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Review

Pure drug nanomedicines - where we are?

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ABSTRACT

Pure drug nanomedicines (PDNs) encompass active pharmaceutical ingredients (APIs), including macromolecules, biological compounds, and functional components. They overcome research barriers and conversion thresholds associated with nanocarriers, offering advantages such as high drug loading capacity, synergistic treatment effects, and environmentally friendly production methods. This review provides a comprehensive overview of the latest advancements in PDNs, focusing on their essential components, design theories, and manufacturing techniques. The physicochemical properties and *in vivo* behaviors of PDNs are thoroughly analyzed to gain an in-depth understanding of their systematic characteristics. The review introduces currently approved PDN products and further explores the opportunities and challenges in expanding their depth and breadth of application. Drug nanocrystals, drug-drug cocrystals (DDCs), antibody-drug conjugates (ADCs), and nanobodies represent the successful commercialization and widespread utilization of PDNs across various disease domains. Self-assembled pure drug nanoparticles (SAPDNPs), a next-generation product, still require extensive translational research. Challenges persist in transitioning from laboratory-scale production to mass manufacturing and overcoming the conversion threshold from laboratory findings to clinical applications.

1. Introduction

Pure drug nanomedicines (PDNs) emerged as a promising field of theranostics, integrating diverse disciplines such as physics, mathematics, materials science, pharmacy, chemistry, biology, and engineering [1–4]. Drug delivery represents the most prevalent application of nanomedicines, accounting for 78% of global sales and 58% of patent applications [5, 6]. Among the various types of nanomedicines approved by the U.S. Food and Drug Administration (FDA), lipid-based nanoparticles, particularly liposomes, are the most common, followed by micelles (primarily polymer-based) and nanocrystals [7–14]. However, liposomes and polymer-based nanomicelles face potential limitations, including relatively low drug loading capacity, high production costs, and diffcuclty of mass production [7, 12, 15]. In contrast, PDNs offer the general advantages of nanomedicines, such as specific loading capabilities, high therapeutic efficacy, and targeted delivery. Additionally, PDNs circumvent issues are associated with immunotoxicity, low loading capacity caused by carriers, and high costs, and facilitate industrial production [16, 17].

Published scientific articles related to PDNs have explored various therapeutic applications, including cancer, infection, autoimmune diseases, inflammation, and others [18–20]. Conventional chemotherapy is restricted by toxicity and drug resistance, but PDNs regulate the balance between efficacy and toxicity through targeted therapy, biodistribution, and lesion accumulation [21]. This approach represents a promising strategy for cancer treatment. PDNs passively target and accumulate at tumor sites based on the enhanced permeability and retention (EPR) effect. Surface stabilizers can promote active tumor accumulation or uptake as targeted or internalized functionalized ligands, respectively [22]. Notably, PDNs modified with target ligands such as antibodies, nucleic acid ligands, and ligand peptides are expected to increase drug accumulation and overcome drug resistance [23–25]. The administration of PDNs is no longer limited to intravenous routes, that can implement systemic or local treatment *via* the gut, eyes, skin, and other routes. For instance, Wang et al. prepared oral 10-hydroxycamptothecin (HCT) nanocrystals with a size of approximately 190 nm using precipitation [26]. *In vitro* release experiments revealed that the drug release at 72 h was 2.5 times higher than that of bulk HCT. Pharmacokinetic data in rats demonstrated that the oral bioavailability of HCT nanocrystals was 8.6 times higher than that of active pharmaceutical ingredients (APIs) [26]. These results indicate that nanocrystal technology can significantly improve solubility and oral bioavailability while preserving the lactone structure of camptothecin. In ocular drug delivery, nanocrystal technology enhances drug solubility, creating a concentration gradient that enables the drug to pass through the physiological barriers of the eye and rapidly release [19]. Paredes et al. prepared dapsone nanocrystals using the media milling technique [27]. Compared to raw dapsone, dapsone nanocrystals exhibited a 3.32-fold improvement in water solubility at pH 4.5. Nanocrystals with a large specific surface area may increase the retention time in the mucosa and exhibit prolonged-re-

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lease behavior in the eye [28]. For example, econazole nitrate nanocrystal suspension was prepared by spray drying [29]. The maximum concentration of econazole nitrate nanocrystal in tears was 2.3 times higher than that of the bulk drug, as the chitosan in the suspension increased the wettability of the nanocrystals. Furthermore, the viscosity and positive charge of chitosan increased the retention time of the nanocrystals in the eye.

In the industrial production of PDNs, the management and control of quality parameters, including size, distribution, shape, and formulation information, are crucial. A combination of techniques or innovative approaches is commenly recommended for laboratory production [30]. For instance, Koseki et al. employed an ultrasound-assisted reprecipitation method to prepare rod-like SN-38 nanocrystals [31]. Furthermore, the preparation methods for self-assembled pure nanomedicines, such as the template-assisted method, dialysis method, film dispersion, and *in vivo* self-assembly method, are more straightforward [32-35]. For industrial production, conventional and mature manufacturing techniques are preferred over new or combined technologies. The carrier-free nature of PDNs allows for the use of traditional and straightforward processes. This review introduces the approved PDN products, including self-dispersible nanocrystals (SDNCs), drug-drug cocrystals (DDCs), and active-targeted PDNs, such as antibody-drug conjugates (ADCs) and nanobodies. They all have appropriate technologies for large-scale industrial production. The opportunities and challenges for promoting PDN application are then discussed.

2. Definition and Characteristics of PDNs

PDNs integrating nanotechonlogy and biomedicine, are free-carrier nanostructures composed entirely of drug molecules [36]. The nanoscale effect of PDNs also enhances the solubility of insoluble drugs. In contrast to carrier-based nanoparticles, PDNs exhibit a high drug-loading capacity (up to 100%) and efficiency while avoiding the immunotoxicity associated with nanomaterial carriers. The design of molecular structural units fundamentally regulates the structural characteristics and physicochemical properties of PDNs [37]. Furthermore, the drug loading sequence can modulate the release rate and sequence of PDNs [38, 39], influencing the drug therapeutic index. Molecular self-assembly and nanoprecipitation are common techniques employed to transform dispersed drug molecules into nanosized aggregates [40]. Various factors drive the formation of PDNs, including intermolecular forces (such as hydrogen bonding, electrostatic interactions, and π-π stacking), environmental conditions (*e.g.*, pH, temperature, and ionic strength), and process parameters (grinding, stirring, and mixing) [41-43].

Although polymer micelles or liposomes protect drugs from the external environment, their loading capactiy is typically less than 10%, often increasing pharmaceutical costs [44-48]. In contrast to other delivery systems, such as liposomes, micelles, and exosomes, PDNs typically comprise APIs without carriers. Drug adsorbed on a carrier has the potential for desorption in non-target sites, precluding the achievement of the ideal therapeutic effect. Under the protection of lattice energy, drug nanocrystals exhibit high drug loading and low desorption rates [49]. *In vitro* release experiments demonstrated that the drug loading of mitoxantrone nanocrystals (approximately 100%) was approximately 12 times that of mitoxantrone liposomes [50, 51]. Simultaneously, PDNs can achieve the clinical therapeutic effect with low doses and high efficacy. PDNs can reduce the administration frequency and improve patient compliance while also avoiding the risk of multiple drug resistance and toxic side effects associated with multiple high-dose medications.

2.1. High drug solubility

Solubility represents a critical parameter for drug delivery systems (DDSs) [52]. Low solubility poses a significant challenge to a drug's bioavailability. Following oral administration, drugs with limited saturation solubility in body fluids often exhibit reduced oral bioavailability [53, 54]. Similarly, in intravenous delivery, poor solubility frequently necessitates low drug doses and large solvent volumes, thereby increasing the risks of toxicity and adverse effects. Consequently, improving dissolution and bioavailability is a crucial consideration when designing preparations for insoluble drugs [55]. Prodrugs, for instance, demonstrate promise in elevating solubility by bonding hydrophilic groups to hydrophobic drugs, thereby enhancing bioavailability [56, 57]. However, supplementary studies on the physical and chemical properties, as well as the *in vivo* behaviors of prodrugs, are inevitable [58]. This strategy is not dominant, considering the shortened time and cost of research and development (R&D), and the extended duration of patent protection. Nanosizing, the reduction of particle size to the nanometer level, significantly increases the surface-to-volume ratio and greatly improves the bioavailability of insoluble drugs. It is an effective, economical, and universal strategy for insoluble drugs. According to the Noyes-Whitney equation, nanosizing accelerates the dissolution rate and increases saturation solubility [59, 60]. The Calvin equation demonstrates that dissolution pressure increases as particle size decreases. Nanoparticles obtain significant solution pressure, shifting the equilibrium toward dissolution [61]. For instance, a baicalein nanocrystal prepared using the high-pressure homogenization method (HPH) with poloxamer 188 as a stabilizer displayed a 2.01-fold increase in dissolution profiles compared to raw baicalein [62]. This suggests that the saturated solubility and dissolution rate of insoluble drugs in body fluids are accelerated. The vast specific surface area increases the residence time and adsorption of drugs in the intestinal mucosa, resulting in improved intestinal absorption [63]. Furthermore, increased saturation solubility creates a more significant concentration gradient for penetration into the skin membrane, enhancing passive diffusion [64]. Additionally, Shen et al. prepared quercetin (QC) hybrid nanocrystals with diameters around 280 nm and 550 nm [65]. Following intravenous administration, the fluorescence intensity of 280 nm hybrid nanocrystals in blood was stronger than that of 550 nm nanocrystals within 30 hours. These results indicate that nanocrystals with smaller particle sizes may dissolve faster. In intravenous injection, the risk of capillary blockage at the micron level is avoided. The unique functional groups in drugs diversify their surface properties, facilitating recognition or ingestion by tissues in the body with continuous or rapid release.

2.2. Hypotoxicity

Safety and toxicity are the primary concerns and the most crucial data for new drug registration. Insoluble drug formulations frequently contain numerous excipients, such as cosolvents and solubilizers, which may precipitate in non-aqueous solutions like blood [66], potentially leading to safety issues. In contrast, PDNs exhibit high solubility without additional excipients, mitigating these concerns.

The advancement of nanomedicine led to the utilization of various nanomaterials, such as polymer nanoparticles and liposomes, in DDSs [67, 68]. However, in toxicity studies, researchers often focus on the toxicity of the entire preparation, neglecting the effects of individual nanoparticles. It is important to note that the toxicity profiles of nanoparticles and nanomaterials are distinct [69]. Consequently, the toxicity of nanoparticles, particularly slow- or non-degradable particles, warrants significant attention [70, 71]. For instance, researchers reported that acid-functionalized single-walled carbon nanotubes, when phagocytosed by macrophages, impaired mitochondrial function and inhibited phagocytic activity [72]. Furthermore, the toxicity of nanoparticles

is dependent on the potential exposure routes in humans. The respiratory system serves as the primary entry point for airborne particles. Yang et al. investigated the association between chronic obstructive pulmonary disease and polystyrene nanoplastics [73]. Their findings revealed that nanoplastics in the lungs induced oxidative stress and inflammatory responses, and polystyrene nanoplastics traversed the alveolus-blood barrier, entering the bloodstream.

The toxicity of drugs is profoundly influenced by the administered dose and frequency [74]. With nanotoxicology advancing, researchers evaluated how nanomaterial properities impact their *in vivo* toxicology, such as shape, charge, pH, and size [75, 76]. For instance, direct genotoxicity was detected in 20% of evaluated genotoxicity tests for metal-containing nanoparticles and up to 70% for nanofibers [77]. In comparison, carbon-based nanoparticles generated higher levels of reactive oxygen species (ROS) than micron particles [78]. This may be attributed to the larger specific surface area of nanoparticles, which provides a greater reaction surface. Moreover, when ovarian granulosa cells were incubated with 10 nm diameter gold nanoparticles (AuNP) for 24 h, the gold nanoparticles significantly infiltrated or damaged the mitochondria [79]. To ensure nanoparticles do not produce unintended effects, *in vivo* toxicology should be investigated during the design and modification process [80].

2.3. Stability

The long-term stability of drugs is a key parameter for quality assurance and plays a crucial role in the manufacturing process. Ensuring the safety and efficacy of drugs during storage and transportation is crucial. However, the nanosize effect presents challenges for the physical stability of nanoproducts. According to the Ostwald ripening effect, in a highly dispersed system, the saturated solubility of small particles is higher than that of large particles [81, 82]. In a study, Zhang et al. explored the addition of small nanoparticles to regulate the growth of larger nanoparticles, achieving product size and shape uniformity [83]. PDNs with extensive interfacial areas are thermodynamically unstable systems susceptible to aggregation and precipitation to reduce Gibbs free energy [84]. When PDNs have poor physical stability, the nanosize effect may no longer exist, resulting in sedimentation, aggregation, crystal growth, recrystallization, and even toxic side effects after medication. The Zeta potential (ζ) can induce crystal aggregation or growth, affecting the absorption process *in vivo* and leading to lower drug therapeutic effects. Physical stability also affects the system's fluidity and compressibility, adversely impacting the R&D of PDN preparations.

PDNs are primarily stored and transported in aqueous suspension. The type, volume, and temperature of the solvent and excipient significantly influence the physical stability of the nanoparticles. For instance, in a study by Nowak et al., silver nanoparticles were coated with different stabilizers, including naproxen, diclofenac, and 5-chlorosalicylic acid [85]. After 30 days, the silver nanoparticles coated with naproxen exhibited a more pronounced increase in size compared to the other stabilizers. To mitigate the Ostwald ripening effect, the solvent should not dissolve the drug but should have good solubility for the excipient [64].

Chemical and optical stability represent critical factors, particularly for drugs sensitive to light, heat, and humidity. Some drug nanoparticles are dispersed in aqueous media, where hydrolysis and oxidation can produce unpredictable effects. The stabilizer molecules covering the nanoparticle surface protect the internal compounds from oxygen and light [86]. For instance, omeprazole, a poorly soluble compound that degrades rapidly in water-based media, was formulated as a nanosuspension by Möschwitzer et al. [87]. Its content remained relatively stable for one month, suggesting that the surface stabilizer and crystal structure may have shielded omeprazole from decomposition.

3. Commercialization and regulation of PDNs

3.1. Approved PDNs for the clinic

Nanomedicines frequently offer significant social and economic advantages. Over the past two decades, the FDA and European Medicines Agency (EMA) have approved approximately 80 drugs and medical device products related to nanomedicine for commercialization [88]. As the market expands and nanomedicine rapidly develops, the number of approved nanomedicine products is expected to further increase. By 2025, the protein-based nanomedicine market in cancer, inflammation, and the central nervous system (CNS) is estimated to reach $28 ± 14 billion [89]. The nucleic acid-based market will reach $14 ± 7 billion, and the small molecule-based market will reach $6 ± 3 billion [89]. Among the numerous nanomedicine products, PDNs are easily convertible. Drug nanocrystal products accounted for about 30% of all nanodrug products submitted to the FDA [90]. Despite the outbreak of the coronavirus disease 2019 (COVID-19) crisis and subsequent economic recession in 2020, the drug nanocrystals market is projected to reach $83.1 billion [91]. Moreover, biotechnology consistently generates profits, and the global nanomedicine market is remarkably vast. These factors have propelled the pharmaceutical industry towards the R&D of biological nanomedicine products. For instance, Ablynx developed the first nanobody drug, Caplacizumab, which is used to treat acquired thrombotic thrombocytopenic purpura [92]. The first marketing approval has the potential to significantly alter the biomedical and economic landscape of nanobodies, establishing a foundation for nanobodies to become mainstream biological PDN products. Currently, 14 ADCs have been marketed [93], and more than 100 ADC candidates are currently in clinical studies [93]. By 2026, global sales of marketed ADCs are anticipated to surpass $16.4 billion [94], with Trastuzumab deruxtecan leading the way. Its global sales are estimated to reach $6.2 billion in 2026, positioning it as the best-selling ADC [94]. It is envisioned that PDNs will yield substantial market benefits and continue to attract the interest of academic personnel and investors.

3.2. Technical barriers

Securing patent protection from the United States Patent and Trademark Office (PTO) is economically critical for basic research and commercial product development [2]. During the "patent cliff" period, pharmaceutical companies face urgent pressure to develop and launch novel products. PDNs have garnered increasing attention from the pharmaceutical industry. Compared to conventional drugs, the complex nature and specialized knowledge involved in PDNs provide a competitive edge in the market, restricting generic alternatives and mitigating the revenue decline associated with the "patent cliff" [89].

Approximately 30% of all nanomedicine patent applications are related to drug nanocrystals [90]. These nanocrystals can be administered through various routes, such as oral, intravenous, and subcutaneous, and applied in different therapeutic areas, including anti-infection (26%), anticancer (24%), anti-anorexia (11%), and anti-inflammatory (11%) [90]. ADCs present significant challenges and stringent requirements in terms of technology and production for intellectual property protection. However, they also offer greater opportunities for extending patent life and reducing the likelihood of patent breakthroughs. ADCs integrate multiple components, such as the target, antibody, linker, and toxin. Antibody patents and regulations do not fully apply to

ADCs, making the identification of novel antibodies, targets, junctions, and toxins challenging. For instance, researchers filed a patent related to the linker in Trastuzumab deruxtecan [95], describing multiple peptide linkers that include the glycine-glycine-phenylalanine-glycine sequence [95]. Any new component or combination of components (antibody-toxin, antibody-linker, and linker-toxin) can receive specific patent protection and extend the market exclusivity period of the corresponding ADCs. In 2012, AstraZeneca acquired the VA-PABC linker developed by Spirogen [95] and promptly filed a patent describing an ADC that enhances the ability of tesirine to bind to anti-CD19 antibodies.

3.3. Regulatory of PDNs

The rapid advancement of nanomedicines, the urgent demand for related products, and the need for industry progress have presented numerous challenges to regulatory agencies. The FDA is required to clarify the PDNs within its jurisdiction, propose scientifically sound regulatory policies, evaluate appropriate products rationally, and provide technical guidance [96]. In 2012, the draft industry guidance "Considering Whether an FDA-Regulated Product Involves an Application of Nanotechnology" indicated that the FDA had not yet clearly defined nanotechnology. The 2014 industry guidance stated: "whether a material or end product is designed to possess an external dimension or an internal or surface structure in at least one nanoscale range (approximately 1 to 100 nm), or exhibits properties or phenomena, including physical, chemical, or biological effects, that can be attributed to its size that is even up to 1 μm (1000 nm)" [90, 97, 98]. This signified that the FDA regulated nanotechnology products according to specific legal standards and powers. Furthermore, in 2012, the FDA issued two additional draft industry guidelines addressing nanotechnology issues in cosmetics and food [99]. The European Cosmetic Regulation covered insoluble, persistent, or synthetic nanomaterials in the 1–100 nm size range in cosmetics, including nanocrystals, liposomes, and nanoemulsions [100–102].

The physicochemical and biological properties of novel and complex materials are vital for ensuring the reproducibility of the production process and the anticipated biological effect [103]. Their supervision is essential and indispensable. Currently, a clear definition of nanomaterials is lacking. Regulatory agencies have limited experience with emerging nanomaterials, and reliable data sets for developing regulatory strategies are absent. Furthermore, standard nanomaterials for reference and specific tools for adequately characterizing fundamental product properties are unavailable. Experiments conducted without adhering to regulations and guidelines have raised serious concerns about nanomaterials. Regulators, the pharmaceutical industry, governments, and academia are collaborating to develop specific, scientific, and comprehensive research reports, risk assessments, and guidelines for nanomaterials [104].

Current regulatory guidelines are more favorable for the development of PDN products. PDNs offer an optimal alternative approach, reducing the barriers to commercial translation while largely circumventing the need for nanocarrier application and oversight. The US FDA has granted approval to numerous PDN products, encompassing DDCs, ADCs, and nanobodies.

The supervision of complex nanomedicine products involves their key characteristics, and products are sampler, so the evaluation becomes easier [105]. The regulatory process for marketed PDN products is well-established, with numerous available guidelines, International Organization for Standardization (ISO) standards, and approved methods and references. Particle size and distribution, for instance, are critical factors for PDN products. These parameters can be characterized dynamically or statically through various imaging or light scattering techniques, such as dynamic light scattering, laser diffraction, and image analysis [106–108]. The polycrystalline form is another crucial aspect of drug nanocrystals, as it influences dissolution, stability, and bioavailability. This property can be determined using X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), or spectroscopic methods [106, 108–110]. Product safety is inextricably linked to the guidance provided by regulators and industry guidelines, which contribute to the improvement of PDN products by enhancing benefits and mitigating risks [19].

Presently and in the future, regulatory bodies and the pharmaceutical industry are collaborating to establish a comprehensive regulatory framework through the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). In 2010, the EMA conducted a scientific workshop on nanomedicines [111]. Participants discussed the advantages and challenges of nanotechnology in medicine and specific issues, including the characteristics, biological distribution, and interactions of nanomedicines with biological systems [111], facilitating the evaluation of future nanomedicines. Concurrently, major pharmaceutical companies have increased investment in preclinical and clinical research on PDNs, providing reliable data and reference materials to inform the development of regulatory policies.

Comprehensive pharmacoeconomic studies are essential prior to the commercialization of PDNs. The development of a Health Technology Assessment (HTA) will support the introduction of PDNs and enhance their clinical application. Furthermore, it provides regulatory agencies and public health stakeholders with crucial information regarding the safety, efficacy, and cost-effectiveness of PDNs [97, 112]. To successfully integrate PDNs into the public health system, interdisciplinary training for researchers, healthcare professionals, and public health experts is necessary [113].

4. PDN Types

4.1. SDNCs

SDNCs, primarily consisting of drugs and stabilizers, have recently demonstrated significant potential for disease treatment applications. Since their invention in the 1970s, nanocrystals have represented over 20% of nanomaterial-based new drug applications received by the FDA [114]. The formation of SDNCs begins with the regular arrangement of drug molecules into a crystal with a specific structure, followed by the adsorption of stabilizers (amphiphilic compounds) onto the crystal surface to prevent aggregation. These carrier-free SDNCs can achieve nearly 100% drug loading, resulting in enhanced therapeutic effects at lower drug doses. The FDA approved Ryanodex® for the treatment of malignant hyperthermia [118]. A single bottle of Ryanodex® can be dissolved in 5 mL of sterile water within 20 sec and achieve standard therapeutic effects [119, 120]. In contrast, a bottle of traditional Dantrium preparation requires 60 mL of sterile water and takes 8 min to dissolve, with approximately 8–9 bottles needed to achieve the same therapeutic effect [119, 120].

SDNCs can be prepared using by a variety of methods, including bottom-up, top-down, and combination approaches [121–127]. Currently, the majority of commercially available nanocrystal suspensions are produced through a top-down approach. Wet media milling and HPH are the most common technologies in the pharmaceutical industry. Additionally, combination technologies such as Nanoedge, Nanopure XP, CAV-Precipitation, and SmartCrystal have been developed to meet specific production requirements [128]. Manufacturers must determine appropriate fabrication techniques and adjust process parameters to produce uniform and high-quality nanocrystals on an industrial scale. Fig. 1 and Supporting Information Table S1 illustrate the methods used to fabricate drug nanocrystals.

4.1.1. SDNC fabrication

The preparation and stability of highly stable crystals are

closely related to the physicochemical properties of drugs [129, 130]. Drugs with high hydrophobicity and enthalpy values (or cohesive energy) have a greater propensity to form stable nanocrystals [131]. Specifically, drugs with high cohesive energy ($\delta E > 30$ kj·g^{-1}) are more likely to produce stable nanocrystals, while those with low cohesive energy ($\delta E < 25$ kj·g^{-1}) tend to agglomerate and grow within nanosuspensions. Furthermore, the addition of solvent or additive molecules can increase viscosity and suppress diffusion, thereby altering supersaturation levels and inducing crystallization. Previous research from the Weizmann Institute has demonstrated that adding chiral serine to a solution caused glycine crystals to grow in a pyramid shape rather than the typical bipyramid [132, 133].

The stabilizer plays a pivotal role in maintaining the stability of SDNCs, with the appropriate type and concentration being key factors. Stabilizers can be categorized into four groups: ionic surfactants, non-ionic surfactants, polymers, and other stabilizers [134–141]. The suitable stabilizer and its concentration are essential to counterbalance the detrimental effect of viscosity. Bernard et al. classified hypromellose (HPMC), methylcellulose (MC), hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), and carboxymethylcellulose sodium (CMC-Na) as high-viscosity stabilizers, while PVP30, PVP90, and TPGS were identified as low-viscosity stabilizers [145, 146]. Moreover, the zeta potential (ζ) is a determinant of the physical stability of the nanocrystal suspension system [86]. Findings demonstrated that increasing the molecular weight and concentration of Poloxamer reduced its ζ potential (Poloxamer ζ (F127) > ζ (F68)). Notably, a significant decrease in ζ potential was observed with increasing Poloxamer F127 concentration [147]. Furthermore, the behavior of nanoparticles in living organisms can be modified by the coated polymers, which offer stabilization, release control, and other functionalities [143, 144]. Sharma et al. discovered that combining Poloxamer F68 with a small quantity of chitosan derivatives enhances the stability of paclitaxel (PTX) nanocrystals [142]. The accumulation of PTX nanocrystals in Caco-2 cells was higher compared to PTX alone, as chitosan inhibited P-glycoprotein (P-gp) and reversibly opened tight cell junctions [102].

4.1.2. *In vivo* fate of SDNCs

Comprehending the fate of SDNCs) within living organisms is crucial for their R&D, significantly accelerating the advancement process. The primary method for obtaining pharmacokinetic and biodistribution data of SDNCs involves monitoring the *in vivo* concentration of free drugs. However, this approach overlooks the *in vivo* behavior of intact SDNCs. The release behavior of SDNCs in physiological *in vivo* environments differs from *in vitro* conditions. The lack of agitation and fluid *in vivo* likely leads to slow and persistent release, allowing intact nanocrystals to interact with biological tissues [19]. Researchers have proposed that the *in vivo* behavior of certain SDNCs may resemble that of nanocarrier particles [148, 149].

The behavior of SDNCs in living organisms may vary depending on the route of administration [150]. Studies have shown that SDNCs dissolve rapidly in gastrointestinal fluid after oral administration, creating a high concentration gradient. The area under the curve (AUC) of nimodipine nanocrystals at 12 h post-oral administration was 2.5 times higher than that of their solid dispersions [151]. Recent findings suggested that intact nanocrystals may adhere to the gastrointestinal mucosa and subsequently be absorbed through epithelial transcellular pathways, leading to an improved oral AUC [152]. In contrast, SDNCs do not dissolve immediately following intravenous injection. Smaller nanocrystals are subject to rapid dissolution due to fluid shear force, while larger nanocrystals may be engulfed by macrophages, resulting in swift distribution to the liver and spleen [153, 154]. Furthermore, nanocrystals with specific shapes have demonstrated enhanced efficiency or improved biosafety in drug delivery applications [155, 156]. Zhou et al. developed rod-shaped and spherical pegylated hydroxycamptothecin nanocrystals (HCPT-NRs) with an average diameter of 200 nm [157]. In cellular uptake studies using 4T1 and MCF-7 cells, rod-shaped hydroxycamptocampine nanocrystals (HCPT-NRs) exhibited higher uptake efficiency compared to spherical HCPT-NRs, indicating superior anticancer potential. Similarly, Weiss et al. prepared both non-functional and functionalized cellulose nanocrystals (CNCs) [158]. *In vivo* biocompatibility experiments revealed that charged CNCs are non-immunogenic, while uncharged CNCs elicited undesirable inflammation at high concentrations, leading to tissue damage and disease responses. Moreover, the dissolution rate, penetration, and uptake efficiency of SDNCs are significantly influenced by their size. Small-sized curcumin nanocrystals (approximately 240 nm) demon-

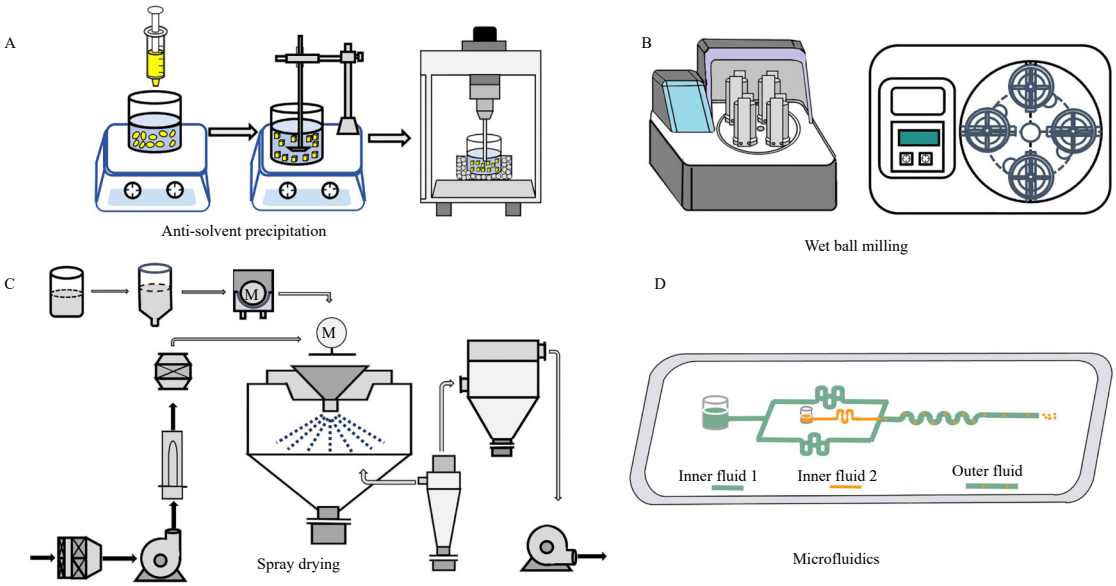

Fig. 1 Methods used for fabricating SDNCs. (A) The anti-solvent precipitation method mixes the drug solution with the antisolvent, blocks crystal growth, and reduces the size of drug particles. Ultrasonic sound is used to induce crystallization. (B) Wet ball milling prepared drug nanocrystals by the interaction between milling beads and drug particles. (C) The spray drying method converts a fluid drug into small droplets through an atomizer, then transforms them into drug particles. (D) Microfluidic technology manipulates different microchannels and fluid flow speeds to create uniformly size-controlled drug particles.

strated a faster dissolution rate and higher diffusion percentage in simulated pulmonary mucus compared to large-sized curcumin nanocrystals (approximately 500 nm) [115]. Additionally, the permeability of fenabemide nanocrystals from donor to acceptor cavities decreased with increasing particle size [116]. The transport pathways of nanocrystals were different based on their size, as observed in the larval zebrafish model [117]. Nanocrystals measuring 70 nm are internalized into lysosomes and the endoplasmic reticulum, while 200 nm nanocrystals accumulate more in lysosomes. The proteins adsorbed onto nanocrystals with different stabilizers can change delivery routes, and interactions between nanocrystals and cell layers. For instance, Qin et al. successfully employed polyvinylpyrrolidone (PVP) K17, D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS), and poloxamer F68 as stabilizers to develop celecoxib nanocrystals (CXB-NCs) [159]. The peak concentration of CXB-NCs/TPGS and CXB-NCs/F68 was 5 and 3.5 times higher than that of CXB-NCs/PVP K17, respectively. The pharmacokinetic curve of CXB-NCs/PVP K17 was significantly flatter, indicating slower drug release. Tight junctions (TJs) between cells pose a challenge in delivering drugs to the systemic circulation and specific organs. Certain stabilizers can interact with TJ proteins and regulatory molecules, substantially improving the delivery efficiency of SDNCs [160, 161].

4.1.3. Application of SDNCs

Nanocrystals represent a feasible and practical approach for delivering APIs through various administration routes. Over 20 nanocrystal-based products have been approved and commercialized, with numerous new products in various stages of clinical research [9]. Oral administration is the most appropriate and preferred route, as well as the first choice for product commercialization [162]. When exposed to gastric and intestinal fluids, nanocrystals rapidly dissolve and absorb, improving the total AUC and reducing the impact of eating or fasting on drug absorption [163]. *In vitro* release tests have demonstrated that ibuprofen nanocrystals released 90% of the total drug within 1 h, while pure drugs and commercially available products released only 58% and 63%, respectively [106]. Extracenteral administration, including intravenous, intramuscular, and transdermal routes, is another practical approach that offers higher patient compliance, avoids first-pass effects, and allows for rapid treatment termination. Transdermal drug delivery is a typical example [64, 148, 164, 165], where drug nanocrystals produce a high drug load in skin furrows and hair follicles, promoting drug penetration through the skin barrier and maintaining continuous drug release [166, 167]. For instance, studies have shown that nanosuspensions of Apreminast, used for psoriasis treatment, exhibited 2.6- and 3.2-fold higher penetration rates in the stratum corneum and viable layers compared to micropowder suspensions [168]. *Ex-vitro* skin penetration studies demonstrated that the dermal deposition of fumaric acid (FA) from FA nanocrystals was 2-fold compared to raw FA. *In vivo* results exhibited that the *in vivo* distribution of nanocrystals was improved, enhancing therapeutic effects compared to the commercially available Fucidin cream [169]. Considering the high sensitivity of eye tissue, organic solvents, extreme pH, and complex materials should be avoided. SDNCs can reduce irritation to the eye, improve solubility, and prolong retention time [170]. For example, the ophthalmic anti-inflammatory drug flumetholone has been formulated into eye drops, and after administration of nanocrystalline eye drops, the average concentration of flumetholone in the aqueous humor was 2−3 times higher than that of microcrystal eye drops within 60 min [171].

SDNCs are also employed as a carrier for the delivery of biopharmaceuticals, such as proteins and nucleic acids, enabling combinatorial therapy. SDNCs consistently exhibit specific morphologies, including rod-like and disk-like shapes [135, 136, 172]. Notably, rod-like nanoparticles demonstrate the ability to target the pulmonary circulation following intravenous administration[149]. The research group utilized rod-shaped PTX nanocrystals as carriers to develop a pulmonary artery-targeted co-delivery system of PTX and caspase-3 (Cas-3) for the alleviation of monocrotaline-induced pulmonary hypertension [136]. The system was fabricated by loading the protein onto PTX-nanocrystals, followed by a coating of glucuronic acid (GlcA) for targeting the glucose transporter-1 (GLUT-1) on pulmonary artery smooth muscle cells (PASMCs). The findings revealed that nanoparticles with a diameter of 170 nm exhibited prolonged circulation in the blood, accumulation in the lungs, targeted pulmonary arteries (PAs), induced regression of PA remodeling, and improved hemodynamics, resulting in decreased pulmonary arterial pressure and Fulton's index (Fig. 2).

4.2. DDCs

DDCs are composed of two or more distinct drug molecules within a single crystalline lattice, maintained in specific stoichiometric ratios through non-covalent interactions [173]. This approach offers a cost-effective strategy by reducing production expenses and facilitating the development of novel drug combinations. Notably, DDCs enable the systematic enhancement of drug properties without altering their core chemical structures, which has garnered considerable attention in the field of pharmaceutical eutectics.

In recent decades, the FDA has approved and successfully commercialized various DDC products. Furthermore, preclinical and clinical research on DDCs has received increased incentives, leading to a substantial rise in capital investment from researchers and companies (Supporting Information Table S3) [175-186]. Additionally, the rising number of DDC patents granted by the European and US patent offices reflects a growing interest in advancing technologies for more complex and efficient DDC formulations [174].

4.2.1. Distinctiveness of DDCs

DDCs are a distinctive solid-state form derived from the modification of the physicochemical properties of drug molecules. This is achieved by altering the molecular arrangement and intermolecular interactions within a shared crystal lattice. This novel approach offers the potential to enhance the properties of one or both drugs without requiring changes to their chemical structures.

4.2.1.1 Changing the melting point, hygroscopicity, solubility, and mechanical strength of drug molecules

DDCs can significantly impact key properties such as melting point, hygroscopicity, solubility, and mechanical strength. Studies demonstrated that the melting point of a DDC generally was between those of the individual components [187–190]. In cases where one drug has a particularly high melting point, the DDC typically exhibits a higher melting point overall. Additionally, shifts in the molecular packing within the lattice can alter the mechanical properties of the DDC, affecting parameters such as tensile strength, breaking force, elasticity, and compressibility [191, 192]. The solubility of a DDC is closely related to the solubility of the coformer, with the coformer's characteristics playing a crucial role in the dissolution behavior of the entire system [193]. For instance, the DDC formed by dihydromyricetin (DMY) and PTX reduced the solubility difference between the slightly water-soluble DMY and the highly water-soluble PTX [194]. This resulted in a significant reduction in the equilibrium solubility of PTX and a slight increase in that of DMY. The findings indicated that DMY and PTX were released synchronously and continuously from the cocrystal, enabling the simultaneous release of two drugs with significantly different solubilities and synergistic therapeutic effects [194]. Temozolomide, a well-dissolved anticancer drug with rapid clearance from the body, was cocrystallized with hesperid-

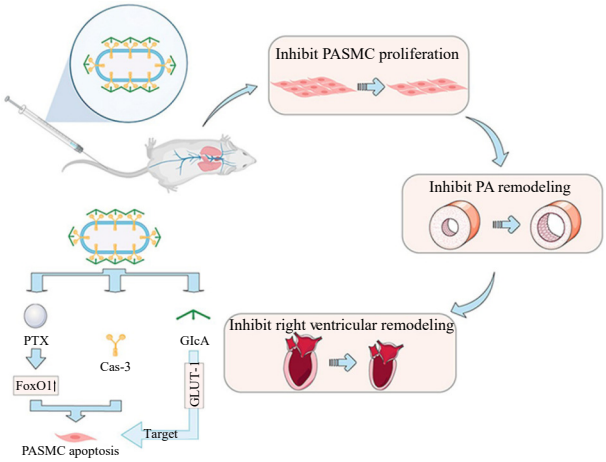

Fig. 2 Self-assembly PTX nanocrystals loading with Cas-3 targeting PASMCs restore the FoxO1 expression and promote cell apoptosis, alleviating PA remodeling and improving cardiopulmonary functions (Ref. 136, Copyright by Elsevier B.V 2024).

in, a less soluble natural anti-tumor component. The temozolomide-hesperidin cocrystal reduced the solubility of temozolomide from 7600 (pH 1.2) and 6424 (pH 6.8) to 483.9 (pH 1.2) and 193.5 μg·mL^{-1} (pH 6.8) [195]. Hesperidin effectively slows the release and absorption of temozolomide, extending its retention time in the body. The lyotropic behavior of DDCs offers economic benefits to both producers and consumers by reducing the required dose and production and marketing costs, and improving patient compliance.

4.2.1.2 Improving drug stability: Physical, chemical, and optical stability

DDCs are a multi-component system in which the active groups of drug molecules in the lattice interact non-covalently. This structural arrangement protects the drugs from environmental factors such as water, oxygen, and light. For instance, levofloxacin (LVFX) is a spectral antimicrobial agent commonly used in antibacterial treatment. Under light exposure, LVFX's hydrogen bond receptor (-C=O) is susceptible to degradation. The experiment found that the LVFX-metacetamol cocrystal (LVFX-AMAP) formed hydrogen bonding (-N-H$^{\cdots}$O), which enhances its photostability [196]. Additionally, isoniazid is prone to oxidative degradation. Gallic acid (GA) is commonly used as a natural antioxidant. The DPPH method demonstrated that the isoniazid-GA cocrystal exhibited scavenging activity against DPPH radicals [197].

4.2.1.3 Enhancing bioavailability and displaying synergistic effect

DDCs are employed for synergistic therapy and can potentially reduce production and application costs [198]. For instance, with the widespread and persistent use of antibiotics, antimicrobial resistance has become a significant global concern. Shemchuk et al. utilized a ciprofloxacin (synthetic antibiotic)-thymol (natural antibiotic) cocrystal for infection treatment [199]. While *Escherichia coli* is sensitive to ciprofloxacin alone, thymol alone or in a physical mixture with ciprofloxacin did not inhibit the growth of *Escherichia coli*. However, the ciprofloxacin-thymol cocrystal exhibited considerable antibacterial activity, significantly surpassing that of ciprofloxacin alone [199]. Interestingly, DDCs serve as a potential and promising bridge, ideally linking drugs with nutraceuticals to develop novel, safe, and effective cocrystal products [200]. For nutraceuticals, soluble drugs in DDCs can promote the absorption of these insoluble nutraceuticals [200]. Similarly, nutritional health products may provide adequate patient nutrition and assist DDCs in achieving a more significant curative effect while potentially reducing side effects.

4.2.2. Fabrication of DDCs

Selecting appropriate conformers is crucial for constructing a stable cocrystal structure and a rational drug combination. In virtual screening, researchers typically commence by analyzing the molecular structure of drug compounds. Subsequently, in conjunction with computer-aided virtual screening, researchers identify common hydrogen bond motifs through the examination of X-ray crystal structure data in the Cambridge Structural Database [201]. The relevant drug supramolecular synthons are then screened *via* high-throughput co-crystallization or supramolecular crystal engineering. Furthermore, the intermolecular interactions and stacking arrangements between drug molecules are ascertained [202]. Based on these intermolecular interactions, drug molecules can be linked and recombined in a reversible and dynamic manner.

The supramolecular interaction module enables the programmatic combination of multiple drugs into a single delivery system, circumventing the need for time-consuming and complex synthesis processes. This supramolecular synthonic approach identifies common and reproducible intermolecular interactions, such as van der Waals forces, halide bonds, and π-π stacking. Hydrogen bond interactions, in particular, exhibit favorable strength and spatial flexibility [203-205]. The most frequently observed hydrogen bond receptors include carbonyl oxygen and aromatic nitrogen, while hydrogen bond donors can be ranked by their activity: -COOH > -NH- > R-OH [174]. The -COOH group has emerged as the most prevalent hydrogen bond and is commonly observed in DDCs. Drug molecules typically contain functional groups (*e.g.*, acid, acid pyridine, acid amide, amide, and pyridine) that function as proton acceptors or donors. These groups form supramolecular synthons, such as carboxylic acid dimers, acid-pyridine, phenol-pyridine, and phenol-carboxylic acid, which facilitate the formation of DDCs.

Researchers employed various computational methods to screen potential supramolecular synthons and predict the formation of DDCs. These include crystal structure prediction (CSP) [206], molecular electrostatic potential surface energy (MEPSE) [207, 208], electrostatic mode evaluation [173, 209], solution-based δpKa [204], and Hansen solubility parameter (HSP) calculation [210, 211]. CSP serves as a valuable in silico tool for predicting all possible crystal forms of APIs, and it is the sole virtual screening method that directly considers the impact of crystallinity on DDC formation. The co-crystallization reaction energy (ΔE_{cc}), calculated through the sublimation thermodynamic cycle, represents the difference between the cocrystal lattice energy (E_{latt}) and its pure components [201, 212, 213]. ΔE_{cc} indicates the contribution of the thermodynamic co-crystallization enthalpy and enables the measurement of the co-crystallization tendency [201, 212, 214]. For instance, Sun et al. utilized CSP to predict the indometacin-paracetamol cocrystal and investigated the effect of crystallinity on its formation [201].

The MEPSE value represents the strength of hydrogen bond donors or acceptors in various functional groups to a significant degree [215]. This value is employed to predict the likelihood of forming DDCs. A higher negative MEPS value indicates a stronger hydrogen bond receptor, while a higher positive value signifies a more potent hydrogen bond donor [216]. Musumeci et al. combined 19 compounds with bicalutamide in a 1∶1 molar ratio to create cocrystals [209]. The pairing energy (δE) at interaction sites was calculated and ranked based on MEPS values. The compounds that form stable cocrystals are positioned near the top of the list [209]. The researchers discovered that the possibility of cocrystal formation was predictied through the change in pairing energy (ΔδE) from the MEPS in pure solid phases.

Salt and cocrystals are multi-component crystalline materials. The distinction between them lies in the transfer of a proton between an acid and a base. In salts, the proton transfer is complete, whereas in DDCs, no proton transfer occurs [217]. The pharmaceutical industry generally accepts the p*Ka* rule, which states that salt formation is expected when the pKa difference between

acid and base exceeds 2 or 3 (Δp*Ka* = p*Ka*[protonated base] − p*Ka*[acid] > 2 or 3) [217, 218]. At low Δp*Ka* values (Δp*Ka* < 0), acids and bases almost exclusively form cocrystals. However, when ΔpKa falls between 0 and 3, this parameter is insufficient for accurately predicting solid salts [219, 220]. Jie et al. found that pKa calculation offers a practical approach to designing stable DDCs. They synthesized four -NH-rich isomers —H_2BT (1*H*,1'*H*-5,5'-bitetrazole), DATr (4,5-diamino-4*H*-1,2,4-triazole), 1MAT (1-methyl-5-aminotetrazole), and 2MAT (2-methyl-5-aminotetrazole)—into two salts and one cocrystal [221]. TDATr's p*Ka* value lies between the p*Ka* values of H_2BT, enabling them to form a 1∶1 molar ratio salt. The p*Ka* values of 1MAT and 2MAT are lower than those of H_2BT, indicating reaction trends and cocrystal formation. 2MAT's p*Ka* value is notably lower than 1MAT's, suggesting that 2MAT is more suitable for cocrystal formation with H_2BT. Experimental results from PXRD, DSC, and Hirshfeld surface analysis confirmed the construction of the 2MAT-H_2BT cocrystal [221].

At the molecular level, cocrystal systems exhibit miscibility. The probability of cocrystal formation can be predicted based on the solid-state miscibility of drug molecules [210]. HSPs provide a valuable tool for estimating the miscibility of drugs with other drugs, excipients, and carriers. HSPs can divide the resultant force of various interactions into partial solubility parameters, which represent the likelihood of interactions between molecules [222]. A trend exists between drugs and supramolecular synthons. When the total solubility parameter difference (Δδt) is less than 0.7 $MPa^{0.5}$, it indicates that the materials are miscible and may form DDCs, while a Δδt greater than 0.7 $MPa^{0.5}$ suggests immiscibility [223].

4.2.3. Opportunities and challenges for DDCs

Over the past decade, a significant number of DDCs have transitioned from laboratory development to commercial availability as a non-toxic and controlled-release DDS. Notably, DDCs have found applications in the treatment of various diseases, such as viral infections, inflammatory conditions, and cancer. Interestingly, one-third of DDCs are classified as either non-steroidal anti-inflammatory drugs or anti-tuberculosis agents.

The innovation and development of DDCs are significantly encouraged by rational and up-to-date drug regulatory frameworks during the R&D phase. In April 2011, the FDA first issued guidelines for DDCs, defining them as "a crystalline substance consisting of two or more molecules in the same lattice" [202] and classifying DDCs as a drug intermediate, thereby affecting their development [202]. However, in 2018, the FDA revised the classification of DDCs from drug intermediates to APIs [224]. The guideline emphasized that the drug structure in DDCs is not altered but exists as a new solid form of the drugs. Improvements in patent applications and regulatory systems reduced the financial burden on pharmaceutical enterprises in R&D, offering developers new intellectual property opportunities.

The industrial-scale production methods of DDCs significantly impact their successful commercialization. Crystallization is the most critical process, determining physical properties such as crystal shape, size, distribution, structure, and crystallinity. These factors also affect subsequent production stages, including filtration, drying, and milling [173]. Controlling crystal nuclei and polycrystalline transformation are crucial steps in achieving the desired final product. Industrial-scale crystallization is performed using two methods: batch operation and continuous crystallization. Traditionally, the pharmaceutical industry relie on batch crystallization processes, which continue to be practiced [225]. Well-established batch crystallization methods include solvent evaporation [226], grinding [227], cooling crystallization [228], and antisolvent crystallization [229]. However, these methods have drawbacks, such as batch-to-batch variabilities in particle size distribution and morphology, complex scale-up production, energetic inefficiency, and the need for manual intervention [230, 231]. In contrast, continuous crystallization enables higher yield and uniform purity [232]. The continuous process unit offers flexibility in controlling internal temperature, supersaturation, nucleation, crystal growth, and other parameters [233]. For example, hot melt extrusion, a continuous process, facilitates cocrystal formation without solvents. Karimi et al. employed hot melt extrusion to produce an ibuprofen-nicotinamide cocrystal [234]. Additionally, favipiravir and theophylline cocrystals have been prepared using the spray-dried method for treating respiratory viral infections [235]. Furthermore, Nandi et al. developed a microchannel reactor-based continuous liquid antisolvent crystallization setup with downstream processing, providing a reproducible homogeneous crystallization environment [230]. Over the past decade, continuous crystallization has become the preferred standard in the pharmaceutical industry [236, 237]. Crystal engineering aids in selecting suitable supramolecular synthetic materials. Enhanced regulatory measures, reduced R&D resource consumption, and advancements in production technology encourage pharmaceutical companies to invest more in DDC development.

The development of DDCs faces several notable challenges. Firstly, the design and synthesis of DDCs pose a significant hurdle. High-throughput cocrystal screening is commonly conducted through hydrogen bond supramolecular interactions, limiting the assembly of DDCs from alternative supramolecular interactions such as electrostatic interactions and π-π packing [200]. Furthermore, the selected DDC combinations may not represent a pharmaceutically acceptable pairing with the desired therapeutic effects. Secondly, from the perspective of production conversion and practical treatment, selecting API combinations from existing market or development products is economically and logically sound. However, the limited selection range of APIs increases the complexity of DDC design [238]. Generally, the synergistic efficacy of multiple drugs is significantly influenced by the precise proportion and dosage of the constituent drugs [239]. In DDCs, there is typically a fixed stoichiometric ratio between the drugs, usually 2∶1, 1∶1, or 1∶2. Nevertheless, this stoichiometric ratio does not always align with the optimal dose ratio for clinical efficacy.

5. Self-assembled pure drug nanoparticles (SAPDNPs)

SAPDNPs represent a carrier-free DDS that harnesses interaction forces, such as electrostatic interactions, hydrogen bonding, and hydrophobic interactions, between drugs or drug-drug conjugates [172, 240, 241]. Through the process of self-assembly, single or multiple drugs aggregate into nanostructures with a narrow size distribution. These nanostructures exhibit a high drug encapsulation rate (> 92%), excellent stability, co-delivery of different free drugs, and controlled release behavior, making them a promising candidate for the next generation of PDNs [242]. The production of SAPDNPs does not necessitate carriers or complex technologies and tools, and the assembly process is characterized by simplicity, environmental friendliness, low cost, and high repeatability, facilitating large-scale production [243]. Self-assembly is a self-improving process in which components automatically organize into patterns or structures without human intervention. During this process, drug molecules spontaneously form well-defined and stable aggregations driven by non-covalent interactions. By adjusting and combining assembled elements and driving forces, a diverse array of supramolecular self-assembly structures and functions can be derived.

SAPDNPs represent an emerging field, particularly in cancer treatment and diagnosis. Many clinical anticancer drugs have been criticized for their narrow therapeutic window and high toxicity. SAPDNPs demonstrated unparalleled advantages over traditional DDSs in terms of drug loading capacity, target site ac-

cumulation, production, and preparation. Notably, the application of SAPDNPs has extended to other therapeutic areas, including anti-inflammatory, antibacterial, Parkinson's disease (PD), and immunotherapy. For instance, the NLRP3 inflammasome serves as a key drug target for PD treatment [244]. QC and polyethylene glycol (PEG) were self-assembled into carrier-free nanomedicines (NanoQC) to inhibit NLRP3 inflammation-mediated neurodegeneration [245]. Similarly, osteoarthritis (OA), the most common joint disease, exhibits increased incidence and prevalence with age [246, 247]. Curcumin and icariin, two natural small-molecule drugs, were self-assembled into Cur/ICA NPs through π-π stacking [248]. Compared with the OA group, the expression levels of IL-1β and IL-6 in the Cur/ICA NPs group were down-regulated by 49 and 126 times, respectively. SAPDNPs also seem to be showing their strength in anti-infective therapy. It is well known that antibiotic abuse has further increased the resistance of bacteria, and the public urgently needs new antimicrobial treatment options. The natural antibacterial agents GA and berberine (BBR) were self-assembled into spherical nanoparticles (GA-BBR NPs) [249]. GA-BBR NPs exhibited a more potent *in vitro* antibacterial effect compared to the free BBR and GA-BBR mixture. However, the non-covalent or covalent combination of drugs and functional molecules purposefully modulates the overall properties of the drug formulation. Variations in drug formulations and preparation methods influence the supramolecular interactions that govern self-assembly and treatment efficacy.

5.1. Design and fabrication of SAPDNPs

The self-assembly ability of drugs is a critical factor in the development of SAPDNPs. For instance, HCPT self-assembled nanoparticles with an irregular and uneven structure aggregated and precipitated in aqueous conditions. It meant that HCPT was not suitable to produce stable HCPT self-assembled nanoparticles [250]. Establishing a computational and design method that can accurately and quantitatively predict the formation of stable SAPDNPs from APIs is essential. Various techniques, such as quantitative structure-nanoparticle assembly prediction (QSNAP), molecular docking, simulation, and supramolecular engineering, are employed to predict the self-assembly capability of APIs. Shamay et al. utilized the QSNAP model to design SAPDNPs [251]. The nanoparticle assembly and size were highly predicted by electrotopological molecular descriptors (SpMAX4Bh(s) and GetAway R4e), respectively. Nineteen compounds with SpMAX4_Bh(s) > 6.99 and 25 compounds with SpMAX4_Bh(s) < 6.99 were selected for nanoparticle formation experiments [251]. Remarkably, all but one drug (avasimibe) behaved as predicted by the SpMAX4_Bh(s) value. The discrepancy between the size of these nanoparticles and the value predicted by GETAWAY R4e is within 15 nm [251]. This discovery demonstrates the potential of QSNAP in designing SAPDNPs. However, significant advancements are still required for it to become a practical tool. Building upon existing technologies and expertise, researchers are conducting extensive investigations into the mechanisms and novel formulations of SAPDNPs. Numerous studies showed that the formation and stability of SAPDNPs were primarily influenced by the proportion of free drugs, the linkers of amphiphilic prodrugs, and the self-assembly method.

SAPDNs can form through non-covalent interactions among the drug molecules themselves. The quantity and variety of functional groups present, which is determined by the number of drugs involved, influence the hydrophobic-hydrophilic or electrostatic balance between molecules. This balance dictates the intermolecular interactions that drive the self-assembly process. A study demonstrated that the addition of a hydrophobic near-infrared emitting element transformed camptothecin-Gemcitabine carrier-free amphiphilic prodrugs (CPT-ss-GEM) from nanowires to uniform spherical structures [32, 252]. The primary reason may be the replacement of π-π interactions by hydrophobic interactions as the dominant force in self-assembly. The ratio of drugs can impact the particle sizes and shapes of SAPDNPs [252]. For instance, deprotonated Ce6 can co-assemble with HCPT, which has a limited self-assembly capacity [250]. In this system, the morphology of the co-assembled structure is significantly influenced by the proportion of hydrophilic components. The 1∶1 and 2∶1 (HCPT∶Ce6) co-assembled systems, which have higher hydrophilicity, form irregular needle-like nanostructures. In contrast, the 4∶1 and 8∶1 (HCPT∶Ce6) co-assembled systems, with lower hydrophilicity, yield uniform rod-like nanostructures or even mutually nested structures [166]. Altering the molar ratio of multiple drugs can affect the structure of self-assembled systems, resulting in formations such as brick mud structures or core-shell structures [206]. The varying shapes of SAPDNs significantly influence their *in vitro* release, *in vivo* pharmacokinetics, and efficacy. PTX nanocrystals and amorphous indomethacin (IDM) form a "core-shell" structure through intermolecular interactions, with IDM assembly on the surface [39]. Based on the structure and *in vitro* release data, researchers hypothesized that IDM in IDM-PTX would be released rapidly to modulate the immune system, while the PTX nanocrystals would effectively target tumor tissues and prolong biological half-life [39].

The self-assembly process of amphiphilic prodrugs involves a competition between drug-water and drug-drug interactions. The formation and stability of amphiphilic prodrug self-assembly are driven by the hydrophobic-hydrophilic equilibrium. Specifically, the ratio between hydrophilic and hydrophobic segments can influence the formation and stability of the self-assembly, which can be modified by altering chains and functional linking groups [253]. Linkers altered the spatial position and rotational degrees of freedom between prodrugs to affect the driving force and energy barrier of the self-assembly and modified the micro or macro properties of supramolecular material [254]. For instance, PTX typically forms needle-like crystals due to crystal growth, which hinders the formation of self-assembly. Pei et al. introduced a soft and freely rotatable σ-bond (a bicarboxylic acid bond) between PTX dimers to provide more flexible space, preventing the orderly and stable lattice arrangement of PTX and facilitating self-assembly [255].

SAPDNPs can be prepared using various methods, including antisolvent precipitation, template-assisted techniques, and *in vivo* self-assembly [256]. The conventional nanoprecipitation method has limitations such as low productivity, relatively large particle size, and significant batch-to-batch variance [252]. In contrast, the template-assisted method offers a novel and size-controllable preparation strategy, utilizing templates such as anodized aluminum oxide (AAO) and ice [252, 257]. Zhang et al. developed a technique where a drug organic solution is loaded into an AAO or ice template [252, 257]. Upon removal of the organic solvent, the drugs self-assemble, and the template is subsequently stripped away, yielding uniform SAPDNPs. The ice template-assisted method is particularly advantageous, as it produces SAPDNPs with high reproducibility and adjustable size while avoiding the use of inorganic template materials. This green, low-cost, and high-yield production method represents a significant advancement in SAPDNP preparation [257].

5.2. Types of SAPDNPs

5.2.1. SAPDNPs composed of a single drug

Nanoparticle preparation *via* nano-precipitation may result in drug molecules dissolving or precipitating into large aggregates. Interestingly, certain APIs possess the ability to independently and spontaneously self-assemble into SAPDNPs [242]. Fan et

al. designed a self-assembled ursolic acid nanoparticle (UA-NPs) system utilizing electrostatic and hydrophobic interactions [258]. UA-NPs demonstrated higher cellular uptake rates and toxicity compared to ursolic acid in A549 cells. Furthermore, Li et al. developed self-assembled spherical nanoparticles composed of dihydroartemisinin (DHA NPs) [259]. In a neutral environment (pH 7.4), DHA NPs released only 20% of their dihydroartemisinin payload over 48 h. Conversely, in an acidic environment (pH 5.0), more than 65% of DHA was released from the DHA NPs. These results suggest that DHA NPs have the potential for anti-tumor therapy in the weakly acidic tumor microenvironment (TME). Additionally, numerous other chemotherapy drugs exhibit self-assembly capabilities, including PTX [260], 6-mercaptopurine, and curcumin [261]. These self-assembly systems share similar advantages, such as simple preparation, ultra-high drug loading efficiency, and significantly enhanced delivery efficacy.

5.2.2. SAPDNPs composed of multiple drugs

The co-assembly of multiple drugs exhibits a higher level of complexity compared to single-drug systems. To achieve equilibrium, a greater number of drug molecules must balance numerous molecular interactions [262]. Co-assembly systems involving multiple drugs encompass two primary modes: the co-assembly of hydrophilic and hydrophobic drugs and the co-assembly of hydrophobic-hydrophobic drugs. For instance, various natural active compounds or novel chemical entities, such as BBR and cinnamic acid, demonstrated the ability to co-assemble into nanoparticles, presenting potential applications in precision therapy [263–266].

Monotherapy often exhibits significant limitations in the treatment of diseases. As a combination therapy strategy, multidrug self-assembly offers enhanced functionality, particularly in tumor therapy, by improving efficacy, reducing side effects, and increasing patient compliance. In the middle and late stages of cancer, chemotherapy frequently becomes the sole conventional treatment option. Considering the potential toxicity of chemotherapeutic agents, SAPDNPs composed of chemotherapeutic agents with different anti-tumor mechanisms represent a promising co-delivery strategy. This approach may achieve rapid tumor eradication while avoiding long-term toxicity. For instance, mitoxantrone, PTX, and HCPT are three commonly used chemotherapy drugs [267, 268]. Interestingly, co-assembly did not occur between any two of the three drugs but was observed when all three were combined. This co-assembled combination therapy demonstrated significantly higher cytotoxicity compared to the three free drug groups and the mixture of the three drugs [269]. Moreover, it substantially enhanced cytotoxicity against resistant cells [269]. Similarly, tumor complications, including inflammation, pain, and infection, are often associated with further cancer progression. These complications are evidently detrimental to tumor treatment and long-term survival. Co-assembling symptomatic drugs and chemotherapeutic agents represents a beneficial anticancer strategy.

Minimizing adverse effects, alleviating pain, and enhancing patient compliance are crucial aspects of effective disease management. Photodynamic therapy (PDT), a non-invasive therapeutic and diagnostic approach approved by the FDA, has the potential to reduce the likelihood of tumor recurrence and drug resistance [270–274]. As reported by Stapleton et al., heat and radiation can modulate fluid dynamics, enhance the EPR effect and improve the transport efficiency of nanomedicines [275, 276]. Building upon this foundation, researchers have developed self-assembled carrier-free nanomedicines that incorporate a photosensitizer (PS) and additional components, such as phototherapy enhancers and chemotherapeutic agents [277]. To achieve optimal phototherapy outcomes, efforts focused on reducing oxygen consumption, disrupting antioxidant defense mechanisms, and combining chemotherapeutic drugs. For instance, Li et al. engineered a self-assembly delivery system comprising the photodynamic synergist TH588 and the PS Ce6 [278]. TH588 interfered with the ROS defense system in tumor cells, potentiating the DNA oxidative damage induced by Ce6. Similarly, Zhang et al. developed SAP-DNPs containing genistein, a GLUT-1 inhibitor flavanone, and Ce6 [279], achieving synergistic effects through starvation therapy and PDT without significant cytotoxicity associated with chemotherapeutic agents. PA imaging provided visual guidance and monitoring for PDT, demonstrating the high tumor accumulation efficiency of the nanoparticles [280]. Additionally, various phototherapy enhancers, such as vitamin B [281], iron apoptosis [282], oxidative phosphorylation inhibitors [283], and glutathione transferase inhibitors [284], were explored to disrupt the ROS system in tumor cells, thereby enhancing the anti-tumor and imaging effects of PDT. Furthermore, Guo et al. utilized hydrophobic ursolic acid, PTX, and indocyanine green, an amphiphilic tissue-penetrating agent, to create a dual anti-tumor self-assembled nanodrug [285]. This spherical nanodrug significantly improved the solubility of ursolic acid and PTX, maintained the photostability of indocyanine green, and achieved prolonged accumulation at tumor sites.

Conventional cancer treatments, such as surgical resection and chemotherapy, cure less than 50% of patients [286, 287]. Immunotherapy, which aims to enhance immune defenses to eliminate malignant cells, revolutionized cancer treatment and led to a deeper understanding of tumors [288]. A study combining an immune checkpoint blocker (anti-CTLA-4) and a chemotherapeutic sensitizer (lamonidin) in a co-delivery liposome system demonstrated an enhanced immune response to tumor cells [289]. As SAP-DNPs have a higher loading capacity than liposomes, the researchers suggest that combining immune checkpoint-blocking therapy and adoptive T-cell transfer with chemotherapy and phototherapy could be an effective approach to treating tumors using a self-assembled carrier-free delivery system. For instance, the study incorporated the immune adjuvants metformin and 7-ethyl-HCT into self-assembled nanoparticles (MS-NPs) [290, 291]. Treatment using MS-NPs demonstrated enhanced chemotherapy and immunotherapy effects in mice compared to monotherapy, resulting in a higher survival rate.

5.2.3. SAPDNPs composed of prodrugs

SAPDNPs can be formed by amphiphilic precursor drugs, integrating the benefits of both nanoparticles and prodrugs. This approach involves linking the active drug to other drugs or active components *via* cleavable bonds. In drug-conjugate delivery systems, conjugates with distinct properties impart specific functionalities to the system, such as sustained and controlled release, immunogenicity reduction or elimination, and biological half-life extension [292, 293].

APIs, functioning as hydrophobic or hydrophilic components, can be modified by small molecules or high polymers to form amphiphilic prodrugs, providing opportunities for the self-delivery of drugs [253]. Hydrophilic groups appear to be indispensable for the construction of amphiphilic prodrugs. Long et al. designed a self-assembled nanodrug (Nano DOPA) consisting of an amphiphilic block copolymer [PEG-b-P (L-DOPA $(OAc)_2$)] [294]. In the behavioral test of L-DOPA-induced dyskinesia mouse models, abnormal involuntary movement scores in the Nano DOPA group showed a more significant reduction compared with the L-DOPA group, suggesting that Nano DOPA may be a potential drug for Parkinson's treatment. Similarly, the PTX-succinic conjugate (PTX-SA) joined PTX and succinic acid together *via* an ester bond [295]. Parkinson's treatment. Similarly, the PTX-succinic conjugate (PTX-SA) self-assembled into nanofibers in aqueous solution [295], achieving a drug-loading of PTX as high as 89%. With the hydrolysis of ester bonds, PTX was slowly released from PTX-SA, which enhanced its anti-tumor efficacy [295].

The incorporation of small hydrophobic moieties can enhance the balance and interplay of intermolecular interaction forces, enabling the modification of water-soluble drugs to exhibit spontaneous aggregation behaviors. This approach facilitates the design of self-assembly systems for water-soluble drugs. An effective strategy involves combining water-soluble drugs with a series of fatty acid or sterol analogs to form amphiphilic prodrugs and promote their aggregation behaviors. For instance, Jing et al. developed a conjugate of docetaxel and oleic acid connected *via* thioether bonds [296]. This conjugate was utilized to obtain oxidation and reduction-sensitive SAPDNPs through nanoprecipitation. Similarly, amphiphilic molecules (SQdFdC) have been synthesized [297]. SQdFdC demonstrated a prolonged blood half-life and enhanced anti-tumor activity compared to gemcitabine.

A crucial aspect of self-assembled pro-DDSs is the ability to release APIs from their carriers, enabling them to exert therapeutic effects on the organism. Self-immolation linkers represent a powerful tool for developing targeted pro-DDSs that conjugate multiple drugs. These linkers are designed to respond to specific chemical or physical stimuli, such as acidic environments, enzymes, or redox conditions, facilitating targeted delivery of multiple drugs [298, 299]. Homodimeric prodrugs based on self-immolation linkers can self-assemble into nanomedicines with high drug loading capacities [300, 301]. For instance, a novel paclitaxel-s-s-paclitaxel (PTX-s-s-PTX) conjugate was synthesized using a disulfide bond, which self-assembled into uniform nanomedicines (PTX-s-s-PTX NPs) [302]. The high drug loading (78%) and redox-sensitive disulfide bonds of PTX-s-s-PTX NPs enabled rapid and extensive release of PTX within tumor cells. Similarly, conjugates of different drug molecules can self-assemble into heterodimeric prodrugs, which offer advantages for combination therapy compared to homodimeric prodrugs. The FDA has approved irinotecan (CPT-11) and topotecan for the treatment of colorectal and small-cell lung cancer, respectively. However, the clinical application of camptothecin (CPT) was significantly limited by its poor solubility, high systemic toxicity, and instability [303]. Ao et al. conjugated the hydrophobic CPT with the hydrophilic photothermal agent neoindocyanine green, which self-assembled into IR820-SS-CPT NPs [304]. The disulfide bond of IR820-SS-CPT was cleaved in response to reduced glutathione in the TME, releasing IR820 and CPT for combined chemo-photothermal treatment. Recently, several SAPDNPs consisting of imaging agents and drugs were designed to respond to the acidic TME through pH-responsive linkers, such as hydrazones, acetals, esters, and imines. For example, Yu et al. synthesized ketone-linked amphiphilic glucose-etoposide prodrugs that self-assembled into nanomedicines activated by dual enzyme and acid stimulation, resulting in the effective release of acetone and glucose [305].

Water-soluble biomacromolecules were conjugated with APIs through hydrophilic parts or self-immolation junctions [306]. Drug-peptide amphiphilic conjugates that self-assemble into nanostructures are widely used to deliver various anticancer drugs for tumor treatment [307–309]. For instance, Man et al. developed cathepsin B-cleavable peptide (Phe-Arg-Arg-Gly, FRRG)-doxorubicin prodrugs (FRRG-DOX). FRRG-DOX self-assembles into stable targeted SAPDNPs, which target tumors and enhance therapeutic efficiency [310]. Interestingly, some water-soluble peptide-drug conjugates cannot self-assemble *in vitro*. However, they can undergo supramolecular self-assembly [311] and biocompatible condensation reactions under enzyme induction [312, 313]. Compared to normal cells, certain enzymes often exhibit abnormal activity in cancer cells. Guided by these enzymes, amphiphilic prodrugs self-assemble into stable nanostructures through supramolecular interactions (π-π interactions, hydrogen bonds, and intermolecular charge interactions) [314]. These nanostructures demonstrate enhanced cellular uptake and drug retention in cancer cells, which is particularly beneficial for overcoming multidrug resistance [34]. Liang et al. combined PMI, a functional peptide that induces cancer cell apoptosis, with HCPT to produce a self-assembling drug-peptide conjugate [315]. This amphiphilic conjugate modulated peptide folding and self-assembly behavior to obtain self-assembled nanomedicines, exhibiting enhanced cellular uptake and nuclear accumulation capacity. Similarly, Miao et al. designed the acetyl-Arg-Val-Arg-Arg-Cys(StBu)-Tyr(I-125)-2-cyanobenzothiazole conjugate [313]. Overexpressed furin in tumor cells regulates the biocompatible condensation reaction between the 1,2-amino-mercaptan group of cysteine and the cyanide group of 2-cyanobenzothiazole, allowing the conjugate to self-assemble into radioactive nanoparticles (125I-NPs) *in vivo*. The cellular enrichment of 125I-NPs also prevents cell clearance, rendering them a promising *in vivo* imaging technique [313].

Nucleic acids, such as messenger RNA (mRNA) and small interfering RNA (siRNA), are highly hydrophilic and negatively charged natural biological macromolecules. DDSs, including lipids and polymers, are necessary for their *in vivo* and *in vitro* delivery [316, 317]. In comparison to PDNs, the potential side effects of polymer materials and synthetic lipids as carriers have not been thoroughly elucidated [318]. However, the presence of phosphoric acid and bases in nucleic acids provides the opportunity to develop nucleic acid-API conjugates and SAPDNPs [317]. For instance, a cationic PS was conjugated with the siRNA targeting Polo-like kinase 1, which then self-assembled into siRNA-photosensitizer nanoparticles (siPLK1-NB NPs) through electrostatic attraction [319]. Upon light exposure, siPLK1-NB NPs effectively inhibited the growth of external tumor cells by downregulating the expression of PLK1 and inducing photodynamic cell death.

6. Active-targeted PDNs

Active targeting demonstrates superior precision and delivery efficiency compared to passive targeting. This approach significantly enhances the internalization and accumulation of drugs at target sites, resulting in improved therapeutic outcomes [320]. The modification of antibodies or the combination of antibodies with drugs yields carrier-free nanomedicines with high selectivity for the target, enabling the simultaneous delivery of both therapeutic agents [321, 322]. Active-targeted PDNs, which include nanobodies and ADCs, exhibit promising potential in disease treatment.

6.1. Nanobodies

Antibodies are widely employed in the treatment of solid tumors. However, their efficacy is limited by their large size, inadequate tumor penetration, and instability within solid tissues. Nanobodies, derived from the unique functional heavy chain in camel serum, represent a novel and distinct antigen-binding fragment [323]. With a molecular weight of 90 kDa, nanobodies exhibit improved tumor penetration properties [324, 325]. Furthermore, their variable antigenic-binding domain (VHH) possesses a prolate shape with dimensions of 4 nm × 2.5 nm × 3 nm [326]. Nanobodies combine the specific targeting ability of antibodies with the drug delivery systems of PDNs. They possess excellent characteristics such as small size, high stability, strong antigen-binding affinity, water solubility, and natural origin, which generate significant interest in their potential for disease diagnosis and treatment. Notably, Caplacizumab received approval from the EMA and FDA in 2018 for the treatment of thrombotic thrombocytopenic purpura [92]. Additionally, numerous nanobodies are currently undergoing clinical studies for the treatment of various diseases, particularly cancer [327, 328]. As a result, nanobodies have bright prospects in the treatment and diagnosis, which inspires enthusiasm for investment by many pharmaceutical companies.

Consequently, nanobodies demonstrate promising prospects

in both treatment and diagnosis, garnering enthusiasm and investment from many pharmaceutical companies. Nanobodies possess unique structural characteristics that differentiate them from monoclonal antibodies. These distinct features include: (1) Strong affinity: The antigen-binding loop of nanobodies facilitates interaction with concave paratopes on the antigen surface [324], exhibiting an affinity equal to or surpassing that of conventional antibodies [329, 330]; (2) High stability and solubility: Additional disulfide bonds between CDR1 and CDR3 confer high stability to nanobodies, enabling them to retain full binding capacity after one week at 37 °C [331–333]. Furthermore, crystal data of VHH and its antigen complex confirmed the conversion of its frame-2 region from a hydrophobic to a more hydrophilic region [334, 335], contributing to the high solubility of nanobodies. (3) Low immunogenicity: The gene encoding VHHs shares high homology with human VH families 3 and 4 [336, 337]. Moreover, humanized nanobodies further reduce immunogenicity [338]. Temple et al. generated a series of humanized anti-CD72 nanobodies for B-cell malignancies [339]. (4) Rapid tissue penetration and blood clearance: Compared to monoclonal antibodies, small-sized nanobodies do not exhibit a barrier effect at the binding site [340], resulting in more uniform tumor distribution. Tumor interstitial fluid pressure and the EPR effect facilitate the penetration of small-sized nanobodies into tumors [335]. However, free nanobodies are rapidly cleared from the bloodstream [341], which is advantageous for diagnostic applications but detrimental for nanobody-drug conjugates. (5) Construction and production: Nanobodies can be readily expressed in microbial systems (*e.g.*, bacteria, insects, and fungi) and quickly selected from display libraries. This process eliminates the need for cell culture, screening, and purification, thereby reducing production costs [342, 343]. Fig. 3A illustrates the nanobodies produced by phage display libraries.

Nanobodies are versatile tools utilized in various domains, including scientific research, disease diagnosis, and treatment. Nanobodies can accurately identify and quantify clinical biomarkers [344]. The elongated VHH in nanobodies exposes convex paratopes well-suited to bind the fusing or cryptic epitopes of antigens [345]. Leveraging this principle, nanobodies were employed to block the active site in lysozyme [346, 347], identify the pathogen *Trypanosoma* [348–350], successfully distinguish *Brucella* and *Yersinia* genera [351–353], and detect taeniasis solium infection [354]. Furthermore, researchers successfully developed a sensitive sandwich enzyme-linked immunosorbent assay (ELISA) to detect porcine reproductive and respiratory syndrome viruses using two specific nanobodies [355]. The sensitivity of this assay was comparable to that of a real-time polymerase chain reaction assay, demonstrating the potential of nanobodies for ELISA applications [356]. Moreover, high stability and water solubility expand the scope of nanobodies as research tools. For instance, producing a high-quality diffractive crystal of the target molecule is a critical factor for the structural analysis of biological macromolecules [326]. Crystallography studies showed that the nanobody VHH-antigen complex was a crystallization chaperone [357]. It can promote intermolecular interactions in the lattice and reduce conformational heterogeneity to increase the crystallization ability of the targeted molecule [357–359].

Nanobodies, compact antibody fragments, represent a prominent application in high-resolution imaging [329]. When conjugated with functional molecules such as dyes, radionuclides, or biotin, nanobodies exhibit specific binding to biological targets. This complex generates signals at the target site, enabling non-invasive visual diagnosis. For instance, nanobodies targeting the ALFA-tag proved suitable for super-resolution imaging, intracellular detection, immunoprecipitation, and Western blotting assays [360]. The nanobody E8, which targets CDH17, has demonstrated efficacy as an imaging probe for gastric cancer [361]. Following the injection of E8-IR800 into tumor-bearing mice, significantly stronger fluorescent signals were observed at the tumor site. Notably, E8 exhibited minimal presence in the heart, brain, lungs, or kidneys. Furthermore, staining of critical organs such as the brain, heart, lung, and stomach following E8 nanobody injection revealed no discernible positive staining, with the exception of liver tissues, confirming E8's specific binding activity for CDH17-overexpressing tumors. Similarly, radionuclide-labeled nanobodies were employed for imaging atherosclerotic lesions [362, 363]. However, nanobody-based imaging techniques encounter certain limitations, including rapid renal clearance, challenges in penetrating the blood-brain barrier, and insufficient soluble targets.

Nanobody-based therapies are divided into two primary strategies: he use of pure nanobodies as receptor antagonists and the conjugation of nanobodies with functional molecules. These novel therapeutic approaches optimize efficacy and expand the range of potential applications, including cancer [364, 365], inflammation [366, 367], viruses [368], Alzheimer's disease [369], toxins [370], parasites [246], and autoimmune diseases [92]. For instance, when the nucleotide-binding domain (NBDs) of the adenosine triphosphate (ATP)-binding cassette transporter P-gp binds to ATP, the conformation of the P-gp transmembrane domain changes [371, 372], resulting in P-gp transporting substrates extracellularly. The high-affinity nanobody Nb592 binds to the NBDs, inhibiting the ATP-driven conformational transformation of the P-gp transporter, demonstrating that nanobodies can effectively inhibit ATP-hydrolyzed P-gp. Furthermore, as receptor antagonists, nanobodies can block receptor-mediated life activities, thereby interfering with disease progression and development. Esparza et al . designed a nanobody (NIH-CoVnb-112) that binds to the spike protein receptor-binding domain of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [373]. The NIH-CoVnb-112 prevented the interaction between the spike protein and angiotensin-converting enzyme 2 to treat SARS-CoV-S and simplify antiviral vaccine production. Clinically, serum therapy remains widely used to treat patients with poisoning. Nanobodies are ideal serum toxin scavengers for detoxifying natural toxins, such as snake and scorpion venom [370]. Darvish et al. developed a nanobody (Nb12) for black scorpion venom [374], and after intraperitoneal injection of lethal toxin doses, mice administered intravenous injections of Nb12 after 20 minutes all survived successfully. Due to their high specificity and low toxicity, nanobodies are becoming effective therapeutic agents for autoimmune diseases, as exemplified by the 2018 approval of caplacizumab by the EMA and the FDA for the treatment of thrombotic thrombocytopenic purpura [92].

Nanobodies can be conjugated with a variety of functional molecules, including small-molecule drugs, toxins, enzymes, and imaging agents, to facilitate combined therapeutic approaches [375] (Fig. 3B). For instance, Raimond et al. developed an anti-epidermal growth factor receptor (EGFR) nanobody-PS conjugate that integrates immunotherapy with PDT [376]. These conjugates precisely deliver PS into tumors *via* the anti-EGFR nanobodies, inducing cell apoptosis in EGFR-overexpressing tumor [376]. Additionally, Maza et al. synthesized nanobody-natural killer (NK) cell conjugates [377], which effectively eliminate tumor cells due to the specific binding and killing effect of NK cells. Notably, anti-programmed cell death ligand 1 (anti-PD-L1) nanobodies combined with Toll-like receptor 7 (TLR7) agonists form double-targeted nanomedicines [364]. TLR7 agonists activate immunity and upregulate programmed cell death ligand 1 (PD-L1) levels, while anti-PD-L1 nanobodies serve as immune checkpoint blockers that target tumors. This conjugate activates both innate and adaptive immunity against tumors.

6.2. ADCs

To address the limitations of therapeutic antibodies, such as low activity, poor stability, and short circulation time, research-

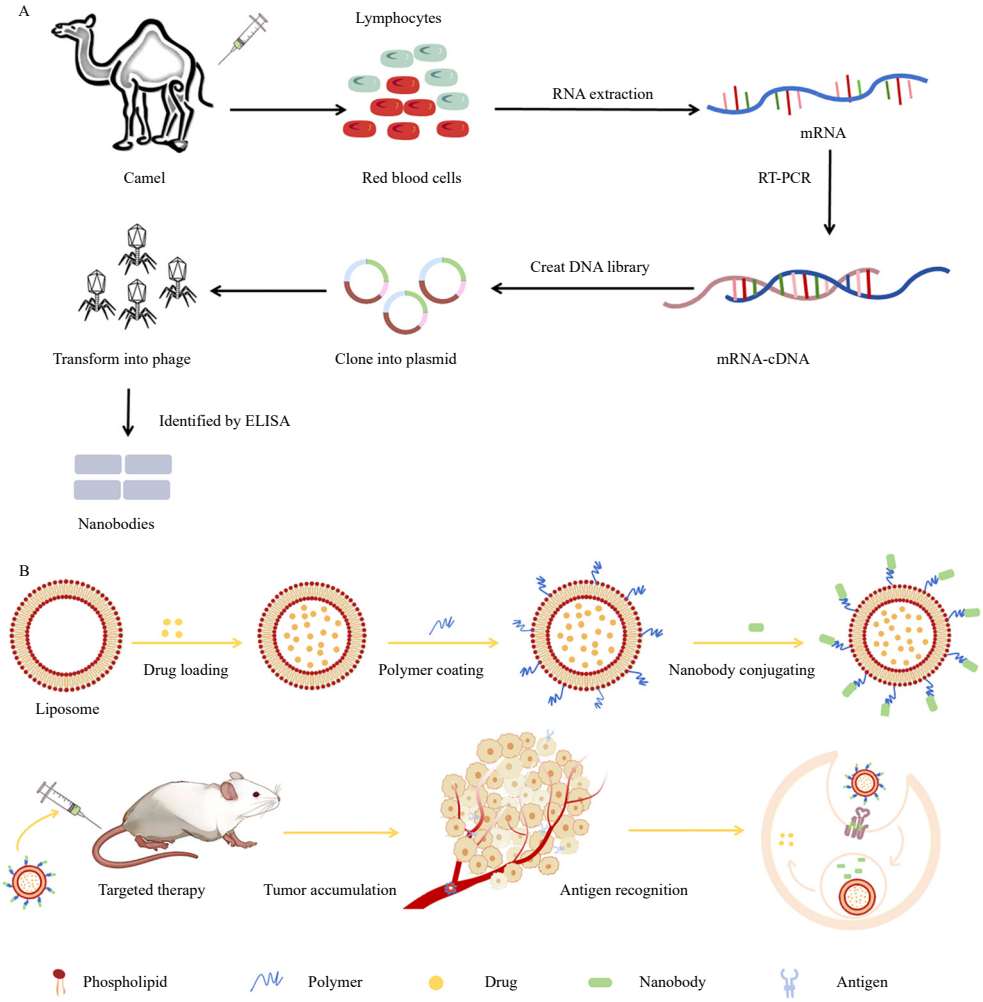

Fig. 3 The generation and application of nanobody. (A) Phage display libraries are used to produce nanobodies. Nanobody generation includes extracting mRNA, reversely transcribing into cDNA, and inserting plasmid into phage. (B) Nanobodies are used to prepare the functionalized liposomes for the treatment of cancer.

ers have modified conventional immunoglobulins. ADCs are attracting increasing attention in the antibody market, with global sales projected to exceed $16.4 billion by 2026 [94, 378]. ADCs are selective and carrier-free anti-tumor nanomedicines that can achieve effective cytotoxicity and drug loading. ADCs consist of three components: monoclonal antibodies (mAbs), cytotoxins, and chemical linkers. After the mAb specifically binds to antigens on the surface of tumor cells, ADCs selectively deliver cytotoxic drugs, inducing tumor cell death [276] (Fig. 4). To date, 14 ADCs have been approved for clinical use (Supporting Information Table S4). By combining the specificity of antibodies with the high potency of cytotoxins, ADCs can be effectively applied to treat various diseases, particularly cancer.

The development of ADCs meets significant challenges due to the required combination of tumor antigens, antibodies, linkers, and cytotoxins [379]. Firstly, the selected antigen must be overexpressed on the surface of target cells to enable recognition and binding of ADCs in the systemic circulation. Subsequently, ADC-antigen complexes depend on receptor-mediated endocytosis for the delivery of cytotoxic drugs into target cells [380]. For instance, in the multicenter phase II trials with GO/Mylotarg®, Jedema et al. discovered that GO-induced cell death is partially CD33-mediated [381], establishing a foundation for the effective treatment of GO tumors. The surface expression level of antigens also plays a crucial role in ADCs. Numerous antigens targeted by approved ADCs include blood tumor targets (CD33, CD30, CD22, and CD79b) and solid tumor targets (human EGFR-2 (HER2), Nectin-4, tumor-associated calcium signal transducers 2 (TACSTD2), tissue factors, and FRα) [382, 383]. Moreover, an ideal antibody with a strong affinity (KD ≤ 0.1 nmol·L^{-1}) and targeting specificity is essential for ADCs [384]. For example, the human/mouse chimeric antibody ch10D7 exhibits a strong affinity for overexpressed CDCP1 in cancer cells. Khan et al. developed ADCs that link the antibody and cytotoxin MMAE *via* an enzymatically cleaved linker [385]. Following internalization by tumor cells, the ADC-antigen complex significantly inhibited tumor cell growth [385, 386]. Furthermore, many antibodies in ADCs were derived from highly immunogenic mice. To reduce immunogenicity and extend plasma half-life, modifying and adjusting the Fc fragments of immunoglobulin G (IgG) through antibody engineering is necessary [387]. The next generation of ADCs encompasses chimeric, humanized, and fully human antibodies.

Linkers between drugs and antibodies play a critical role in ADCs. Ideal linkers allow ADCs to remain stable in blood circulation and rapidly release APIs upon cell entry. Linkers are generally categorized as cleavable or non-cleavable. The cleavable linker responds to physiological and environmental stimuli within the cell, including enzymes and acid. The non-cleaved linker forms strong bonds with monoclonal antibodies (mAbs) and undergoes lysosomal degradation. Caculitan et al. investigated the impact of the VC(S) linker, cleaved by protease, on ADC efficacy, demonstrating cytotoxin release through various mechanisms and inhibition of cathepsin B expression [388]. Drug selection in ADCs is based on disease type and therapeutic mechanism, with

molecules required to maintain antibody biological activity and exhibit high efficacy (IC_{50} within 0.01–0.1 nmol·L^{-1}) [384]. Furthermore, immune stimulatory molecules, such as toll-like receptors (TLRs) 7/8 or their stimulators, can also be utilized in ADC fabrication [389, 390].

7. Challenges of PDN commercialization

Pharmaceutical companies made significant progress in characterizing the physicochemical properties of polymeric drug nanoparticles during R&D. Key parameters such as particle size distribution, half-life, drug loading efficiency, *in vitro* dissolution rates, and *in vivo* biodistribution have been extensively studied [391]. These efforts are enhanced by integrating data from proteomics, metabolomics, and epigenetics, which together contribute to the development of a sophisticated Big Data framework for evaluating PDNs [263, 264]. Leveraging this Big Data approach, pharmaceutical stakeholders can predict the clinical and commercial viability of PDN formulations, assess profit margins, and address critical considerations like reproducibility, technical feasibility, and the financial demands of clinical trials and production [392-394]. Although nanomedicines are rapidly developing, commercializing PDNs remains a significant challenge. The process of licensing novel PDN technologies and patents to established or startup companies is particularly difficult due to the high development costs and regulatory complexities [395]. The increasing costs of development hinder the approval process for the production and marketing of PDNs [395-398].

7.1. From the lab to market

The progression from laboratory discovery to commercially viable PDN products remains an arduous and complex process. Despite the extensive research on PDNs reported in academic literature, only a small fraction have successfully transitioned to the commercial market, primarily due to the prohibitive costs associated with their development and manufacturing [399, 400]. To address these challenges, the pharmaceutical industry must implement advanced manufacturing technologies capable of mass-producing PDNs at reduced expenses [401]. A critical reevaluation of the pharmaceutical industry's role and contribution to PDN development is essential for bridging the gap between research innovation and large-scale commercial production [115].

7.1.1. Supervision of the production line

Mass production methods that satisfy regulatory standards while maintaining low costs are critical for pharmaceutical manufacturing [402]. Reports suggest that the costs of goods sold constitute 20%–25% of total sales [401]. *Rosenberg* further proposes that the development of manufacturing techniques and clinical production expenses collectively account for 40%–60% of the total development costs [401, 403].

There is a pressing need to design and develop a more rational manufacturing facility, encompassing production equipment,

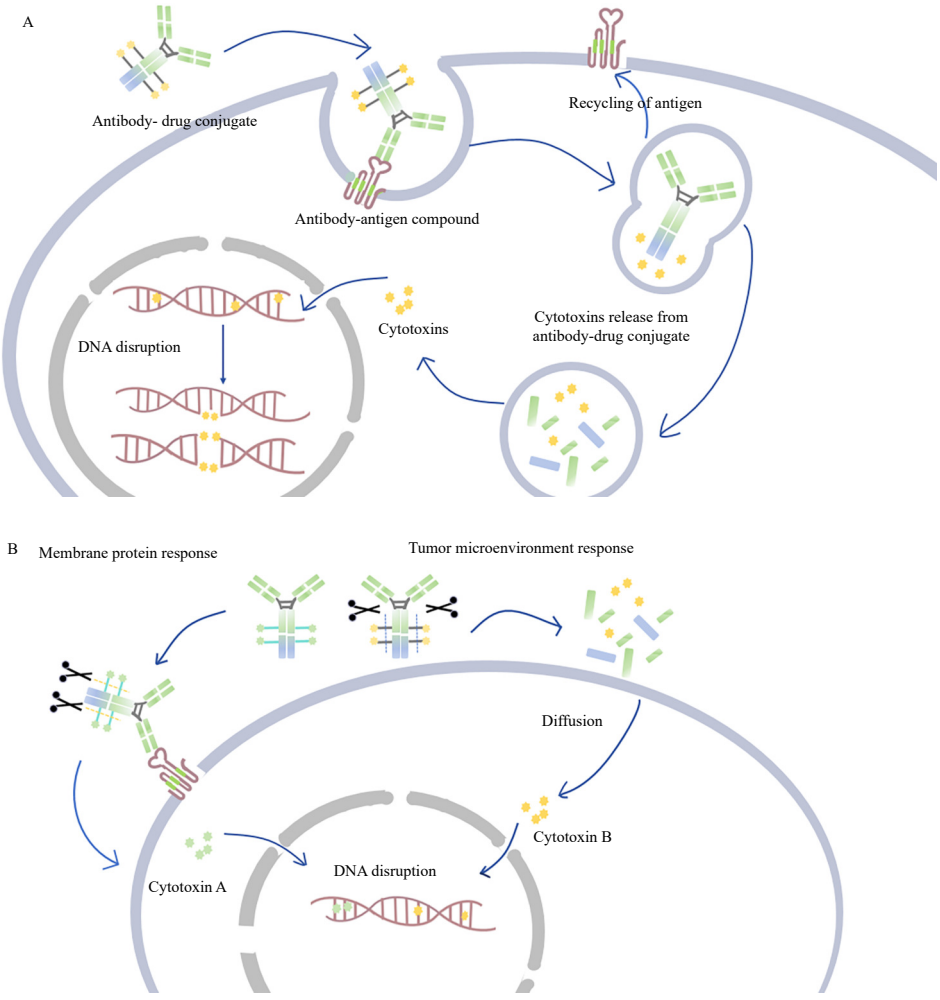

Fig. 4 The internalized and non-internalized mechanisms of ADCs. (A) Internalized ADC. ADC binds to the surface antigen and undergoes internalization, followed by lysosomal capture and degradation to release the cytotoxic payload. The released cytotoxin then interacts with its intracellular target or DNA, inducing apoptosis. (B) Non-internalized ADCs. ADCs release payloads in response to the TME, which subsequently enter cancer cells by diffusion and interact with DNA.

environmental control systems, and personnel [404]. Increased capital investment is crucial for quality and risk management in the production process, including induced crystallization, solvent evaporation, plasmid transfection, and antibody screening. Additionally, numerous databases containing clinical and marketing information are undergoing refinement and development, aiding investors in evaluating market potential and profit scale [405]. Streamlined and secure production lines are vital for the pharmaceutical industry. Advanced manufacturing techniques enhance production efficiency and reduce costs associated with energy, raw materials, and labor.

7.1.2. Dimension control

Particle size and distribution are determined by the inherent properties of drugs and the production process [406]. At the nanoscale, any deviation may alter the *in vivo* behavior of the product, particularly for smaller particles [407]. Top-down methods, such as wet milling, are commonly employed in industry to reduce particle size to the nanometer range. However, this process often necessitates extended processing times and may introduce metal residues [408]. In contrast, nanoprecipitation, a bottom-up approach, can make nanoparticles with a narrow size distribution, simple operation, and minimal equipment requirements. This technique has the potential to produce nanoparticles with sizes below 100 nm, which exhibit enhanced penetration into biofilms [409, 410]. Nevertheless, the solute may undergo further precipitation during the removal of the organic solvent [408]. Additionally, the selection and disposal of organic solvents are critical considerations in this process.

The characterization of PDNs necessitates the establishment of rigorous scientific methods and techniques. Light scattering technology provides a means to measure particle sizes and zeta potential without yielding morphological information [411, 412]. In contrast, imaging techniques, such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM), enable the acquisition of data pertaining to the morphology, surface properties, and other characteristics of PDNs. However, these imaging methods are time-intensive and require the sample to be dry, clean, and conductive [106]. Furthermore, the number of samples that can be observed is limited [107]. The integration of light scattering and electron microscopy is widely accepted as a comprehensive approach to measuring the size and morphology of PDNs [30].

7.2. From the lab to the patient's bedside

The annual approval rate of PDNs is disproportionately low compared to the extensive patent filings. Furthermore, preclinical efficacy findings for numerous PDNs demonstrate significant discrepancies from clinical outcomes, occasionally yielding adverse results. To effectively address these challenges, a comprehensive and profound understanding of the interactions between PDNs and biological systems, the pathological mechanisms underlying complex diseases (particularly cancer), and relevant ethical considerations is imperative [413].

7.2.1. Nanotoxicity

The examination of drug safety and toxicity remains a crucial focus within the pharmaceutical industry. Approximately 20% of nanoparticle failures in clinical trials are attributed to safety concerns [414]. During the decades of rapid development in PDNs, scientists have made notable advancements in safety research and the field of nanoparticle toxicology. However, persistent safety issues continue to hinder the transition from laboratory to clinical application [75]. Certain unique properties of PDNs can contribute to toxicity. For instance, rod-shaped drug nanoparticles exhibit prolonged retention times in systemic circulation compared to spherical drug nanoparticles [149, 415, 416]. Furthermore, the surface charge of PDNs influences their pharmacokinetic profile. Positively charged clarithromycin nanocrystals, for example, demonstrated enhanced mucosal adhesion compared to uncharged and negatively charged nanocrystals [417]. In single-layer CaCO-2 cells, the charged nanocrystals exhibited superior drug transport efficiency compared to uncharged nanocrystals. Additionally, the toxicity of PDNs is dependent on the route of administration and sites of accumulation. Inhaled PDNs may deposit in the trachea and alveoli, potentially leading to inflammation, fibrosis, cyst formation, and necrosis. Following intravenous injection, PDNs may form protein coronas, which alter their surface properties and influence their *in vivo* behavior [418, 419].

7.2.2. Limited understanding of disease pathology

Considering the potential benefits of PDNs to the social economy, nanomedicine is vital in the successful transformation from laboratory to product to solve human health problems, especially in oncology [420]. Cancer is one of the most complex and dynamic human diseases, as its occurrence and development depend on numerous variables [421]. When the DNA sequence in a normal cell mutates, cancer may develop. Sequencing the genomic DNA of cancer cells facilitates the identification of genes that drive and inhibit cancer, as well as the understanding of the role of mutated genes in the disease [422]. Sequencing also benefits the discovery of oncogene blockers and the prediction of cancer development [423, 424]. Although gene sequencing technology has made significant advancements, its cost remains high, and not all genetic mutations have been cataloged. Consequently, obtaining a comprehensive genomic DNA sequence that covers all human cancers remains an elusive goal.

In anti-tumor therapy, drug resistance often arises from multiple factors, including variations in drug targets, alterations in cytopharmacology, and changes in local cancer physiology, either individually or in combination [425, 426]. Research on tumor drug resistance primarily consists of static biological investigations. Due to technological constraints, exposing tumor tissue is necessary, which may pose potential risks to patients and raise ethical concerns [427]. Furthermore, the complex TME plays a crucial role in tumor development, garnering increasing attention from researchers [428–430]. The efficacy of many anti-tumor PDNs relies heavily on the EPR effect. For instance, head and neck tumors and Kaposi's sarcoma exhibit robust EPR effects, making them preferred treatment targets [276]. However, not all tumor blood vessels are leaky, and the EPR effect can vary over time within the same patient or even within the same tumor [431]. Consequently, not all anti-tumor PDNs can be successfully translated into clinical practice.

Despite recent advancements, significant knowledge gaps remain regarding the evolution of the TME throughout cancer progression and treatment [432]. For instance, the differences in TME composition between various cancer types and the potential regulatory role of oncogene mutations on TME composition are poorly understood [433]. Additionally, the biological characteristics and functions of non-malignant components within the TME warrant further investigation [434]. Furthermore, the *in vivo* behavior of PDNs is primarily studied in animal models, and their performance in human subjects remains largely unexplored [435]. The development of comprehensive animal models capable of profiling all tumor types presents a significant challenge. Although several preclinical and clinical studies investigated the pharmacokinetics (PK) of nanotherapeutics across different species [436, 437], there is a paucity of relevant cross-species data. Consequently, the safety and efficacy of PDNs in humans cannot be reliably predicted based on preclinical animal models [436].

7.2.3. Ethical supervision

Clinical trials require that investigators fully inform parti-

cipants or their legal representatives about the study's objectives, potential risks, and benefits. However, issues often arise, including misunderstandings about the experimental nature of treatments, deliberate misrepresentation of risks, or overemphasizing the potential benefits [438]. A particular challenge with PDNs is the difficulty of monitoring long-term toxicity during early-stage clinical trials (Phases I-III). Once PDN products are on the market, unanticipated adverse effects or long-term side effects may emerge. While the FDA encourages post-market studies to monitor such effects, it does not mandate them, which complicates long-term safety evaluations. Another significant challenge is the high cost of PDNs upon market entry, driven by the need to recover R&D investments and protect intellectual property. These high prices often result in healthcare inequities, as only those with greater financial means can afford these cutting-edge treatments, creating increased pressure on public health systems [439]. A critical ethical issue related to PDNs is the growing debate over "enhancement" versus "treatment" [440]. While PDNs are developed for therapeutic purposes, they also hold the potential to enhance human capabilities beyond the treatment of disease, blurring the line between medicine and enhancement [440]. The accessibility of these enhancements is often dictated by wealth, allowing more affluent individuals to gain competitive advantages that are unavailable to those with fewer resources. This imbalance not only raises questions about fairness but also risks creating a societal divide, where access to medical advancements reinforces existing inequalities and threatens social stability [441].

8. Summary and Prospect

PDNs have been extensively employed in the diagnosis, prevention, and treatment of diseases. Furthermore, novel PDN products, developed through innovative technologies and creativity, are currently undergoing preclinical and clinical investigations. Drug nanocrystals, nanobodies, and ADCs are notable examples of PDNs. Drug nanocrystals with specific size and surface properties demonstrate greater potential for delivering substantial quantities of insoluble drugs to targeted sites within living organisms. However, the *in vivo* fate of drug nanocrystals remains unclear. The *in vitro* dissolution tests inadequately simulate the *in vivo* environment. For instance, AZ68 nanocrystals exhibited reduced solubility and dissolution rate compared to their amorphous nanosuspensions [119, 442]. Nevertheless, following intravenous administration, they displayed similar PK performance. Moreover, according to the mononuclear phagocytic system hypothesis, drug nanocrystals with small particle sizes dissolved rapidly. Drug nanocrystals with larger sizes and specific shapes were transported by macrophages into the liver. After intravenous administration, the transport of drug nanocrystals to the tumor site may pose a significant challenge. Stabilizers were highly diluted or formed protein corona in blood circulation, potentially weakening the targeted ability of drug nanocrystals. Additionally, utilizing standard top-down processes to reduce particle sizes below 100 nm presents a considerable challenge. DDCs enhanced the physicochemical properties of drugs, including mechanical properties, hygroscopicity, stability, dissolution rate, and bioavailability. Particularly for DDCs, the conformers possessed their own pharmacological activity. The interaction between drugs and conformers complicated the study of the correlation between *in vitro* properties and *in vivo* responses. For example, compared to free GA, supramolecular GA-glutamic acid cocrystals exhibit stronger binding to α-glucosidase [443, 444]. For DDCs, the study of transport, distribution, and metabolism is often limited to free drugs. The conformers frequently possess pharmacological activity and may cause adverse effects. Furthermore, during cocrystal preparation, contact with solvent leads to dissociation, crystal transformation, and stoichiometric changes. This imposes stringent requirements on scale-up techniques and the production of DDCs.

SAPDNPs, representing the next generation of PDNs, have garnered increasing attention. Primarily prepared through nanoprecipitation, SAPDNPs present challenges for large-scale industrial production. By incorporating functional molecules such as fatty acids, polymers, cytotoxins, photothermic agents, and peptides, SAPDNPs achieved targeted drug release and synergistic therapy. Although SAPDNPs demonstrate great potential in cancer treatment, their exploration of other disease applications remains limited. Research on SAPDNPs has primarily focused on therapeutic effects, with minimal discussion of their assembly mechanisms, hindering the development of suitable tools for screening optimal SAPDNP formulations. Furthermore, the lack of *in vivo* pharmacokinetic and toxicological data impeded their clinical application and commercialization.

ADCs have achieved remarkable success in oncology therapy, continuing to excite investors. However, selecting and optimizing ADC modules, including targets, payloads, antibodies, and junctions, is a complex and challenging process. Pharmaceutical companies must also develop new modules beyond their existing patents, involving multiple production steps that lead to increased complexity and costs. Nanobodies are expected to be an excellent tool in tumor treatment and diagnosis, but their application in other diseases requires further exploration. Moreover, faster and simpler production techniques are needed to meet society's demand for nanobodies.

In summary, SDNCs are highly effective in enhancing the solubility of poorly soluble drugs, offering significant advantages such as well-established manufacturing technology and supportive regulatory policies. DDCs are frequently employed as a combination therapy approach, substantially improving drug efficacy while minimizing side effects. DDCs are also extensively utilized to enhance the oral bioavailability of insoluble drugs. Moreover, SAPDNPs enable the co-delivery of multiple drugs without crystal formation, exhibiting remarkable synergistic therapeutic benefits for cancer treatment. Compared to ADCs, nanobodies possess unique characteristics, including small size, high stability, high specificity, and low immunogenicity, making them favorable for targeted therapy and *in vivo* imaging. However, the limited utilization of nanobodies as therapeutic agents against diseases may be attributed to the scarcity of preclinical and clinical research and the absence of large-scale production capabilities. In contrast, ADCs demonstrate promising potential for cancer treatment, as the payloads in ADCs exhibit superior tumor cell-killing efficacy compared to nanobodies. The cleavable conjugates can more effectively respond to the pathological characteristics of tumors and facilitate payload accumulation at the tumor site. Nevertheless, numerous ADC resistance mechanisms are being identified, and bispecific ADCs designed to overcome drug resistance have not yet achieved clinical application.

Abbreviations

PDNs, pure drug nanomedicines; DDCs, drug-drug cocrystals; ADCs, antibody-drug conjugates; Nanobodies, nanobodies; SAPDNPs, self-assembled pure drug nanoparticles; FDA, U.S. Food and Drug Administration; EPR, enhanced permeability and retention; HCT, 10-hydroxycamptothecin; R&D, research and development; APIs, active pharmaceutical ingredients; HPH, high-pressure homogenization method; ROS, reactive oxygen species; EMA, European Medicines Agency; CNS, central nervous system; COVID-19, coronavirus disease 2019; IOS, International Organization for Standardization; XRPD, X-ray powder diffraction; DSC, differential scanning calorimetry; ICH, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; HTA, Health Technology Assessment; SDNCs, self-dispersible nanocrystals; TPGS, vitamin E polyethylene glycol 1000

succinate; PVP, polyethylpyrrolidone; PTX, paclitaxel; HPMC, hypromellose; MC, methylcellulose; HEC, hydroxyethyl cellulose; HPC, hydroxypropyl cellulose; CMC-Na, carboxymethylcellulose sodium; ζ, Zeta potential; AUC, area under curve; TJs, tight junctions; TEM, transmission electron microscopy; DMY, dihydromyricetin; TMZ, temozolomide; LVFX, levofloxacin; CSP, crystal structure prediction; MEPSE, molecular electrostatic potential surface energy; HSP, Hansen solubility parameter; PD, Parkinson's disease; QC, Quercetin; PEG, polyethylene glycol; OA, osteoarthritis; GA, gallic acid; BBR, berberine; QSNAP, quantitative structure-nanoparticle assembly prediction; HCPT, hydroxycamptothecin; IDM, indomethacin; AAO, anodized aluminum oxide; PDT, photodynamic therapy; PA, photoacoustic; CPT, camptothecin; TME, tumor microenvironment; DDSs, drug delivery systems; siRNA, Small interfering RNA; VHH, variable antigenic-binding domain; ELISA, enzyme linked immunosorbent assay; EGFR, epidermal growth factor receptor; ATP, adenosine triphosphate; P-gp, P-glycoprotein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; PD-L1, programmed cell death ligand 1; HER2, human epidermal growth factor receptor-2; TACSTD2, tumor-associated calcium signal transducers 2; frα, folate receptor α; IgG, immunoglobulin G; SEM, scanning electron microscopy; AFM, atomic force microscopy.

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Declaration of competing interest

These authors have no conflict of interest to declare.

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