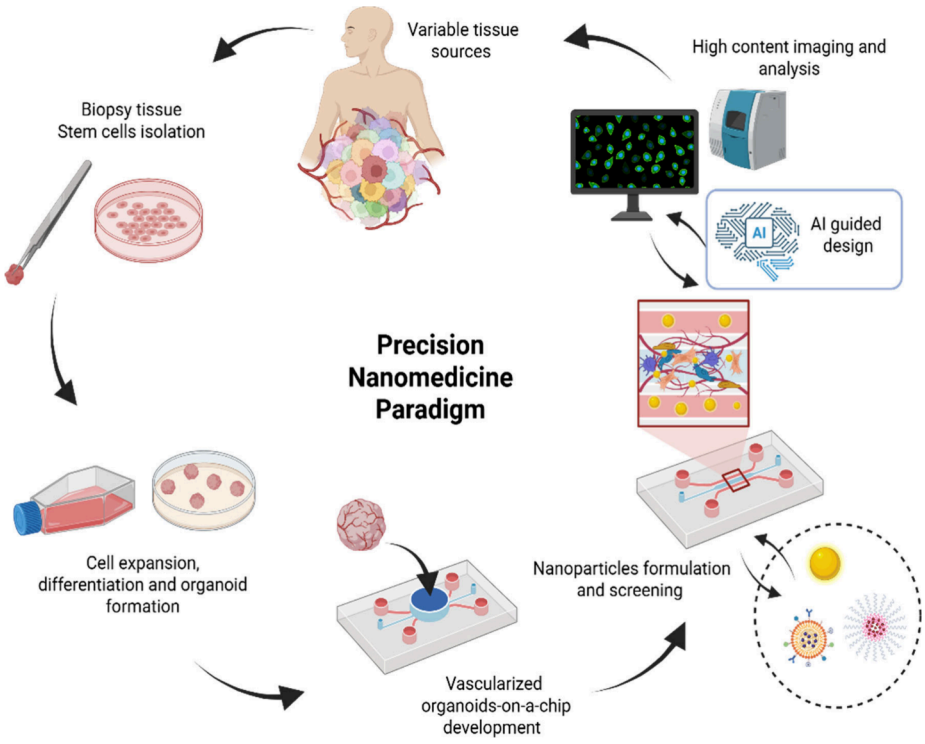

Figure 1. Schematic depicting the emerging paradigm of nanoparticle development for precision nanomedicine.

rapid rate of advances in "omics" technologies (e.g., genomics, proteomics metabolomics, etc.), alongside a decrease in assay time and cost, there is a provocative possibility that profiling an individual's unique biology is close at hand. However, a significant gap remains between drug selection and the vast data sets generated through multiomics analyses;[21] that is, we are still trying to understand how individual molecular profiles relate to therapeutic efficacy, toxicity, and side effects. In most cases, this understanding only emerges after therapy, as individual responses are retrospectively analyzed, largely due to the fact that most preclinical data are derived from standardized cell lines and nonhuman animal models.[22,23]

Precision nanomedicine will not only require robust individualized multiomics technology, but the establishment of an individualized preclinical pipeline for testing therapies to evaluate their suitability. While there have been notable successes in developing individualized therapies on patient cells, and in patient-derived xenograft (PDX) models, critical limitations remain that preclude the timely reproducible analysis of an individual's response to drugs.[22,24] First, conventional cell lines cultured on tissue culture plasticware rarely mimic the native state of human tissue, which is invariably comprised of a complex microenvironment with form and context underlying function. Rarely can the precise cells of interest be stably isolated and expanded from an individual while remaining viable and maintaining proper *in vivo* functional activities.[25,26] Next, PDX models while more physiological in form, suffer from disadvantages associated with nonhuman interference, e.g., integration of animal cells with the implanted human tissue, which can confound downstream analyses.[27] Additionally, these models neglect the immune component, as the animals must be immunocompromised to allow the foreign tissue to grow. This is a major limitation, given the significant role of immune cells in disease. Furthermore, PDX models are time-consuming, expensive, and often only allow limited tissue sources, e.g., implanted material from resected/biopsied tissue.[28]

An emerging paradigm that is poised to solve these challenges and pave the way to precision nanomedicine is the advent of organotypic models (so-called "organoids") and microphysiological systems (Figure 1).[29−32] In contrast to the limitations of conventional cell culture and PDX models, organoid models can be coaxed to display tissue-mimetic features and propagate in culture in ways that closely align with native biology. Early discoveries of the self-organization of tissue, for instance, mammary cells assembling into duct-like structures with functional secretion of milk proteins,[33] have catalyzed a paradigm shift toward 3D tissue models in lieu of 2D cell culture. Seminal work by Sato et al. demonstrated the stable *in vitro* growth of stem cell-derived intestinal organoids, obviating the need for a functional *in vivo* niche.[33] Since then, there have been examples of organoids and microphysiological systems that mimic the complexity of a huge assortment of tissues.[34] In parallel, the development of synthetic matrices, bio fabrication techniques, and advanced microfluidic technologies have accelerated the pace of discovery in the field, with new tissue models being reported each day.[35,36] These advanced *in vitro* models align with the concept of new approach methodologies (NAMs), which aim to reduce reliance on animal testing while improving human-relevant predictive power in nanomedicine safety and efficacy assessment.

In this review, we argue that the full potential of precision nanomedicine will require the simultaneous development of accurate and reproducible models of tissue systems that reflect individualized biology and disease states. We start by surveying the background associated with nanoparticle design and how tuning properties can be employed to overcome biological barriers. The current state of the field with respect to clinical efficacy and toxicity is discussed in the context of nanoparticle design criteria and testing using conventional models. Next, we

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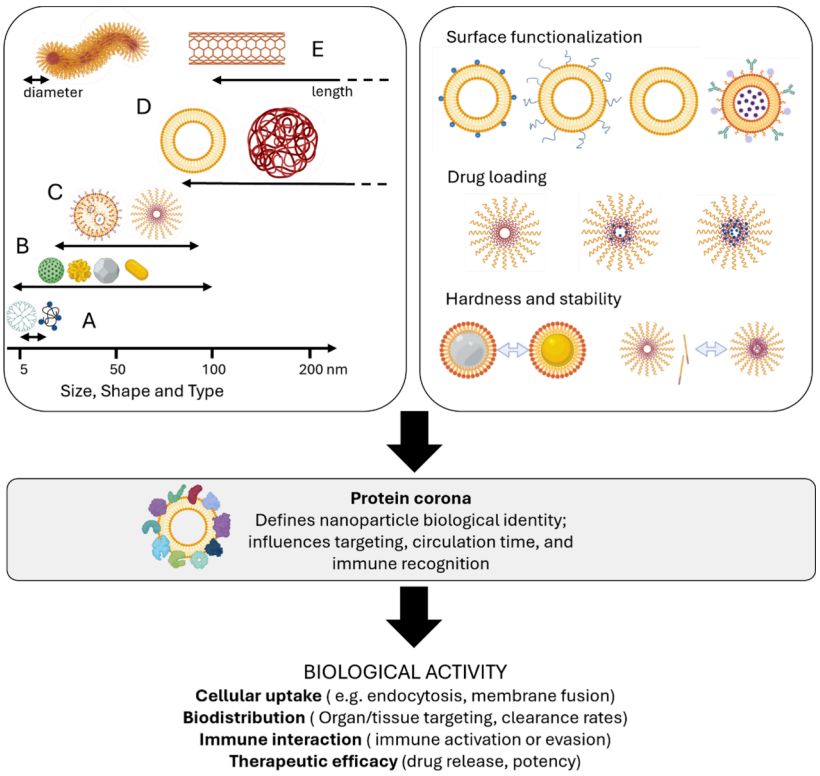

Figure 2. Survey of nanoparticle design criteria for effective bioactivity.

introduce the shift from 2D cell culture to multicellular spheroids and organoids and outline the progression toward accurate and reproducible organotypic systems. We then highlight the emerging role that these systems have played in optimizing nanoparticle formulations in recent years, with a view toward their future use in more predictive assessments of efficacy and toxicity. Several specific examples of how perfusable microphysiological systems will open more opportunities for nanodrug development are presented. Finally, we conclude with a discussion of the remaining barriers that must be addressed to establish reproducible preclinical pipelines for fundamental biology, disease diagnosis and prognosis, and future drug development.

2. NANOMEDICINE DESIGN: TUNING PARTICLE PROPERTIES TO OVERCOME BIOLOGICAL BARRIERS

2.1. Motivation for the Use of Nanoparticles. The delivery of therapeutic agents via nanoparticles presents several key advantages.[20,37] One primary reason is of a practical nature, as drugs usually have low solubility or poor hydrolytic stability. Many pharmacologically active compounds are poorly water-soluble, which limits their bioavailability.[38] Encapsulation within water-dispersible nanoparticles can enhance solubility by several orders of magnitude and protect labile drugs from premature degradation.[39] Additionally, nanoparticle-based systems can enable controlled, sustained release, providing temporal control over drug availability.[40] The structure of nanoparticles can also suppress drug crystallization, further enhancing bioavailability.[41] Consequently, the pharmacokinetics of the drugs are governed by the properties of the nanoparticles rather than by the drugs themselves.[42,43] This allows even drugs that do not adhere to Lipinski's rule to be effectively delivered to target cells. Nanoparticles dictate the fate of the drugs and can therefore enhance circulation time, bypass drug clearance, and evade efflux mechanisms in multidrug-resistant cells, improving therapeutic efficacy and target-site accumulation. The holy grail of drug delivery, however, remains the targeted accumulation of therapeutics at diseased sites.[44−46] A landmark concept in this pursuit is the enhanced permeability and retention (EPR) effect, first reported by Matsumura and Maeda.[47] The EPR effect describes the tendency of nanoparticles to accumulate in tumor tissue due to leaky blood vessels and impaired lymphatic drainage, thus facilitating targeted delivery and prolonged retention of anticancer agents. While this paradigm fuelled much of nanomedicine's development from the 1990s to the 2010s, it is now recognized as variably expressed in human patients and highly dependent on tumor type, vascular architecture, and individual physiology.[48,49] More recent studies have challenged the notion that passive extravasation through endothelial gaps is the predominant mechanism of tumor accumulation.[50] Instead, active transport processes, such as endothelial transcytosis, may account for the majority of nanoparticle entry into solid tumors, with retention and clearance further influenced by tumor−stroma interactions and lymphatic drainage.[51] This "active transport and retention" (ATR) principle offers a complementary or alternative mechanistic framework for nanoparticle delivery, with design implications that include engineering nanoparticle−endothelium interactions, modulating lymphatic flow, and tailoring retention via extracellular matrix remodelling. Incorporating both EPR-dependent and active transport mechanisms into nanoparticle design strategies may therefore improve delivery efficiency and clinical translation.

Understanding that nanoparticle delivery to tumors is governed by both passive mechanisms and active biological processes underscores the importance of strategies that can exploit and complement these pathways. One such approach is

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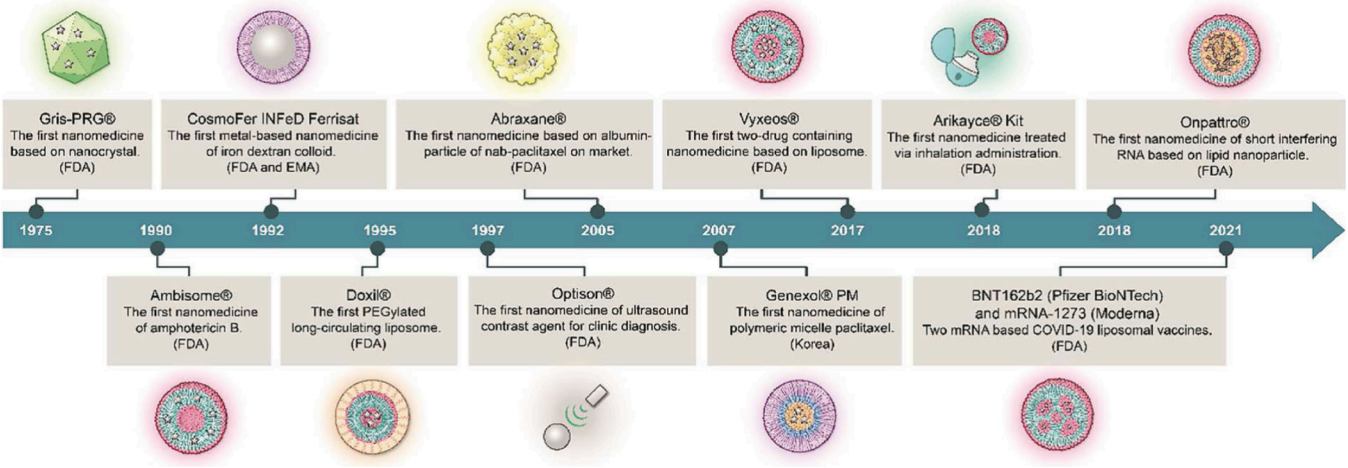

Figure 3. Historical timeline of major nanomedicine development. Reproduced from ref 3. Copyright 2023 Elsevier.

active targeting, in which nanoparticles are functionalized with ligands designed to selectively recognize and bind to overexpressed receptors on tumor or stromal cells. Targeting ligands range from small molecules, peptides, aptamers, to antibodies and have shown *in vitro* and animal models that can enhance cellular uptake and tumor accumulation.[20] However, to date, there is only a handful of such nanoparticles in clinical trials.[52] Progress in active targeting has been hampered by several key challenges. These include the complex design requirements of nanoparticles functionalized with targeting ligands, potential immunogenicity, tumor heterogeneity (often resulting in insufficient target receptor overexpression), and unfavorable interactions between nanoparticles and biological media. Notably, despite significant investment in nanomedicine, the proportion of injected dose accumulating in tumors remains very low. Recent meta-analyses confirm that delivery efficiency has not substantially improved over the past decade, with median values of ~0.76% from 2005−2018[53] and ~0.67% from 2008−2021.[54] Despite the promise of active targeting, there are still several challenges to overcome before clinical translation can fully realize its potential.

2.2. Types of Nanoparticles and Their Properties. In nanomedicine, nanoparticles typically range from 10 to 100 nm, although particles well above 100 nm have also been described (Figure 2).[20] Nanoparticles are categorized not only by their size but primarily by their material. Nanoparticles used in nanomedicine can be classified into three categories: organic and inorganic nanoparticles, as well as natural carriers, such as viruses. Inorganic nanoparticles[55] can be derived from metals (e.g., gold,[56] silver[57]), metal compounds (e.g., upconverting nanoparticles,[58] zinc oxide, cerium oxide or iron oxide),[59] or carbon-based material, such as carbon nanotubes,[60] hydroxyapatite,[61] mesoporous silica[62] and ceramics. Organic nanoparticles encompass a wide range of structures, reflecting the chemical diversity of organic molecules. These include polymeric micelles,[63] dendrimers,[64] polymer-drug conjugates,[65] liposomes,[66] and related structures like lipid nanoparticles,[67] solid polymer nanoparticles,[68] and natural polymers, such as alginates,[69] nanocellulose,[70] and chitosan,[71] as well as protein-based nanoparticles.[72] In addition, natural carriers like viruses, particularly lentiviruses, have gained attention as drug delivery vehicles due to their narrow particle size distribution and ideal size range for cellular uptake and biodistribution.[73,74]

Nanoparticle size plays a crucial role in drug delivery.[75−77] However, identifying the ideal size is complex since nanoparticles face various challenges throughout their delivery journey. They need to avoid clearance by the mononuclear phagocytic system in the bloodstream, facilitate extravasation into tumors, and ensure cellular uptake. A review suggested that a size of 100 nm supports extended circulation, while both theoretical and experimental findings indicate that nanoparticles sized at 30 nm are optimal for efficient endocytosis.[78] Smaller particles are also generally more effective at penetrating tumor tissue due to improved extravasation.[78]

The type of nanoparticle often dictates both size and shape (Figure 2). Dendrimers and polymer-drug conjugates measure under 5 nm, while polymeric micelles range around 50 nm, depending on polymer block lengths. Liposomes exceed 100 nm owing to membrane tension, although the associated lipid nanoparticles can be as small as 30 nm. In contrast, the synthesis of metal nanoparticles, such as gold, can be synthesized across a broad size range, from just a few nanometers to over 100 nm, and are readily produced in various nonspherical forms, including rods, shells, and stars.[79]

Other nanoparticles, including carbon nanotubes and nanocellulose, naturally possess a nonspherical, rod-like structure. These materials typically measure just a few nanometers in diameter but can extend several hundred nanometers in length.[80,81] Despite their elongated shape, they exhibit prolonged circulation *in vivo*, likely due to reduced phagocytic uptake.[81,82] Interestingly, large anisotropic nanoparticles can still be efficiently internalized by cells. For example, platelets measuring up to 5 μm were shown to be readily taken up by the macrophage cell line RAW 264.7,[83] whereas spherical nanoparticles larger than 1 μm generally exhibit limited uptake.

Nevertheless, the link between the shape of nanoparticles and their *in vitro* cellular uptake remains unclear, as some studies indicate that anisotropic nanoparticles achieve better cell internalization while others report the contrary. This conflicting finding may be attributable to variations in other physical properties, such as particle stiffness, softer, rod-like structures may bend and adapt more easily to cellular membranes, influencing uptake efficiency.

The fate of nanoparticles is determined not only by their size and shape, but also by their surface characteristics.[78,84] The conjugation of targeting ligands has been proposed to redirect biodistribution and enhance cellular uptake. However, even

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subtle changes in the surface properties of nanoparticles can significantly impact biological activity. Surface charge, whether negative or positive, is often manipulated to improve the colloidal stability of nanoparticles but also plays a key role in biological behavior.[84]

In the past, researchers have attempted to link the zeta potential of nanoparticles with biological outcomes.[85] Highly charged nanoparticles were thought to elicit a strong response from the mononuclear phagocyte system, leading to rapid clearance. Positively charged nanoparticles were associated with increased cellular uptake, due to favorable interactions with negatively charged cell membranes, which can also lead to adverse effects.[86] In contrast, neutral nanoparticles coated with poly(ethylene glycol) (PEG) were observed to have a long circulation time, albeit at the cost of reduced cellular uptake by all cells, including cancer cells (the "PEG dilemma").[87] However, there is now an increased understanding that nanoparticle behavior is dictated not solely by surface charge, but by the formation of a surrounding protein corona, which is often mixed with other molecules found in the environment.[88,89] The quantity of proteins, their composition, and their state of denaturation ultimately determine where the nanoparticles accumulate. Adding further complexity, several other parameters can also impact nanoparticle performance. For instance, drug loading has been shown to alter the physicochemical properties of nanoparticles, sometimes resulting in reduced cellular uptake as drug loading increases.[90,91] Therefore, it needs to be emphasized that nanoparticles need to be characterized in depth using a range of analytical techniques to fully understand their structure before linking it to biological outcomes.

2.3. Clinical Trials. Despite their complexity, numerous nanoparticle systems are now available on the market, with over 500 nanoformulations currently undergoing clinical trials (Figure 3).[92] Initially concentrated on cancer treatment, nanoformulations are now also being utilized for a wide range of diseases, including autoimmune disorders, neurological conditions, cardiovascular issues, and inflammatory diseases, among others,[93] yet there is still a focus on cancer and infections.

The simplest form of nanoparticles, nanocrystals, have been used to enhance drug delivery for over 50 years, although today they make up only a small fraction of nanoparticles in clinical trials.[94] Today, the field is dominated by liposomes and lipid nanoparticles. Following Doxil's approval in 1995, which is a liposomal formulation of doxorubicin, several additional liposomal formulations have reached the market. More recently, lipid nanoparticles, which have similar building blocks to liposomes, have gained significant prominence as carriers for mRNA-based COVID-19 vaccines, further solidifying their role as a preferred platform for addressing formulation challenges. Collectively, liposomes and lipid nanoparticles now account for more than one-third of all nanoparticle formulations in clinical trials.[93,95,96]

More than 10 years ago, protein-based nanoparticles comprised a relatively high proportion of clinical trials, largely due to the success of Abraxane—the small molecule paclitaxel covalently bound to albumin.[97] However, interest in this class has waned in recent years. In contrast, polymer-based drug delivery systems have gained prominence. These include a range of platforms such as drug-loaded micelles, cyclodextrin-based polymers, dendrimers, solid polymer nanoparticles, and polymer-drug or polymer−protein conjugates, including polymer-antibody conjugates.[93]

Among the limited number of actively targeted nanoparticles that have reached clinical trials, nearly half utilize antibodies to direct the therapeutic to cancer sites. Notably, this area appears to be dominated by approaches that employ transferrin to target its receptor.[52] Inorganic nanoparticles like hafnium oxide are now FDA-approved as radiosensitizers, while others, such as gold capsules and silicon nanoparticles, are being tested in clinical settings for tumor photothermal therapy or tumor radiotherapy, but not for drug delivery.[98] Viruses[99] and bacteria[100] are increasingly being tested as drug delivery carriers in clinical trials. After the approval of Gendicine in 2003, a p53-expressing adenovirus, several viruses have been approved for drug delivery, including gene delivery.[93,101] Overall, there is a growing trend toward the use of natural carriers in drug delivery, reflecting a broader interest in leveraging biologically derived systems for improved targeting and biocompatibility. Exosomes, nanovesicles naturally released from cells, have made it to early stages in clinical trials.[102] Biomimetic nanoparticles, which are synthetic carriers coated with cell membranes, have also shown promising preclinical results, although they have not yet progressed into clinical evaluation.[103] Another area that has received huge investment in fundamental research is the development of stimuli-responsive materials.[104] Although these "smart" drug carriers have been under investigation for decades, only a few have made it onto the market. Notable examples include ThermoDox, a thermally responsive liposomal formulation, and LiPlaCis, an enzyme-degradable liposome designed to release its payload in response to secretory phospholipase A2, an enzyme commonly overexpressed in tumors. For further details, readers are referred to a recent review on nanoparticles in clinical trials, which provides comprehensive analyses and tabulated summaries of clinically relevant formulations.[93]

It is well established that merely a small percentage of developed nanoparticles progress to clinical trials, with an even smaller number reaching the market. For example, while nanomedicines have an estimated success rate of approximately 94% in Phase I clinical trials, this drops to 53% in Phase II and further declines to just 18% in Phase III.[105] Several practical challenges contribute to this limited translational success. As discussed by Vicent and colleagues,[106] these include difficulties in scaling up production while maintaining acceptable costs, achieving high purity, and addressing batch-to-batch variability, issues that are often compounded by the complexity of nanoparticle designs.

Early engagement with regulatory agencies can help define development strategies with greater clinical relevance. Nevertheless, a major barrier to translation remains the lack of reliable preclinical models capable of predicting the performance of nanoscale drug delivery systems in human patients. Addressing this gap requires rigorous toxicology studies to evaluate nanoparticle safety before progressing to *in vivo* testing. Yet, these assessments also have significant limitations, as outlined below.

2.4. Toxicology Studies. Following in-depth characterization of nanoparticles, using scattering, spectroscopic, chromatographic and microscopy techniques as per literature recommendations[107] and by government organizations,[108] their toxicology profile can be evaluated, as reviewed in detail elsewhere.[109,110] This characterization step is a prerequisite for establishing reliable structure−activity relationships, which are critical to understanding the features that enable, for example, high cellular uptake. At this stage, it is also recommended to

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identify suitable control, such as the current gold standard within the relevant field. Preliminary assessment of metabolic activity, which often relies on colorimetric assays such as sulforhodamine B (SRB) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, can provide quick answers to whether a nanoparticle formulation inhibits cell proliferation. These assays typically operate under the assumption that the nanoparticles are indeed internalized by the cells.[110] Cell proliferation assays must therefore be complemented by cellular uptake studies, typically performed using fluorescence microscopy or flow cytometry. However, it is important to note that flow cytometry primarily measures nanoparticle association with the cell surface, rather than true internalization.[111] Cell uptake studies, combined with temperature changes or the addition of inhibitors, can help identify the endocytic pathway involved. While most nanoparticles are taken up by endocytosis, their uptake route, whether via micropinocytosis, clathrin-mediated endocytosis, caveolae-mediated endocytosis, or alternative pathways, is influenced by particle size, shape, and surface chemistry. Generally, higher cellular uptake is directly correlated to higher toxicity,[112] assuming the nanoparticles belong to the same material class. Thus, grasping the factors that influence high cellular uptake is essential to comprehend cellular toxicity. Nanoparticles in the size range of approximately 30−50 nm have often exhibit the highest cellular uptake,[113] although particle shape also plays a role.

As discussed earlier, while surface chemistry plays a role in cellular uptake, the most direct determinant is the absorption of proteins onto the nanoparticle surface. Nanoparticle aggregation, driven by protein adsorption and high degrees of denaturation, can hinder uptake by certain cell lines, making these nanoparticles appear nontoxic. Conversely, specific adsorbed proteins can enhance cellular uptake in other cell lines, leading to different toxicity outcomes. Importantly, the protein corona can also cover ligands that were initially functionalized on the nanoparticle surface, making specific delivery futile.[114]

Care needs to be taken when choosing the right metabolic assay to assess nanoparticle toxicity. Nanoparticles can interfere with colorimetric, plate-based assays, potentially resulting in false values. This highlights the need to use at least two independent tests to validate findings.[115] In contrast, flow cytometry-based assays appeared to exhibit minimal interference from nanoparticles, as shown by a study comparing different metabolic assays in cells stained with Propidium Iodide (PI) or Calcein-AM following nanoparticles incubation.[116] Once nanoparticles cause cell death, it is also essential to determine the mode of cell death, whether via apoptosis or necrosis,[117] which can be determined by an array of assays.[118]

While metabolic activity assays can indicate whether cells are affected by nanoparticle exposure, they do not reveal the underlying mechanisms of toxicity. Cells can respond to nanoparticles by producing more reactive oxygen species (ROS). When the cellular antioxidant defense system, such as glutathione peroxidase, is overwhelmed, this can lead to oxidative damage of cellular components, including membranes, DNA, proteins, and organelles. Molecular probes, including 2′,7′-difluorescein-diacetate (DCFH-DA), are frequently used to detect ROS. Upon oxidation by ROS, DCFH-DA is converted into a fluorescent compound, providing a quantitative measure of oxidative stress. Alternative assays are also available to assess oxidative stress and a catalogue of recommended assays can be found elsewhere.[119]

Nanoparticles are known to potentially cause inflammatory responses in cells, which can be detected by measuring inflammatory biomarkers.[120,121] Various assays, including ELISA (enzyme-linked immunosorbent assay) and Western blotting, are available for measuring produced cytokines. However, it is important to note that nanoparticles may interfere with the analysis, as certain cytokines can adsorb onto the nanoparticle surface, potentially leading to underestimation of their concentration.[110] Given these potential adverse effects, significant efforts have focused on engineering nanoparticles to minimize toxicity and improve their safety profile. In parallel with toxicity evaluation, a range of nanoparticle design strategies have been developed to reduce or prevent adverse effects.[122,123] Surface modifications such as PEGylation, zwitterionic coatings, or cell membrane camouflaging can minimize protein adsorption, immune recognition, and off-target accumulation.[124,125] Incorporating active targeting ligands, such as peptides, aptamers, or antibodies, can further enhance selective delivery to diseased tissues, thereby reducing systemic exposure. The use of biodegradable and bioresorbable materials, including lipids, polysaccharides, and FDA-approved polymers such as PLGA, enables controlled degradation and clearance, lowering the risk of long-term accumulation.[126,127] Additionally, stimuli-responsive systems that release their payload in response to specific physiological triggers (e.g., pH, enzymes, or temperature) can improve therapeutic precision and minimize exposure to healthy tissues.[128,129] Collectively, these approaches represent complementary strategies to improve nanoparticle safety profiles while maintaining or enhancing therapeutic efficacy.

Advancements in "omics" technologies have made it possible to gain a greater understanding of the mechanisms underlying nanotoxicology.[130] However, their widespread use is still limited. In addition to proteomics, transcriptomics can provide valuable insight into gene expression changes following nanoparticle exposure, revealing activation or suppression of specific biological pathways related to inflammation, stress response, or apoptosis.[131] Metabolomics, by profiling small-molecule metabolites, can capture functional consequences of nanoparticle-induced perturbations in cellular metabolism, oxidative balance, and energy homeostasis.[132] Together, these omics approaches offer a systems-level view of cellular responses, enabling the identification of early toxicity signatures and potential predictive biomarkers. When integrated with computational modeling, transcriptomic and metabolomic data sets can help link physicochemical nanoparticle properties to adverse biological outcomes, ultimately accelerating the development of safer and more effective nanoparticles for nanomedicine.[133]

These approaches help us to learn more about single cells, but they cannot serve as comprehensive models for complex diseases. Consequently, researchers often turn to animal models; however, species-specific differences in physiology, immune responses, and nanoparticle dynamics frequently result in poor translation to human outcomes. Moreover, assessing nanoparticle distribution, cellular uptake, and clearance *in vivo* remains technically challenging and ethically constrained. As a result, there has been a growing interest in human-relevant *in vitro* platforms that can better recapitulate tissue architecture and function. While physicochemical optimization of nanoparticles can address issues such as stability, biodistribution, and targeted delivery, these advances must be matched by equally sophisticated testing platforms capable of replicating the

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Table 1. Comparison of preclinical models for anoparticle development

Model	Advantages	Disadvantages
2D Cell Culture *Fast, cheap, but oversimplified*	◆ High-throughput Nanoparticles screening ◆ Simple and cost-effective ◆ Easy to analyze uptake and toxicity ◆ Standardized protocols across labs	▼ No tissue-like barriers or ECM ▼ Overestimates Nanoparticles uptake ▼ No gradients (oxygen, nutrients) ▼ Poor predictor of in vivo behaviour
3D Spheroids *Tumor-like, scalable, semi-physiological*	◆ Nanoparticles penetration and retention modelling ◆ Tumour resistance and hypoxia mimicking ◆ Spatial toxicity distribution ◆ Compatible with imaging and HTP platforms	▼ Limited cell diversity ▼ Lacks vasculature and immune components ▼ Difficult to control spheroid uniformity ▼ Still lacks systemic response modelling
Organoids *Patient-relevant, architecturally complex*	◆ Personalized Nanoparticles efficacy testing ◆ Genetic background and disease state modelling ◆ Organ-specific Nanoparticles transport mimicking ◆ Long-term tracking of Nanoparticles fate	▼ Batch-to-batch variability ▼ Time- and resource-intensive ▼ Lower throughput than 2D/3D ▼ Limited vasculature and immune crosstalk
Microphysiological Systems *Dynamic, systemic, human-relevant*	◆ Real-time monitoring of Nanoparticles biodistribution ◆ Flow, shear stress, and organ–organ interactions simulation ◆ Immune and vascular Nanoparticles interaction studies ◆ Reduced reliance on animal models	▼ Technically complex ▼ High cost and specialized equipment ▼ Not yet standardized ▼ Low throughput, limited scalability

Usability ↑ / Relevance ↑

complexity of human biological environments. In this context, organotypic models and microphysiological systems have emerged as powerful next-generation tools. By integrating human-derived cells into architecturally and functionally relevant 3D systems, often combined with dynamic microfluidic control, these platforms provide an opportunity to assess nanoparticle performance under conditions that closely mimic *in vivo* physiology, thereby bridging the gap between conventional *in vitro* assays and clinical translation.

3. ORGANOTYPIC ASSAYS AND MICROPHYSIOLOGICAL SYSTEMS

The integration of organotypic assays and microphysiological systems has revolutionized *in vitro* research by enhancing physiological relevance, improving predictability, and reducing reliance on traditional animal models. This section will explore the evolution of these systems, their applications, and their potential for advancing nanomedicine development (Table 1).

3.1. From 2D Cell Culture to 3D Spheroids: Moving toward Complexity. Traditional 2D cell culture systems have long served as the backbone of *in vitro* biology and drug testing due to their simplicity, scalability, and cost-effectiveness. However, the structural and physiological simplicity of monolayer cultures has significant drawbacks. These systems lack the spatial and biochemical complexity found *in vivo*, leading to discrepancies in cell signaling, gene expression, and, critically, drug response. This mismatch frequently contributes to poor predictive power during nanoparticles screening, with therapeutic agents that perform well in 2D often failing *in vivo* or in clinical trials.

To address these shortcomings, 3D cultures models, such as spheroids, have gained traction.[134] These self-organizing cellular aggregates exhibit architectural and functional features of tissues, including spatial organization, cell−cell and cell-matrix interactions, and the establishment of nutrient and oxygen gradients. These properties are especially critical in nanoparticle drug development, where penetration, uptake, and efficacy are highly dependent on tumor-like architecture and microenvironmental conditions.

One of the earliest and most widely adopted protocols for generating multicellular tumor spheroids was published by Friedrich et al. in *Nature Protocols*,[135] which provided a robust and reproducible method for generating size-controlled spheroids for drug screening and toxicity assays. The study showed that spheroids respond to chemotherapeutics in a more clinically predictive manner than 2D cultures, including exhibiting drug resistance behaviors observed *in vivo*, a crucial feature for evaluating nanoparticle-based formulations that rely on penetration and release kinetics within dense tumor masses.

Since then, advancements in 3D culture platforms have continued to support their use in nanoparticle testing. For example, LaBarbera et al. demonstrated differential uptake and cytotoxicity of polymeric nanoparticles in breast cancer spheroids compared to monolayers, underlining the importance of evaluating nanoparticle diffusion and cellular access within a tissue-mimetic environment.[136] More recent studies, such as those by Vinci et al., have emphasized the value of spheroid-based high-throughput screening platforms to identify nanoparticle formulations that can overcome penetration barriers and hypoxic resistance, two hallmarks of the tumor microenvironment.[137] A growing body of experimental work from 2020 onward continues to illustrate the unique advantages of spheroid models in nanoparticle research, building on foundational methods for generating uniform tumor spheroids. For instance, recent work by Pratiwi et al. employed fluorescence imaging combined with machine learning to quantify the intratumoral distribution of mesoporous silica nanoparticles within tumor spheroids,[138] offering a refined spatial analysis of nanoparticle behavior that exceeds the capabilities of conventional 2D culture systems. Further supporting this shift, Bromma et al. demonstrated the utility of 3D tumor spheroids for evaluating nanoparticle-based cancer therapy, specifically showing how these models can reliably capture the therapeutic effects of combined gold nanoparticle and docetaxel treatment by mimicking *in vivo*-like gradients and tissue architecture that critically influence response (Figure 4).[1]

Beyond efficacy, 3D spheroids have proven especially valuable in nanoparticle toxicity screening by replicating the structural and biochemical complexity of solid tumors more accurately than 2D monolayers. Recent experimental studies have increasingly highlighted these advantages. Li et al. systematically evaluated how nanoparticle size influences penetration and cellular uptake in tumor spheroids.[139] Their results demonstrated that 15 nm nanoparticles penetrated more efficiently into spheroid cores via an energy-independent transcellular pathway,

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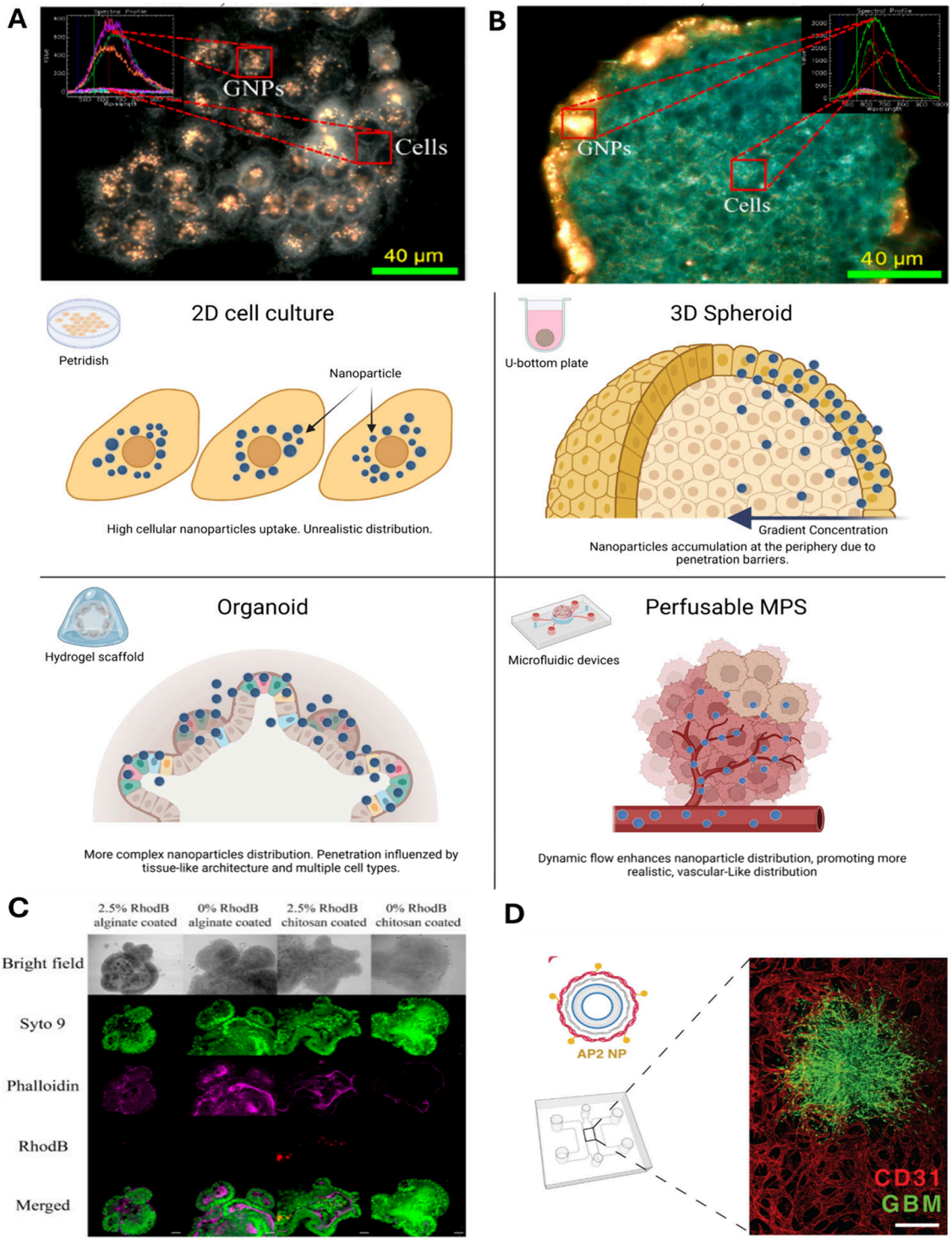

Figure 4. Preclinical models for testing efficacy of nanoparticles. (A) Gold nanoparticles uptake in 2D monolayer of HeLa in a 96-well microplate. Reproduced from ref 1, Copyright 2021 MDPI. (B) Gold nanoparticle uptake in 3D spheroid of HeLa in a U-bottom 96-well microplate. Reproduced from ref 1, Copyright 2021 MDPI. (C) Nanoparticles accumulate in gut organoids with different surface coatings. The higher Rhodamine B signal is observed with chitosan-coated nanoparticles compared to alginate-coated nanoparticles. Reproduced from ref 8. Copyright 2021 MDPI. All animal procedures were conducted with the approval of the Iowa State University Institutional Animal Care and Use Committee IACUC 9-04-5755-M. NIH guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985) have been observed. (D) A microfluidic model for predicting human glioblastoma response to blood–brain barrier-penetrant nanoparticle trafficking. Reproduced from ref 16. Copyright 2022 The Author(s), licensed under CC BY-NC-ND 4.0. No changes were made.

while larger particles showed restricted diffusion and limited uptake, highlighting the importance of tailoring nanoparticle design to the structural complexity of 3D tumor models. In another study, Hashemi et al. investigated the cytotoxic effects of doxorubicin (DOX) delivered via natural killer (NK) cell-derived exosomes on triple-negative breast cancer spheroids.[140] The study demonstrated that DOX-loaded NK exosomes significantly reduced tumor cell viability in 3D spheroid models

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compared to 2D monolayer cultures, highlighting the enhanced drug resistance observed in 3D systems. Further emphasizing the translational potential of 3D systems, Bromma et al. conducted a comparative study of nanoparticle accumulation in 2D monolayers, 3D spheroids, and *in vivo* xenograft models using prostate cancer cells.[141] Their results showed that 3D spheroids more closely mimicked *in vivo* nanoparticle accumulation patterns, especially in the presence of docetaxel, thus serving as a more reliable intermediate for predicting drug delivery behavior in solid tumors. A complementary study by Kelly et al. employed single-cell resolution imaging within 3D spheroids to quantify nanoparticle-induced cytotoxicity across different spheroid layers.[142] Their approach revealed spatially distinct toxicity profiles not observable in 2D cultures, demonstrating that peripheral and core cells can respond differently due to diffusion limitations and microenvironmental gradients.

Finally, integrating functional readouts within spheroid models is becoming increasingly feasible. Ning et al. developed a nondestructive imaging method to monitor spheroid formation, migration, and fusion in real time, enabling dynamic assessment of tumor behavior without disrupting the 3D culture.[143] Debruyne et al. introduced multimodal near-infrared-emitting nanosensors into live multicellular spheroids,[144] enabling real-time imaging of oxygenation gradients, a critical factor in tumor physiology and nanomedicine efficacy. El-Sadek et al. introduced a label-free imaging approach using dynamic optical coherence tomography (OCT) to monitor drug responses in patient-derived tumor spheroids.[145] By capturing microstructural and functional changes in real time, the method allowed for noninvasive assessment of treatment effects without requiring dyes or molecular probes. All together, these tools represent a significant advancement for investigating how microenvironmental dynamics influence nanoparticle performance under physiologically relevant conditions.

Together, these studies show that 3D spheroids are a key bridge between simple 2D cultures and *in vivo* complexity. By preserving key features of tissue architecture, such as spatial gradients, mechanical constraints, and cell−cell/matrix interactions, spheroids offer significantly improved physiological relevance without the species-specific limitations inherent to animal models. They provide a scalable and increasingly sophisticated platform for nanoparticle screening, toxicity evaluation, and mechanistic insight. Importantly, beyond serving as a screening tool, spheroid models have been instrumental in uncovering critical nanoparticle characteristics that govern effective drug delivery. Through these models, key parameters such as penetration depth, surface charge, and particle size have been linked to therapeutic efficacy. Studies demonstrated that achieving high nanoparticle penetration throughout the spheroid is essential, with smaller nanoparticles and optimized surface properties enhancing diffusion. Interestingly, while cationic surfaces or targeting ligands promote cellular uptake, they often result in nanoparticle accumulation at the spheroid periphery, limiting deeper penetration. In contrast, negatively charged nanoparticles, despite lower uptake in 2D systems, show superior penetration into the spheroid core. Additionally, spheroid-based research has explored the impact of nanoparticle architecture, highlighting benefits of cross-linked micelles and rigid particles, although conclusions regarding optimal particle shape remain inconclusive due to variable findings across models. These insights emphasize the unique value of spheroid systems in guiding nanoparticle design strategies that may not be apparent in conventional monolayer cultures.[146]

However, despite these advantages, spheroids still fall short in capturing the full spectrum of tissue function and systemic interactions observed in the human body. They lack the cellular diversity, long-term maturation, and structural compartmentalization characteristic of native organs. Functional features such as organ-specific vasculature, immune interactions, and multilineage differentiation remain limited in traditional spheroid systems.

To bridge the gap between simple spheroids and more physiologically relevant models, several studies have employed 3D organotypic coculture platforms that incorporate multiple primary cell types and extracellular matrix components. For example, advanced biomimetic models have been developed to evaluate nanoparticle-based therapies targeting metastatic ovarian cancer.[147,148] These systems, which better replicate tumor−stroma interactions and invasion dynamics, have proven valuable for assessing both drug-loaded micelles and siRNA-delivering nanoparticles designed to inhibit cancer cell migration and invasion. Such approaches highlight the growing role of tailored organotypic models in enhancing the predictive power of nanoparticle screening by better replicating key aspects of the tumor microenvironment. However, while these models provide an important bridge between simple spheroids and advanced systems, they still lack features such as organ-level architecture, long-term functionality, and patient-specific biology.

To address these limitations, the next step in modeling complexity lies in the emergence of stem cell-derived organoids and induced pluripotent stem cell (iPSC)-based platforms. These systems offer not only greater architectural fidelity but also the ability to model developmental processes, genetic backgrounds, and patient-specific disease phenotypes, thereby providing a more physiologically relevant platform for evaluating nanomedicine behavior and therapeutic response.

3.2. Toward Physiological Relevance: Organoids as the Future of Nanodrug Testing. The evolution from spheroids to stem cell−derived organoids mark a pivotal shift in the development of physiologically relevant *in vitro* models for nanomedicine. While both spheroids and organoids offer a 3D architecture that more accurately mimics tissue environments compared to 2D monolayers, they differ significantly in origin, structure, and complexity.[149,150] Spheroids are typically formed through the aggregation of established cancer cell lines and excel at reproducing basic tumor features such as hypoxia, nutrient gradients, and limited drug penetration. In contrast, organoids are derived from stem cells, either adult tissue-resident stem cells, cancer stem cells, or induced pluripotent stem cells (iPSCs), and are capable of self-organizing into more complex, tissue-like structures that recapitulate aspects of organ development, lineage diversity, and function.

Recent methodological advances have made these models increasingly accessible and reproducible. For example, Driehuis et al. developed a robust protocol for generating genetically stable, long-term expandable human adult stem cell-derived organoids, enabling scalable applications in drug testing and disease modeling.[151] These advanced models provide a robust platform for investigating nanoparticle biodistribution and organ-specific toxicity, while their ability to replicate the architecture and functionality of organs such as the liver, brain, and gastrointestinal tract enhances their potential for

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patient-specific therapeutic screening, offering more predictive and human-relevant data than traditional models.

Organoid generation typically begins with the isolation of stem or progenitor cells from patient biopsies, resected tissue, or induced pluripotent stem cell (iPSC) cultures. These cells are embedded within a supportive extracellular matrix (ECM) scaffold, most commonly Matrigel or defined synthetic hydrogels, which provides structural cues and a 3D environment for self-organization.[152] Culture media are tailored with lineage-specific growth factors and small molecules to direct differentiation and maintain stemness where required; for example, Wnt, R-spondin, and Noggin supplementation for intestinal organoids, or EGF and FGF for neural organoids.[34,153] Long-term expansion and stability are often supported by mechanical protection from the gel, controlled oxygenation, and periodic passaging to preserve viability and genetic integrity. Emerging protocols are replacing Matrigel with chemically defined matrices and microfabricated scaffolds to reduce variability and improve reproducibility, an essential step for quantitative nanomedicine testing.[154−157]

In oncology, organoids derived from patient tumors or cancer stem cells offer a uniquely powerful platform for precision medicine, as they preserve individual genetic, epigenetic, and histological characteristics.[158] Importantly, the inclusion of cancer stem cell populations within organoid models addresses a key limitation of simpler systems, as these cells are often responsible for tumor recurrence and resistance to treatment yet are underrepresented in conventional 2D and spheroid cultures. Recent advancements in glioblastoma organoids (GBOs) have revolutionized drug screening and therapeutic development, enabling more personalized treatment approaches.[159,160] GBOs derived from patient tumors retain the genetic and phenotypic diversity of the original malignancies, making them powerful models for testing the efficacy of targeted therapies based on specific tumor subtypes. For instance, Jacob et al. demonstrated the utility of GBOs in identifying tailored therapies by testing a variety of chemotherapy agents, such as Gefitinib, Trametinib, and Everolimus, in response to mutations commonly found in glioblastoma, including EGFR and PI3K mutations.[161] Building on these findings, Loong et al. highlighted the potential of patient-derived tumor organoids in predicting drug responses for glioblastoma, providing further evidence that GBOs can assist in personalized treatment planning by reflecting the molecular heterogeneity of the tumor.[162] This work laid the groundwork for applying GBOs to guide clinical decisions.

More recently, Chen et al. advanced the clinical application of GBOs by examining tumor samples from multiple glioma patients,[163] including a 29-year-old male with IDH-WT glioblastoma, a particularly aggressive subtype that lacks mutations in the isocitrate dehydrogenase (IDH) gene and is associated with poorer prognosis and resistance to treatment. In this case, GBOs revealed a BRAFV600E mutation, and subsequent testing with Vemurafenib and Trametinib demonstrated significant efficacy, leading to clinical treatment with these agents. The patient achieved complete tumor regression and remained in remission for over a year, underscoring the potential of organoid-based drug testing to guide personalized treatment strategies. In addition to testing traditional therapies, GBOs have proven instrumental in assessing anti-invasive and antiangiogenic treatments. Goranci-Buzhala et al. used GBOs to evaluate GI254023X, an inhibitor targeting the PI3K-mTOR pathway, which successfully reduced glioma invasion.[164] Additionally, Bevacizumab, an antiangiogenesis agent, was tested in combination with VAL-083 chemotherapy, showing promising results in improving treatment outcomes.

Following the success of GBOs in personalized drug testing, a variety of other organoid models have emerged for testing drug efficacy across different organ systems. Intestinal organoids, derived from gut stem cells or iPSCs, have emerged as a powerful model for studying drug absorption, metabolism, and toxicity in the gastrointestinal tract.[165,166] These 3D organoids replicate the structure and functionality of the intestinal epithelium, including key features such as cellular differentiation, microvilli, and the intestinal barrier. They are particularly useful for evaluating how oral drug formulations are absorbed in the gut and how nanoparticles or drugs interact with the intestinal epithelium. For example, Kasendra et al. utilized human intestinal organoids to study mucosal penetration and inflammatory responses induced by oral nanoparticle formulations.[167] Their study emphasized how these organoids can model both drug absorption and intestinal toxicity, which are critical for understanding how nanomedicines are processed by the gastrointestinal system. Similarly, Sato et al. demonstrated the ability of intestinal organoids to simulate the effects of cytotoxic drugs and identify adverse gastrointestinal effects, such as intestinal inflammation or epithelial damage, that might not be observed in simpler models.[33] These models are especially valuable for nanomedicine, where the biocompatibility and toxicity of nanoparticle-based drug delivery systems can vary significantly due to the complex interactions between nanoparticles and the gut barrier.

Pancreatic cancer is notoriously difficult to treat, and pancreatic cancer organoids have become key models for understanding drug resistance and evaluating combination therapies.[168−170] Recent research has demonstrated the remarkable potential of patient-derived organoids (PDOs) to bridge the gap between laboratory models and clinical reality in pancreatic cancer treatment. Kim et al. have advanced the field by establishing a diverse biobank of pancreatic cancer organoids that capture the molecular signatures associated with tumor metastasis.[171] Their work highlights how PDOs not only reflect tumor heterogeneity but also reveal distinct drug sensitivity patterns, underscoring the potential for tailoring treatments based on individual tumor profiles. Similarly, Frappart et al. demonstrated that PDOs, when compared to patient-derived xenografts, provide a faster and more cost-effective means of predicting drug responses, making them highly suitable for integration into clinical workflows.[172,173] Building on these foundations, Beutel et al. showcased the clinical relevance of PDO pharmacotyping through a prospective trial, where *ex vivo* drug sensitivity testing accurately forecasted patient responses to chemotherapy.[174] Their findings suggest that PDO-guided treatment selection could become a powerful tool to enhance patient outcomes by avoiding ineffective therapies. Adding to this, Demyan et al. emphasized the dynamic nature of PDOs in monitoring treatment response over time, particularly in the neoadjuvant setting, where rapid drug screening could inform adjustments in therapeutic strategies.[175] Together, these studies illustrate a clear trajectory toward the incorporation of pancreatic cancer organoids into personalized medicine.

Breast cancer is another area where organoids have significantly impacted drug testing, particularly in understanding the interplay between tumor cells and the stroma. In a study by Camorani et al.,[176] patient-derived breast cancer organoids were used to test bispecific aptamer-decorated nanoparticles for targeted photothermal therapy. These nanoparticles were

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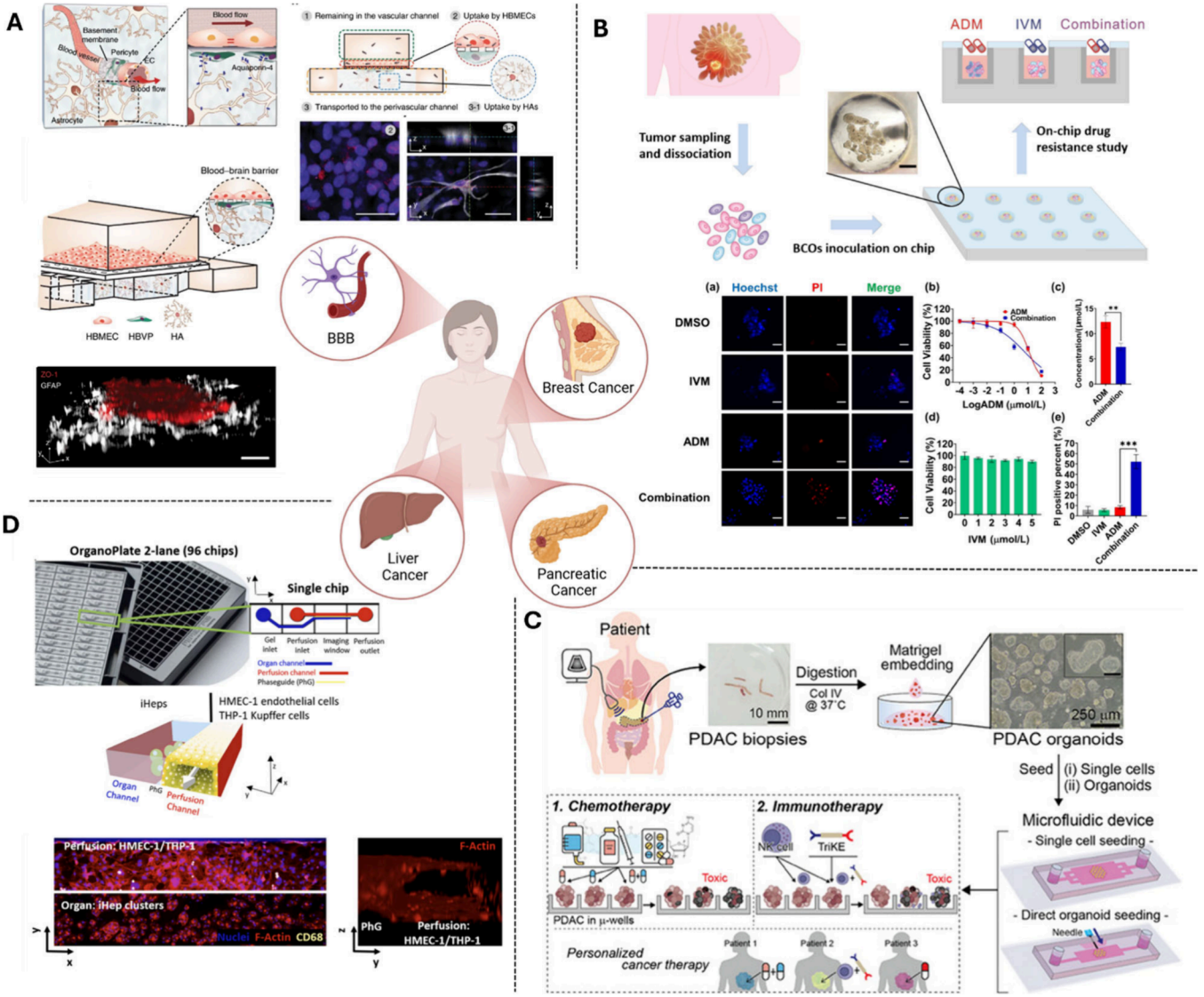

Figure 5. Key studies using organoid/MPS for nanodrug development. (A) Microengineered human BBB model to investigate nanoparticle transport mechanisms. Reproduced from ref 2. Copyright 2020 Springer Nature. All animal experiments were reviewed and approved by the Georgia Tech's Institutional Animal Care and Use Committee. (B) Microarray-based patient-derived organoids for studying drug resistance in breast cancer. Reproduced with permission from ref 4. Copyright 2024 American Chemical Society. (C) Personalized chemotherapy and immunotherapy testing using microfluidic organoid cultures from pancreatic cancer biopsies. Reproduced from ref 6. Copyright 2023 The Authors. Advanced Science published by Wiley-VCH GmbH. (D) High-throughput screening of compound toxicity with a 3D microfluidic liver model. Reproduced from ref 17. Copyright 2021 Elsevier.

designed to target both tumor cells and stromal cells, significantly improving therapeutic efficacy. This study highlighted the importance of considering genetic and architectural variability within organoid models, as these factors can dramatically affect nanoparticle delivery and drug response.

In conclusion, the ongoing integration of organoids into drug testing and nanomedicine highlights a critical step toward creating human-relevant, patient-specific models. By combining the complexity of organ-specific features with the power of stem cell-derived organoids, these models will continue to drive the next generation of personalized therapies, offering improved predictability of drug efficacy compared with traditional models.

At the same time, certain aspects of human physiology remain challenging to capture with organoids alone. Features such as functional vasculature, immune interactions, long-term maturation, and coordinated multiorgan responses are still difficult to reproduce in static cultures. These challenges are increasingly being addressed through integration with microfluidic perfusion, tissue−tissue interfaces, and interconnected organ modules, which create a more dynamic and physiologically relevant environment. The following section explores how such microphysiological systems are being developed and applied to advance nanomedicine testing.

3.3. Advancing Nanomedicine with Perfusable Microphysiological Systems. While organoids provide a major advance in recapitulating native tissue architecture and function, they generally lack physiological fluid flow and multiorgan interactions. The integration of microfluidic systems with organoid cultures addresses these limitations and has emerged as a key innovation in nanomedicine, creating perfusable microphysiological systems that significantly enhance the physiological relevance of these models.[177,178]

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Microfluidic platforms are typically fabricated using soft lithography, in which a master mold patterned via photolithography defines the microchannel geometry.[179] Polydimethylsiloxane (PDMS) is the most widely used elastomer owing to its optical transparency, biocompatibility, and ease of bonding to glass or other PDMS layers via plasma activation.[180] Surface modifications, such as extracellular matrix (ECM) protein coatings (e.g., collagen, laminin, fibronectin) or chemical functionalization with silanes or PEG derivatives, are often applied to promote specific cell adhesion and control protein adsorption. Alternative thermoplastics (e.g., cyclic olefin copolymer, PMMA) may be used when reduced gas permeability or high-throughput manufacturing is desired.[181,182] These fabrication and surface-conditioning steps are critical for recapitulating physiological conditions, as they directly influence shear stress, permeability, and cell−matrix interactions within the microchannels.

By tailoring channel dimensions, flow rates, and surface chemistries, microfluidic devices can be optimized for specific tissue types or experimental goals. This tunability enables reproduction of physiological parameters such as nutrient and oxygen gradients, vascular perfusion, and organ-to-organ communication, while supporting the coculture of multiple cell types in defined spatial arrangements. Such control underpins the development of highly sophisticated microphysiological systems (MPS) that more accurately model nanoparticle transport, diffusion, clearance, and pharmacokinetics in environments that closely mimic human biology. By integrating dynamic fluid flow and mechanical shear stress, these systems provide real-time, in situ monitoring capabilities and a more *in vivo*-like experience than traditional 2D and static 3D culture systems.

Building on these capabilities, the integration of microfluidic systems with organoids has further enhanced their maturity and physiological relevance. For example, liver organoids have benefited from microfluidic platforms, with Wang et al. developing a perfusable liver organoid system that simulates drug metabolism and hepatotoxicity, improving predictions of liver toxicity.[183] This platform has allowed for more accurate testing of drug-induced liver injuries, such as those caused by acetaminophen, a medication commonly associated with liver damage. Similarly, kidney organoids, as demonstrated by Homan et al., have been integrated with microfluidic systems to promote vascularization and maturation, facilitating more accurate nephrotoxicity screening.[184] These systems also enable long-term cultures under fluid shear stress, improving the reliability of toxicological evaluations by replicating renal function more effectively than traditional models.

Brain organoids, when coupled with microfluidic devices, provide valuable insights into blood−brain barrier (BBB) permeability and neurotoxicity. These brain-on-a-chip systems. enable the study of how nanoparticles cross the BBB and their potential neurotoxic effects, offering a more realistic and reliable platform for testing central nervous system (CNS)-targeted therapies. Additionally, the versatility of microfluidic platforms has enabled further advancements in brain organoid modeling. Karzbrun et al. demonstrated the use of microfluidic devices to model cerebral folding, providing deeper insights into brain morphogenesis, a process that traditional static cultures cannot replicate.[185] Moreover, Ao et al. developed a microfluidic platform with an air−liquid interface, which improved oxygenation within cerebral organoids, enhancing their growth and functional maturation.[186] This innovation not only reduced the hypoxic core that often limits organoid maturation but also enabled studies on prenatal exposures, such as cannabis, offering a valuable model for examining the effects of environmental toxins on brain development. More recently, Cho et al. integrated a microfluidic system with human brain organoids to create a vascularized brain-on-chip model.[187] This system facilitated the development of a mature brain organoid with improved neurovascular interactions, while offering a more reliable platform for drug testing and neurotoxicity studies.

These advancements collectively highlight the transformative potential of integrating microfluidic technology with brain organoid culture. By enabling more physiologically relevant models with improved oxygenation, vascularization, and neurodevelopmental features, these systems offer a powerful platform for studying brain function, disease, and drug response. Beyond advancing our understanding of complex neurophysiological processes, their reproducibility also positions them as promising tools for personalized medicine screening and future CNS drug discovery efforts.

4. NANODRUG DEVELOPMENT THROUGH INTEGRATION OF ORGANOIDS AND MPS

Advances in organotypic models and microphysiological systems (MPS) is paving the way to a revolution in preclinical testing of drugs, where individualized precise interventions may be devised in advance of patient treatment. This section explores the applications of organotypic MPS systems in nanodrug development, highlighting key examples from recent research (Figure 5).

4.1. Brain-on-a-Chip for Blood−Brain Barrier Penetration. The blood−brain barrier (BBB) is a highly selective and tightly regulated interface that separates the central nervous system (CNS) from the peripheral circulation.[188] Comprised primarily of brain endothelial cells, pericytes, and astrocytes, it maintains brain homeostasis by controlling the passage of ions, molecules, and immune cells. While this barrier plays a vital protective role, it also presents a significant challenge for drug delivery, particularly in the field of nanomedicine, where the majority of nanoparticle-based formulations are unable to cross the BBB in sufficient quantities to exert therapeutic effects.[188] As a result, understanding and predicting how nanoparticles interact with and traverse the BBB is crucial for the development of effective CNS-targeted therapies.

Earlier studies, such as Cho et al., addressed this by developing three-dimensional BBB spheroid models composed of endothelial cells, pericytes, and astrocytes.[189] These static systems effectively mimicked key barrier features, including tight junction expression and efflux transporter activity. Using this platform, Cho and colleagues demonstrated that ultrasmall gold nanoparticles (2 nm) could cross the BBB spheroids, particularly under hypoxic conditions, highlighting the utility of such models for preliminary nanoparticle screening. However, while BBB spheroids offer valuable insights, they lack physiological flow and mechanical stimuli, key factors that influence barrier function and nanoparticle behavior *in vivo*. This limitation has driven the development of microfluidic BBB models, which incorporate perfusion, shear stress, and spatial organization to simulate the dynamic nature of the BBB more accurately. For instance, Campisi et al. created 3D microvascular networks via vasculogenesis, providing a foundational framework for modeling human brain vasculature *in vitro*.[190] Building on this, Bergmann et al. developed BBB organoids through the coculture of endothelial cells, pericytes, and astrocytes under low-adhesion

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conditions.[191] These organoids successfully recapitulated key BBB characteristics, such as tight junction formation and functional efflux transporters, and offered a valuable tool for studying nanoparticle permeability and neurotoxicity.

A significant leap forward came with the work of Hajal et al., who established a microfluidic human BBB model using iPSC-derived endothelial cells integrated with human pericytes and astrocytes.[192] This triculture system, embedded in a perfusable chip, allowed for real-time, quantitative monitoring of nanoparticle transport under flow conditions. The study evaluated the permeability of PEGylated polystyrene nanoparticles (10 and 70 nm) and Angiopep-2–functionalized nanoparticles, the latter designed for receptor-mediated transcytosis via LRP1. The platform effectively differentiated between passive diffusion and active transport, offering a powerful tool for screening nanomedicine formulations targeting the CNS. Expanding on this approach, Straehla et al. developed a vascularized glioblastoma-on-a-chip model to study the delivery of targeted nanotherapeutics across the BBB into tumor tissue.[16] Their system, which included self-assembled vascular networks formed by endothelial cells, astrocytes, and pericytes, closely mimicked the GBM tumor microenvironment. The authors used modular, layer-by-layer functionalized nanoparticles, engineered with glioblastoma-targeting ligands, to enhance tissue-specific delivery. Cisplatin-loaded nanoparticles exhibited improved accumulation and efficacy in both the *in vitro* platform and orthotopic xenograft models, validating the predictive power of their system for translational nanomedicine research. Another key advancement in BBB modeling came with Song Ih Ahn et al, who developed a microfluidic human BBB model that integrates iPSC-derived endothelial cells with pericytes and astrocytes. This model not only enhanced the vascularization and maturation of brain organoids but also allowed for the real-time study of nanoparticle transport.[2]

Collectively, these advances illustrate the evolution from static spheroid models to dynamic, perfusable, microfluidic platforms, each offering increasing levels of physiological complexity. Among these, microfluidic BBB models are particularly valuable for their ability to replicate shear stress, cellular architecture, and real-time transport dynamics. The insights gained from these systems can directly inform the design of CNS-targeted nanomedicines by guiding choices in particle size, ligand functionalization, and surface chemistry to improve penetration, retention, and therapeutic efficacy.

4.2. Microfluidic Platforms for Precision Therapeutics in Pancreatic Cancer. Pancreatic ductal adenocarcinoma (PDAC) is among the deadliest malignancies due to its aggressive progression, profound heterogeneity, and resistance to conventional chemotherapy.[193] A defining feature of PDAC is its dense desmoplastic stroma, composed of pancreatic stellate cells (PSCs), immune cells, and extracellular matrix components, which collectively promote tumor growth, invasion, and chemoresistance.[194] Traditional 2D culture systems fail to reproduce this complex tumor microenvironment (TME), limiting their translational value. As a result, the integration of PDOs with microfluidic technologies has emerged as a powerful strategy to more accurately model the PDAC landscape and assess therapeutic responses, including nanoparticle-based therapies.

Early efforts to improve *in vitro* PDAC models include the work by Lee et al., who developed a microfluidic coculture platform embedding PDAC spheroids within a 3D collagen matrix alongside PSCs.[195] This model recapitulated key TME characteristics such as epithelial–mesenchymal transition (EMT) and drug resistance. The presence of PSCs was shown to significantly enhance cancer cell motility and reduce responsiveness to chemotherapy, mirroring clinical resistance mechanisms. Building on this, Hwang et al. created a more complex multiplexed coculture system enabling quantitative assessment of stromal effects on drug response and cancer cell invasion.[196] This model demonstrated how PSCs modulate extracellular matrix remodelling and conferred resistance to standard chemotherapeutics like gemcitabine, reinforcing the need to target both cancer cells and their supporting stroma.

In a major advancement, Haque et al. introduced a patient-derived tumor-on-a-chip model incorporating PDOs, PSCs, and immune cells such as macrophages within a microfluidic platform.[197] This setup enabled real-time tracking of cellular behavior and drug response within a faithfully reconstructed TME. Their findings underscored that disrupting stromal and immune interactions could enhance chemotherapy efficacy, supporting the utility of such platforms in personalized treatment design. Recognizing the critical role of hypoxia in PDAC progression, Geyer et al. designed a microfluidic organoid system capable of mimicking oxygen gradients within the TME.[198] Their model revealed that hypoxic conditions drastically alter the response of PDOs to standard therapies, including gemcitabine, stressing the importance of physiological cues in preclinical drug testing.

A notable contribution to nanoparticle-focused research comes from Choi et al., who developed a microfluidic chip that supports the growth of PDOs from minimal biopsy tissue, making it especially suited for personalized nanotherapeutic screening.[6] This platform retained the genomic fidelity of PDOs while enabling real-time drug testing. Significantly, the authors evaluated nanoparticle-based chemotherapies, including glycogen synthase kinase inhibitors encapsulated in polymeric nanoparticles, and biologics codelivered with NK cells. The study demonstrated enhanced tumor-killing activity when nanoparticles were combined with immune cell-based therapies, revealing a promising strategy for overcoming PDAC's immune-evasive behavior.

In summary, these pioneering studies illustrate how the integration of PDOs with microfluidic chips offers a versatile and human-relevant system for dissecting PDAC biology and evaluating new therapies. The incorporation of nanoparticle delivery systems into these platforms adds a vital translational layer, enabling the real-time analysis of particle penetration, drug release, and therapeutic synergy. These insights can be used to design nanoparticles that maintain efficacy under hypoxic, stromal-rich conditions, and to select surface modifications that improve penetration through dense tumor matrices.

4.3. Breast Cancer Organoids: Targeting Tumour–Stroma Interactions. Breast cancer, known for its heterogeneity and resistance to standard therapies, remains a major challenge in oncology.[199] Traditional *in vitro* models fall short in capturing the complexity of the TME, particularly the dynamic interactions between cancer cells and surrounding stromal components. In response to these limitations, the integration of PDOs with microfluidic platforms has emerged as a transformative strategy, proving valuable in the testing of nanoparticle-based therapies targeting tumor–stroma interactions and offering a robust and scalable platform for the development of precision nanomedicine.

The evolution of this field began with pioneering work by Shirure et al., who designed a vascularized tumor-on-a-chip

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platform integrating patient-derived breast cancer organoids with self-assembled 3D microvessels.[200] This system successfully replicated essential tumor functions, including angiogenesis, invasion, and drug response, while allowing real-time observation of drug and nanoparticle diffusion across vascular networks. Importantly, it provided a perfusable microenvironment that maintained long-term organoid viability and was amenable to antiangiogenic and chemotherapeutic screening. Building on these principles, Wu et al. introduced a droplet-assisted microfluidic platform that enabled the rapid and scalable formation of vascularized breast tumor organoids.[201] By coculturing breast cancer cells with endothelial cells and fibroblasts in microgel droplets, their system recapitulated key tumor−stroma interactions and promoted the formation of vascular-like networks. These perfusable structures enhanced drug diffusion and more accurately simulated *in vivo* drug responses, including nanoparticle transport and cytotoxicity. Next, Fang et al. introduced a microarray-based organoid chip containing a 14 × 8 microwell array designed for high-throughput breast cancer PDO culture and drug testing.[4] Their platform preserved tumor heterogeneity and enabled precise assessment of drug responses to Adriamycin and Ivermectin, both as monotherapies and in combination. Notably, Ivermectin enhanced Adriamycin-induced cytotoxicity by downregulating P-glycoprotein (P-gp), a major efflux transporter associated with drug resistance. This study highlighted the potential of miniaturized organoid-chip systems for evaluating nanoparticle-based formulations and drug resistance mechanisms in a personalized context. Most recently, Sun et al. leveraged organoid-on-chip technology to evaluate an engineered oncolytic virus (AD4-GHPE) in breast cancer PDOs.[202] The virus was designed to reduce PD-L1 expression and secrete GM-CSF, thereby enhancing CD8+ T-cell responses and improving tumor-specific cytotoxicity. This study showed the ability of organoid-chip platforms not only to evaluate cytotoxic agents, but also to assess immunomodulatory nanoparticles in a controlled, tumor-relevant microenvironment, further expanding the scope of applications in precision immuno-nano therapy. The ability to profile drug resistance and stromal interactions enables nanomedicine designs that incorporate efflux pump inhibitors, dual-drug strategies, or targeted ligands to overcome resistance.

Together, these studies chart a clear progression in the field, from vascularized drug delivery models to high-throughput resistance profiling and immune-targeting nanoparticle testing. The integration of breast cancer organoids with microfluidic systems continues to pave the way for next-generation precision nanomedicine, offering robust, scalable platforms that bridge the gap between laboratory models and clinical relevance.

4.4. Microfluidic Liver Organoid Platforms for Predictive Drug Toxicity Testing. Liver diseases, including hepatocellular carcinoma and drug-induced liver injury (DILI), represent a major clinical challenge due to the liver's complex architecture, diverse cellular composition, and central role in xenobiotic metabolism.[203] Despite being a primary site for drug metabolism, the liver is also highly susceptible to adverse drug reactions. Current preclinical models—such as 2D hepatocyte cultures or animal testing—often fail to recapitulate human liver physiology, leading to poor predictive power for clinical outcomes. Microfluidic integration with liver organoids provides a highly dynamic and physiologically relevant platform for drug testing and toxicity assessment.

In one of the earliest examples, Au et al. developed the microfluidic organoids for drug screening platform, enabling the formation of hepatic organoids through the coculture of HepG2 (a human hepatocellular carcinoma cell line) and NIH-3T3 fibroblasts within a hydrogel matrix.[204] These organoids exhibited fibroblast-dependent contractile behavior and enhanced liver-specific functions, including albumin secretion and cytochrome P450 enzyme activity. The platform allowed miniaturized, high-throughput hepatic screening, demonstrating the feasibility of dynamic organoid culture for preclinical drug evaluation. Jin et al. engineered vascularized liver organoids by coculturing induced hepatic (iHep) cells, directly reprogrammed from human dermal fibroblasts, with human umbilical vein endothelial cells in a decellularized liver extracellular matrix hydrogel.[205] Cultivated within a perfused microfluidic device, these liver organoids developed complex vasculature and showed improved metabolic and biosynthetic activity, including urea and albumin production. The model proved particularly promising for evaluating drug-induced hepatotoxicity under physiologically relevant flow conditions.

More recently, Bircsak et al. presented a validated, three-dimensional liver microfluidic platform built using the OrganoPlate system, which supports coculture of primary human hepatocytes and hepatic stellate cells under flow.[17] This model was optimized for high-throughput drug screening, showing sustained hepatic functionality and predictive performance for DILI across multiple compounds. Notably, it enabled chronic toxicity testing over extended culture periods—an important feature for assessing long-term therapeutic safety.

Collectively, studies across brain, pancreatic, breast, and liver models illustrate the growing sophistication of organoid-on-a-chip platforms for drug testing. These systems better replicate human tissue physiology, improve the predictive power of preclinical assays, and provide a strong foundation for integrating nanoparticle evaluation. While liver organoid-on-a-chip models have not yet been widely applied in nanomedicine, their success in replicating organ-specific function underscores the transformative potential of MPS in future nanodrug development pipelines.

5. CONCLUSIONS

Collectively, the advancement of *in vitro* models has marked a critical shift in the way nanoparticles are assessed and understood, moving from conventional 2D cultures to more physiologically relevant 3D spheroids and organoids. However, it is the emergence of MPS, or organ-on-chip technologies, which offers the most transformative potential for nanomedicine. By combining organoid biology with microfluidic engineering, MPS integrate dynamic flow, shear stress, tissue−tissue interfaces, and functional biological barriers to replicate human physiology more accurately. Furthermore, the acceleration of biofabrication techniques and 3D printing will facilitate even more complex architectures of cells and materials to be juxtaposed to multiple organotypic systems with perfusable microfluidic control.[36] These systems enable real-time, noninvasive monitoring of nanoparticle behavior, including transport, accumulation, toxicity, and therapeutic action, under conditions that mimic *in vivo* environments. Notably, MPS platforms such as blood−brain barrier, liver, and tumor-on-chip models have shown immense promise for evaluating nanoparticle performance in ways that static models and animal studies cannot. Their ability to recapitulate organ-specific physiology, immune interactions, and systemic

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responses makes them highly valuable for predicting clinical outcomes.

Emerging technologies are poised to further reshape precision nanomedicine when combined with advanced *in vitro* platforms. CRISPR-based genome editing offers the possibility to generate patient-specific organoids carrying precise genetic alterations, enabling the study of nanoparticle responses in relevant disease genotypes. Artificial intelligence can accelerate the analysis of complex data sets generated by MPS, supporting the rapid optimization of nanoparticle designs and predictive modeling of therapeutic outcomes. Finally, digital twin systems, virtual replicas of patient physiology, could integrate experimental and clinical data to simulate nanoparticle behavior in silico, guiding personalized treatment strategies before clinical intervention.

Together, these converging innovations promise to close the gap between preclinical modeling and clinical translation, propelling nanomedicine toward a truly predictive and personalized future. However, several bottlenecks remain, including limited predictive accuracy, lack of standardized protocols, and persistent gaps between model performance and clinical outcomes. Moreover, tracking nanoparticles in 3D organoids or within MPS in situ can be hindered by tissue thickness, device opacity, light scattering, and limited imaging resolution. Furthermore, nonspecific adsorption of nanoparticles to device materials, as well as biofouling from serum proteins used in these systems, can alter nanoparticle concentration, protein corona formation, and distribution profiles, potentially introducing artifacts into dosing studies. Addressing these challenges not only is essential for maximizing the predictive value of MPS-based nanomedicine evaluations but will also require systematic benchmarking of MPS against clinical data sets, the development of harmonized operational and reporting standards, and greater integration of multiorgan platforms to capture systemic effects. Establishing comparisons across clinical data, animal models and MPS/NAMs, through robust *in vitro in vivo* correlation frameworks, is a critical need to ensure effective translation of these advanced systems to drug development infrastructure. Building on these advances, the integration of MPS into the nanomedicine development pipeline could redefine preclinical testing by providing a modular and scalable framework for iterative screening, mechanistic studies, and personalized therapy design.

As technologies mature and standardization improves, MPS stand poised to accelerate the translation of nanoparticle-based therapies from bench to bedside, enhancing precision, reducing failure rates, and ultimately paving the way toward more effective and individualized treatments in nanomedicine.

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Notes

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