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Enhancing Drug Delivery Precision: Development and Optimization of Nanoparticle-Based Formulations for Targeted Therapy in Preclinical Models

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Abstract

In recent years, the utilization of nanoparticles has proliferated across a wide spectrum of clinical domains. Nanoparticles have been engineered to surmount the constraints associated with free therapeutics and negotiate biological barriers—systemic, microenvironmental, and cellular—that exhibit heterogeneity across diverse patient cohorts and diseases. Mitigating this patient heterogeneity has also been facilitated through precision therapeutics, where tailored interventions have augmented therapeutic effectiveness. Nonetheless, current nanoparticle development predominantly emphasizes the refinement of delivery platforms with a uniform approach. As lipid-based, polymeric, and inorganic nanoparticles undergo increasingly nuanced engineering, there arises the potential for tailoring them to drug delivery in a more personalized manner, ushering in the era of precision medicine. In this Review, we deliberate on sophisticated nanoparticle designs employed in both generalized and precision applications, offering insights into their potential for enhancing precision therapies. We

concentrate on advancements in nanoparticle design that surmount heterogeneous barriers to delivery, positing that intelligent nanoparticle design can enhance efficacy in broad delivery applications while facilitating customized designs for precision applications, thereby ultimately enhancing overall patient outcomes.

Keywords: Nanoparticles, Clinical applications, Biological barriers, Systemic barriers, Microenvironmental barriers, Cellular barriers, Patient heterogeneity, Precision therapeutics, Personalized interventions, Optimization

Introduction

Engineered nanomaterials present considerable potential for enhancing the specificity of disease diagnosis and treatment. Nanotechnology offers avenues to address the constraints of conventional delivery methods, spanning from macroscopic issues such as biodistribution to microscale barriers like intracellular trafficking. Strategies such as cell-specific targeting and molecular transport to specific organelles hold promise in this regard. To expedite the realization and clinical application of these promising nano-enabled technologies, the US National Science and Technology Council (NSTC) launched the National Nanotechnology Initiative (NNI) in 2000, outlining well-defined initiatives and grand challenges for the field. These initiatives have underpinned recent endeavors aimed at investigating and enhancing nanotechnology, with nanoparticles (NPs) constituting a significant focus of research and advancement.

NPs have emerged as potential agents to enhance the stability and solubility of encapsulated payloads, facilitate membrane transport, and extend circulation times to bolster safety and efficacy. Consequently, NP research has proliferated, yielding promising outcomes in vitro and in small animal models. However, despite the extensive research driven by the NNI, the actual number of nanomedicines accessible to patients falls substantially below field projections, partly owing to a translational gap between animal and human studies. This gap stems from a limited understanding of the physiological and pathological disparities between animal model species and humans, particularly regarding how these distinctions influence the behavior and functionality of nanomedicines in vivo. Species differences aside, clinical translation faces additional hurdles stemming from patient heterogeneity, with scant research investigating the interactions between nanomedicines and stratified patient populations. Consequently, while a few nanomedicines secure approval, they are seldom recommended as primary treatment options, and improvements are observed in only select patient subsets. This limitation owes partly to the underexplored heterogeneity in

both the biological underpinnings of diseases and among patients, which modulates NP efficacy by influencing NP distribution and functionality in diseased tissues.

Many initial NP iterations struggled to surmount these biological delivery barriers, but recent NP designs have leveraged advancements in controlled synthesis techniques to integrate intricate architectures, bio-responsive moieties, and targeting agents to enhance delivery. Consequently, these NPs can serve as sophisticated systems, including in nanocarrier-mediated combination therapies, to modulate multiple pathways, optimize therapeutic efficacy against specific macromolecules, target distinct cell cycle phases, or overcome drug resistance mechanisms.

The burgeoning focus on developing NPs to overcome biological barriers specific to patient subgroups or disease states can be attributed, in part, to the increasing prominence of precision, or personalized, medicine and the launch of the Precision Medicine Initiative (PMI) in 2015. Precision medicine aims to harness patient data—such as genetic profiles, environmental exposures, or comorbidities—to formulate individualized treatment strategies. Precision approaches mitigate the impact of patient heterogeneity, enabling more precise patient stratification, enhanced drug specificity, and optimized dosing or combinatorial approaches. However, precision therapies encounter similar biological delivery barriers as conventional medicines, constraining their clinical efficacy. Hence, novel NP designs, informed by patient data and engineered to surmount specific barriers in stratified patient populations, hold considerable potential for enhancing the delivery and response to precision medicine therapies.

This Review is dedicated to exploring advancements in nanomedicine that hold the potential to facilitate the clinical translation of precision medicines and enhance patient-specific therapeutic outcomes. It underscores the significance of harnessing biomaterials and innovations in biomedical engineering to surmount biological barriers and address patient heterogeneity. The Review delineates progress towards the objectives outlined by the National Nanotechnology Initiative (NNI) and the Precision Medicine Initiative (PMI) aimed at refining disease treatment at the individual level. While nanoparticles (NPs) have demonstrated success in precision diagnostic applications, this Review centers on their role in delivering precision medicine therapeutics, anticipating their substantial impact on future precision NP endeavors. Moreover, the Review scrutinizes the biological barriers impeding the widespread efficacy of NP applications and critically evaluates rational NP designs engineered to overcome these hurdles. Additionally, it delves into the distribution and delivery trends gleaned from decades of NP research, probing the influence of NP attributes

on therapeutic responses. The emerging themes, coupled with advancements in engineering NPs for specific applications, assume paramount importance as novel prospects emerge for the clinical translation of NP-based precision therapies in realms such as cancer medicine, immunotherapy, and in vivo gene editing.

NP Classes: Lipid-Based NPs

Lipid-based nanoparticles (NPs) encompass a variety of structural subsets, with the most common being spherical platforms comprising at least one lipid bilayer encasing at least one internal aqueous compartment (Fig. 2). As versatile delivery systems, lipid-based NPs offer numerous advantages, including formulation simplicity, self-assembly capabilities, biocompatibility, high bioavailability, capacity to carry substantial payloads, and the ability to modulate their biological characteristics through control of physicochemical properties. Consequently, lipid-based NPs represent the most prevalent class of FDA-approved nanomedicines.

Among lipid-based NPs, liposomes constitute one of the most prominent subsets, characterized by phospholipid compositions capable of forming unilamellar and multilamellar vesicular structures. This structural versatility enables liposomes to encapsulate and transport hydrophilic, hydrophobic, and lipophilic drugs, with the ability to entrap both hydrophilic and lipophilic compounds within the same system, thereby broadening their applicability. The stability of liposomes, both in vitro and in vivo, is influenced by factors such as NP size, surface charge, lipid composition, lamellar structure, and surface modifications (e.g., ligands or polymers), which can be tailored during synthesis. Given their rapid uptake by the reticuloendothelial system, liposomes often undergo surface modifications to prolong circulation and enhance delivery, facilitating their clinical utility.

Another notable subset of lipid-based NPs, termed lipid nanoparticles (LNPs), is widely employed for nucleic acid delivery. LNPs distinguish themselves from traditional liposomes primarily through the formation of micellar structures within the particle core, a morphology modifiable based on formulation and synthesis parameters. Comprising four major components—cationic or ionizable lipids for complexing with negatively charged genetic material and facilitating endosomal escape, phospholipids for structural integrity, cholesterol for stability and membrane fusion, and PEGylated lipids for enhanced stability and circulation—LNPs have demonstrated efficacy in nucleic acid delivery, particularly in personalized genetic therapy applications. Ionizable LNPs, in particular, represent an optimal platform for nucleic acid therapy delivery due to their near-neutral charge at physiological pH, transitioning to a charged state in acidic endosomal compartments, thereby promoting endosomal escape for

intracellular delivery. Despite these advantages, LNP systems may encounter limitations such as low drug loading and biodistribution patterns resulting in elevated uptake in the liver and spleen.

Polymeric NPs: Characteristics and Applications

Polymeric nanoparticles (NPs) can be synthesized from both natural and synthetic materials, including monomers or preformed polymers, resulting in a diverse array of structures and characteristics (Fig. 2). They offer precise control over multiple NP features and serve as effective delivery vehicles due to their biocompatibility and straightforward formulation parameters. Various techniques such as emulsification (solvent displacement or diffusion), nanoprecipitation, ionic gelation, and microfluidics are employed in their synthesis, yielding distinct final products. Polymeric NPs exhibit versatile drug delivery capabilities, allowing therapeutics to be encapsulated within the NP core, entrapped in the polymer matrix, chemically conjugated to the polymer, or bound to the NP surface. This versatility enables the delivery of diverse payloads, including hydrophobic and hydrophilic compounds, as well as cargos of different molecular weights such as small molecules, biological macromolecules, proteins, and vaccines, making polymeric NPs well-suited for co-delivery applications. By adjusting properties such as composition, stability, responsiveness, and surface charge, the loading efficacies and release kinetics of these therapeutics can be precisely controlled.

The most common forms of polymeric NPs include nanocapsules (cavities surrounded by a polymeric membrane or shell) and nanospheres (solid matrix systems). Within these categories, NPs are further classified into shapes such as polymersomes, micelles, and dendrimers. Polymersomes, akin to liposomes, are artificial vesicles with membranes made using amphiphilic block copolymers, offering improved stability and cargo-retention efficiency. Polymeric micelles, comprising responsive block copolymers, self-assemble to form nanospheres with a hydrophilic core and a hydrophobic coating, thereby enhancing circulation times and protecting aqueous drug cargo. Dendrimers, hyperbranched polymers with precisely controlled mass, size, shape, and surface chemistry, exhibit complex three-dimensional architectures suitable for the delivery of nucleic acids and small molecules. Charged polymers like poly(ethylenimine) (PEI) and poly(amidoamine) (PAMAM) are commonly employed in dendrimer-based applications.

Polyelectrolytes, another type of charged polymer, have been integrated into numerous NP formulations to enhance properties such as bioavailability and mucosal transport, owing to their inherent responsiveness and variability in charge with pH. Polymeric NPs possess favorable attributes for drug delivery, including biodegradability, water solubility,

biocompatibility, biomimicry, and stability during storage. Their surfaces are easily modifiable for additional targeting, facilitating the delivery of drugs, proteins, and genetic material to targeted tissues, particularly in cancer medicine, gene therapy, and diagnostics. However, drawbacks of polymeric NPs include an increased risk of particle aggregation and toxicity. While only a limited number of polymeric nanomedicines are currently FDA-approved and utilized in clinical settings (refer to Table 1), polymeric nanocarriers are undergoing extensive evaluation in numerous clinical trials.

Inorganic Nanoparticles (NPs)

Inorganic materials such as gold, iron, and silica serve as foundational components for synthesizing nanostructured materials employed in various drug delivery and imaging applications (Fig. 2). These inorganic NPs are meticulously formulated and can be tailored to exhibit a diverse array of sizes, structures, and geometries. Gold NPs (AuNPs), extensively studied among inorganic NPs, manifest in various forms including nanospheres, nanorods, nanostars, nanoshells, and nanocages. Notably, inorganic NPs possess distinct physical, electrical, magnetic, and optical properties intrinsic to the base material itself. For instance, AuNPs feature free electrons at their surface, facilitating continuous oscillation at frequencies contingent upon their size and shape, thereby endowing them with photothermal properties. Additionally, AuNPs readily undergo functionalization, expanding their properties and delivery capabilities. Iron oxide stands out as another extensively researched material for inorganic NP synthesis, with iron oxide NPs comprising a predominant portion of FDA-approved inorganic nanomedicines. Magnetic iron oxide NPs, composed of magnetite (Fe_3O_4) or maghemite (Fe_2O_3), exhibit superparamagnetic properties at specific sizes and have demonstrated efficacy as contrast agents, drug delivery vehicles, and thermal-based therapeutics. Other prevalent inorganic NPs include calcium phosphate and mesoporous silica NPs, which have been effectively employed for gene and drug delivery. Quantum dots, typically fabricated from semiconducting materials like silicon, represent unique NPs primarily utilized in vitro imaging applications but hold promise for in vivo diagnostics. Owing to their magnetic, radioactive, or plasmonic attributes, inorganic NPs find niche applications in diagnostics, imaging, and photothermal therapies. Most importantly, they demonstrate favorable biocompatibility and stability, filling critical roles in applications necessitating properties unattainable by organic materials. Nonetheless, their clinical utility is constrained by challenges related to low solubility and potential toxicity, particularly in formulations incorporating heavy metals.

Nanoparticles in Precision Medicine

Precision medicine advocates for the development of patient-specific treatments within clinical settings, aiming to circumvent the limitations inherent in traditional one-size-fits-all approaches and enhance therapeutic outcomes. In oncology, patient stratification via biomarkers and companion diagnostics has become standard practice for drug development, given the inconsistent efficacy observed with unstratified studies involving most cancer nanomedicines. Despite the pivotal role of patient stratification in the clinical development of several precision medicines for cancer, NP-based clinical trials currently encompass unstratified patient populations. However, this paradigm is anticipated to shift in the near future, as the significance of stratification becomes increasingly evident and NPs are designed with specific patient populations in mind. The integration of stratified patient populations into clinical trials may expedite the progression of NPs through the clinical pipeline, as these populations are expected to exhibit more consistent responses to treatment. Moreover, NPs are well-positioned to broaden the scope of potential patient populations eligible for precision medicine therapies by mitigating factors such as comorbidities or heterogeneous biological barriers that may have previously rendered patients ineligible. As NPs surmount prevailing limitations to delivery, thereby enhancing the potency and therapeutic efficacy of precision medicines, they hold the potential to enable more patients to qualify for clinical trials and benefit from individualized therapies.

Since the inception of the Precision Medicine Initiative (PMI) in 2015, numerous applications have integrated nanomaterials into precision medicine frameworks. For instance, a blood test for early pancreatic cancer detection relies on analyzing the personalized biomolecular corona adsorbed onto graphene oxide nanoflakes. The distinctive property of graphene oxide, which binds minimal albumin quantities, facilitates robust protein adsorption from low-level plasma constituents. Other studies employ magnetic NPs or AuNPs, known for their simplicity, in biomarker detection assays, thereby streamlining processes and reducing costs compared to existing methods requiring extensive sample processing. In addition to diagnostic screening, several therapeutic NP applications aim to remodel the tumor microenvironment to enhance particle accumulation and penetration, thereby augmenting drug efficacy or sensitizing tumors to specific therapies. For instance, NP-delivered microRNA can manipulate tumor-associated endothelial cells, thereby altering the tumor vasculature and enhancing tumor responsiveness to traditional cancer therapies. Moreover, bio-inspired lipoproteins have demonstrated efficacy in remodeling tumors, enhancing NP accessibility to cancer cells. Furthermore, photothermal NPs can enhance chimeric antigen receptor (CAR) T cell infiltration and activity against solid

tumors. NPs also hold promise in modulating immune activation or suppression to sensitize cancer cells to therapeutics, with the aim of standardizing heterogeneous environments and expanding the pool of patients responsive to or eligible for precision treatments.

In summary, the convergence of nanoparticles (NPs) with precision medicine represents a pivotal avenue for advancing both disciplines. At present, the evaluation of NPs occurs within unstratified patient populations. However, the integration of NPs tailored for specific patient cohorts could substantially expedite the clinical translation of various nanomaterials. Conversely, the efficacy of precision medicine heavily relies on precisely stratified patient populations. Leveraging NPs to enhance delivery across heterogeneous biological barriers holds immense potential to augment the effectiveness of precision medicines. This approach not only broadens the inclusion of patients within stratified populations but also heightens the prospects for successful clinical translation. The progress in genome sequencing and biomarker detection affords the opportunity to meticulously select cargo for treating patient-specific diseases. While this review predominantly delves into therapeutic applications, NP technologies also harbor considerable promise for enhancing diagnostic capabilities.

Circulation, stability, and clearance

During circulation, several factors, including excretion dynamics, blood flow patterns, protein coronas, and interactions with phagocytic cells, can compromise NP stability and hinder effective delivery. The influence of these environmental factors hinges on the nuanced physicochemical properties of the NP platform, prompting the development of overarching design principles to manipulate these characteristics for favorable outcomes. Notably, NP size emerges as a critical determinant, with NPs smaller than 10 nm being swiftly eliminated by renal clearance, while larger counterparts exceeding 200 nm risk activating the complement system unless appropriately engineered. Surface modifications, such as PEGylation, serve to enhance circulation time by altering NP size and solubility while also shielding the NP surface from enzymatic degradation and antibody recognition. However, the emergence of anti-PEG antibodies poses a significant challenge to the sustained circulation of PEGylated NPs, potentially compromising their efficacy. Alternatively, platelet membrane cloaking presents a promising stealth strategy, mitigating cellular uptake and complement activation, albeit with lingering concerns regarding recognition by alternative cell populations. Interaction with the mononuclear phagocyte system (MPS) may precipitate toxicity, with NP characteristics such as size, shape, and surface properties dictating the nature and magnitude of immune responses. Although certain surface modifications, such as PEGylation,

typically mitigate interactions with MPS cells, the advent of anti-PEG antibodies threatens to undermine this stealth property, thereby fostering MPS interactions.

Barriers to biodistribution

Extravasation constitutes the initial crucial step for NPs in circulation to access target tissues, with this process intricately influenced by NP characteristics such as size. Notably, NPs exhibit size-dependent distribution patterns across organs, with organs such as the liver and spleen often exhibiting the highest accumulation. Pathological microenvironments, such as those found in tumor vasculature, may perturb these size-dependent distribution dynamics. The transferrin pathway presents an enticing avenue for trans-epithelial movement in the intestine, holding particular relevance for conditions like colon cancer and irritable bowel disease. However, active targeting strategies within the gastrointestinal tract confront formidable challenges, including the formation of protein coronas in gastrointestinal fluids and mucus production by goblet cells, which impede interactions with intestinal walls. Moreover, these barriers are further compounded by pathological conditions that augment epithelial permeability and alter the composition of the gastrointestinal microbiome, thereby erecting substantial hurdles to achieving desired biodistribution via oral delivery.

Variability in Microenvironments

Microenvironments often exhibit conditions markedly distinct from those within the circulation, leading to significant alterations in the physical attributes and stability of NPs. For instance, the gastrointestinal tract encompasses regions characterized by extreme pH variations and acidity. These conditions, coupled with the presence of degrading enzymes, render the gastrointestinal milieu inherently unstable for many NPs. Furthermore, disease states can diversely modify gastrointestinal microenvironments, resulting in heterogeneous responses to biomaterials. For example, a comparative analysis of microenvironments in colon cancer and colitis revealed disease-specific compatibility with dendrimer/dextran biomaterials, influenced by variations in amine surface group densities on colon tissue.

Uptake and Internalization of NPs

The NP corona, alongside altered NP characteristics such as hydrophilicity and charge, significantly influences cellular uptake across various cell types including macrophages and cancer cells. This corona-coated NP interacts with the cell surface, composed of a negatively charged, selectively permeable phospholipid bilayer with biomolecules distributed throughout in a fluid mosaic structure. Cell membranes exhibit substantial

variability, with heterogeneous distribution of membrane components such as lipid rafts and transmembrane proteins. Over 400 cell surface transporter types have been identified in human cells, further accentuating cellular heterogeneity.

Cellular Heterogeneity

In addition to general cellular barriers, heterogeneous populations of cells exist both within individual patients and across patient cohorts. Cellular variations are contingent upon individual characteristics. For instance, studies have demonstrated that younger human fibroblast cells from fetal lungs and epithelial cells from fetal colons exhibit increased NP uptake compared to older cells, with younger cells displaying lower susceptibility to toxicity. Furthermore, cell sex has been shown to impact the uptake of AuNPs in human amniotic stem cells and saliva-isolated fibroblasts, underscoring the importance of considering multiple factors in NP delivery.

Precision Medicine

Given the extensive heterogeneity of biological barriers and disease states within and across patient populations, there is a pressing need for the development of highly modular and customizable therapeutic delivery methods. This section delves into the impact of various NP properties on delivery, emphasizing how individual NP design elements, such as architecture, material properties, targeting, and responsiveness, can surmount barriers specific to individual diseases and patients.

Chemotherapeutic agents often exhibit off-target toxicity and induce adaptive resistance, posing limitations to efficacy. Moreover, numerous biological barriers associated with cancer, particularly at the tumor site, necessitate improved delivery techniques. Customizing therapeutics and their delivery systems for individual cancer patients holds immense promise in optimizing treatment outcomes. Adapting to the Tumor Microenvironment The tumor microenvironment profoundly influences patient prognosis, affecting the efficacy of chemotherapy. Although the enhanced permeability and retention (EPR) effect and FDA-approved early NP systems have provided optimism for NP-based delivery, substantial work remains to be done using intelligent NP designs to enhance cargo delivery or remodel microenvironments, thereby augmenting the efficacy of existing therapies.

Active Targeting to Cancer Cells

Current chemotherapeutic agents exert their effects through various mechanisms and target sites. Some disrupt DNA within the nucleus (e.g., doxorubicin, platinum drugs), while others act within the cytosol or affect organelles such as mitochondria. Effective NP trafficking to these sites is

imperative to ensure proper drug action at therapeutic levels, underscoring the necessity for precise NP delivery mechanisms.

In conclusion, this Review has elucidated various NP designs tailored for therapeutic delivery, engineered to surmount the heterogeneous biological barriers encountered across diverse patient populations and diseases. These delivery challenges are compounded by patient comorbidities, varied disease stages, and unique physiological conditions. Addressing this spectrum of needs necessitates the development of NPs customized for distinct patient populations or pathologies, or their intersections. NP platforms offer a plethora of modifiable attributes such as size, shape, charge, surface properties, and responsiveness, which can be selectively harnessed to optimize delivery for specific applications, therapeutics, and patient cohorts. This customization holds potential synergies with precision medicine therapies to refine patient stratification methods during NP platform screening, expand access to precision therapeutics by enabling new patients to qualify for existing therapies with enhanced delivery mechanisms, and ultimately enhance the overall therapeutic efficacy of both precision medicines and NP delivery platforms.

Among these NP attributes, size and shape have undergone extensive scrutiny across various biological contexts, with discernible trends identified in some instances for informed NP design. For instance, NP charge assumes particular significance in muco-penetrating and intracellular applications requiring endosomal escape, while targeting surface markers is paramount in applications necessitating specific NP uptake by certain cell types, as observed in many cancer and immunotherapy settings. However, as design considerations grow more intricate, attempts to extrapolate trends across broad populations become increasingly challenging, potentially compromising the precision of findings within smaller cohorts in pursuit of overarching delivery principles. Therefore, comprehensive analyses of NP design and ensuing interactions within the human body must be undertaken to enhance the specificity of such assertions, particularly as efforts intensify towards stratifying patient populations to identify the most suitable NP platforms for these subgroups.

Through ongoing exploration of NP technologies in laboratory settings, researchers possess the opportunity to amass data and scrutinize outcomes, enriching the expanding repository of known design-function relationship trends in nanomedicine. Nevertheless, it is imperative that trends observed in research settings be contextualized before broad generalizations are made, as subtle disparities in NP composition, animal models, and pathology may significantly influence NP performance and necessitate consideration during the transition of NP technology to clinical applications.

Adopting a precision-focused approach to NP screening, thereby limiting the pool of eligible patients for medication, inevitably reduces the potential market size for each NP-based therapeutic. This reduction may raise concerns regarding the high developmental costs associated with advanced NP designs and the heightened financial risks entailed in potential failures during clinical translation. However, NP platforms proven effective in specific patient populations may find utility in delivering numerous therapeutics, both precision-based and generic. Consequently, the development of highly efficacious NP platforms tailored to stratified groups could yield multiple successful clinical applications. Furthermore, precision NP designs hold promise for enhancing therapeutic efficacy compared to NPs developed for broader populations, with potential improvements in survival rates, quality of life, and dosing regimen optimization warranting the premium associated with precision delivery systems.

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