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Review

Drug nanocrystals: Surface engineering and its applications in targeted delivery

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SUMMARY

Drug nanocrystals have received significant attention in drug development due to their enhanced dissolution rate and improved water solubility, making them effective in overcoming issues related to drug hydrophobicity, thereby improving drug bioavailability and treatment effectiveness. Recent advances in preparation techniques have facilitated research on drug surface properties, leading to valuable surface engineering strategies. Surface modification can stabilize drug nanocrystals, making them suitable for versatile drug delivery platforms. Functionalized ligands further enhance the potential for targeted delivery, enabling precision medicine. This review focuses on the surface engineering of drug nanocrystals, discussing various preparation methods, surface ligand design strategies, and their applications in targeted drug delivery, especially for cancer treatments. Finally, challenges and future directions are also discussed to promote the development of drug nanocrystals. The surface engineering of drug nanocrystals promises new opportunities for treating complex and chronic diseases while broadening the application of drug delivery systems.

INTRODUCTION

Nanotechnology has the potential to overcome several limitations of traditional therapeutic approaches. These limitations include lifelong adverse effects, rising medicine resistance, impairment in targeting, and insufficient drug absorption.[1–4] Numerous drug delivery approaches utilizing nanotechnology strategies that involve liposomes, polymeric nanoparticles, solid lipid nanoparticles (LNPs), micelles, nanocrystals, nano-emulsions, and implants, have been obtained for different drugs.[2,3,5–9] Nanocrystals are receiving significant interest in drug delivery systems due to their ability to effectively deliver medicines with low solubility, high drug loading efficiency, improved stability, and the potential to sustain drug release.[8,10] More importantly, nanocrystals are characterized by their composition, which consists entirely of the drug substance. Normally, two solid-state strategies are used in the drug development process, including nanocrystals and nano-amorphous solids. Drug nanocrystals are tiny crystals of a drug compound that have dimensions in the nanometer range.[11,12] Crystalline drugs often display long-range positional and orientational order. On the other hand, nano-amorphous solids are very small amorphous substances with dimensions in the nanometer range, lacking long-range order in their atomic-level structure. However, amorphous and crystalline solids exhibit distinct physicochemical properties, such as mechanical and thermal properties, as well as physical (and chemical) stability and solubility.[13] Nano-amorphous solids have a tendency to transform into a more stable crystalline form due to their thermodynamic unstable.[14] Drug nanocrystals, with sizes ranging from 10 to 500 nm, possess unique characteristics that make them suitable for parenteral administration. For example, drug nanocrystals smaller than 500 nm can naturally accumulate in tumor cells through the EPR effect when they circulate in the bloodstream. The speed of diffusion and penetration into the dense extracellular matrix of solid tumors increases as the size of nanoparticles decreases. Although the size of drug nanocrystals for oral administration may be larger than parenteral administration, it still falls within the submicron range, which demonstrates a significant improvement in drug bioavailability.[15–17] Stabilization techniques, such as the use of surfactants or polymers, are often employed to prevent nanocrystals from agglomerating and to maintain their stability in suspension.[18] The

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suspensions are stabilized colloidal dispersions, which can be considered a formulation type of drug nanocrystals. Drug nanocrystals and their suspensions have a close connection in the field of drug delivery, despite having different definitions.

In the earliest phase of drug nanocrystal development, many studies indicated that drug nanocrystals could improve the water solubility of hydrophobic drugs and their bioavailability.[19] This concept was widely accepted as a solution for hydrophobic drugs to increase their treatment effectiveness. Therefore, the U.S. Food and Drug Administration (FDA) have approved numerous nanocrystal-based pharmaceuticals launched to market.[20,21] Nevertheless, due to the unique characteristics of drug nanocrystals, such as their rapid solubility in the human body, their applications are limited in some aspects. For example, the drugs that completely dissolves before reaching the target organ result in their off-target side effects and lack of specific drug delivery. Consequently, controlled drug delivery systems have been investigated.[22,23]

Currently, surface design techniques have become a popular field of research. The utilization of surface-engineered LNPs for the COVID-19 vaccination is an outstanding example of this drug delivery platform. By integrating various ligands onto the surface of LNPs, these molecules will facilitate the delivery of LNPs to organs and cell types that are difficult to reach.[24] Similarly to the development of drug nanocrystals, ongoing research is focused on enhancing and modifying the nanocrystal surfaces for application in various aspects of *in vivo* applications, diagnosing disease's progression, and enhancing previously established medication efficacy.[18,21]

Until now, the FDA has not authorized any drug nanocrystals for targeted delivery, including cancer treatment, in clinical trials. The primary obstacle to the clinical translation of therapeutic nanocrystals is their poor target effectiveness. Due to the natural limitations of pure drug nanocrystals, such as their inability to completely overcome several physiological barriers (including mononuclear phagocytosis system (MPS) clearance, stochastic extravasation of cancer vasculature, and dense cellular/physical barriers in malignant tissue), the accumulation of drug content at the cancer site is less than 1%. Moreover, the uncontrolled dissolution/release *in vivo* and hydrophobic surface of drug nanocrystals lowered enhancing permeability and retention effects (EPR) efficiency and raised the clearance by MPS. Additionally, the absence of functional ligand modifications on the surface of pure drug nanocrystals reduced their active targeting efficiency. This ligand modification approach promisingly deals with this problem, leading to the extended drug half-life in the systemic circulation and enhancing the active targeting properties.[10,25] Therefore, various strategies have been employed to fabricate nanocrystal surfaces intended for medical applications. Ligands are employed for the purpose of transporting pharmaceutical agents to the expected site of action. Taking advantage of imaging applications for diagnostic purposes is a common practice.[18] Additionally, stabilizers are employed to improve both the chemical and physical stability of nanocrystals by adhering to their surface.[18,26] Furthermore, nanocrystal-based applications are designed to facilitate drug delivery via various routes of administration.[17,27] However, there are some hurdles associated with drug nanocrystals for use in targeted delivery. These challenges include: (1) the core drug nanocrystals should be coated or modified with appropriate polymers that could prevent the drug from being completely dissolved or leaked before reaching the target sites. (2) The strong attachment of targeting ligands to the nanocrystal surface should be developed to ensure that these ligands still attach to the drug surface until reaching target sites. (3) The sterilization process poses a significant challenge to preparing drug nanocrystals for parenteral administration. Typically, drug nanocrystals can be sterilized through methods, such as autoclaving, irradiation, or aseptic filtration. The inappropriate sterilization technique can cause a physical and chemical stability issue.[28,29]

The present review article emphasizes the recent surface modification of the core drug nanocrystals and their utilization in drug delivery systems. Firstly, select an appropriate and effective nanocrystal preparation method. This is an important step for the design and advancement of nanocrystals because each preparation process acts on a different principle. In addition, each has distinct benefits and drawbacks. There were a variety of processing parameters that affected the drug particles that could be generated. Secondly, surface modification is an essential stage for both conceptualization and fabrication of a particular delivery system; therefore, additional effort is required to identify a new targeting ligand. It will be essential to optimize targeting ligands in order to develop systematic methods for preparing smart drug nanocrystals in a practical and cost-effective manner. Thirdly, the application of drug nanocrystals should be broadened to other routes of drug administration, including dermal, lung, nasal, brain, and ocular. This article focuses on the recent development of drug nanocrystal surfaces and highlights the advantages of various surface modification methods in distinct drug delivery modalities. The objective is to demonstrate the potential of surface modification of drug nanocrystals as a strategy for treating complex pathological conditions.

PREPARATION OF DRUG NANOCRYSTALS

The use of nanocrystal for drug formulation is largely due to their advantageous physiological properties. As the size of the nanocrystal decreases, the permeation rate across membrane barrier increases, as well as the solubility, due to higher surface area of the crystal.[19,30] Additionally, further modifications, such as stabilizer, can be applied during nanocrystal formulation, leading to improve in stability.[31] Compared to conventional drug, smaller particles result in a higher surface area, which contribute to an increased dissolution rate and consequently enhanced drug bioavailability.[32] One unique property of nanosized particles is their adhesiveness, which leads to better absorption via the oral route. Therefore, the nanocrystal technology is particularly beneficial for the drug formulations intended for oral administration.[33]

Drug nanocrystals provide several benefits over other matrix nanoparticles, such as polymeric matrix (polymeric nanoparticles) and lipidic matrix (liposomes, nanoemulsions, LNPs). The major advantage is high drug loading and lower toxicity of drug formulation. The matrix particles have drugs distributed throughout the matrix and/or adsorbed onto their surface, so the drug loading will be less than 100%. Therefore, these nanocarrier-based formulations suffered from several problems, such as low drug loading and a high excipient ratio. On the other hand, drug nanocrystals contain core drug particles and/or little stabilizers, so drug nanocrystals possess the advantage of high drug loading (nearly 100%).[10,34] Moreover, toxic side effects from the encapsulating/solubilizing excipients also may be eliminated. For example, organic solvents and solubilizers were often added to enhance the solubility of hydrophobic drugs like paclitaxel (PTX). For the commercial product (Taxol),

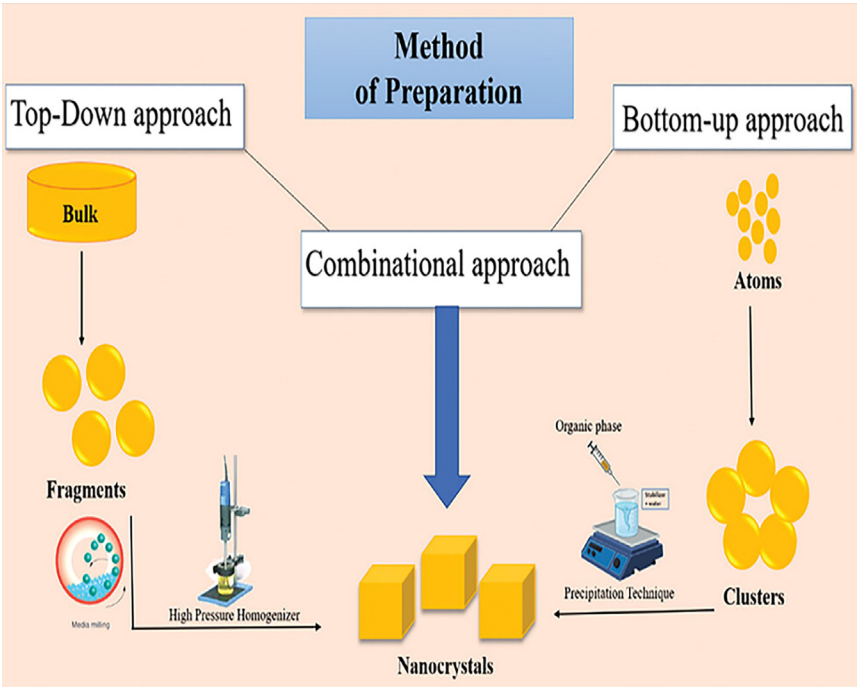

Figure 1. A sequential illustration of the drug nanocrystal manufacturing process
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alcohol and Cremphor-EL were used to enhance the water solubility of PTX. However, the excipients Cremophor-EL could lead to severe side effects, such as allergic reactions and neuropathy in patients. By using nanosuspensions stabilized with intravenously well tolerated surfactants/stabilizers like Tween 80 or Poloxamer 188, this issue can be prevented.[10] For example, PAXCEED is a formulation that contains paclitaxel nanocrystals without Cremophor-EL and is currently undergoing clinical trials. This has the potential to decrease hypersensitivity in patients undergoing treatment for cancer or chronic inflammation.[35] Furthermore, low target efficiency of PTX always causes systemic toxicity. Additionally, with the addition of ligands, the delivery efficiency can also be improved, resulting in lower side effects. Drug specificity can also be adjusted through these modifications to target the appropriate site, a concept known as targeted therapy.[36]

There are three commonly used methods for the production of drug nanocrystals, including bottom-up, top-down, and the combination method. The difference between these methods is based on the initial size of the drug particle. Top-down methods, including high-pressure homogenization and wet milling, apply mechanical force to decrease the drug's size. The large particle will break down into a small particle, whereas bottom-up approaches involve the generation of small particles at the molecular level. The bottom-up method can be divided into two main groups. For example, the solvent evaporation method (spray drying and lyophilization) and the antisolvent methods (solvent-antisolvent methods and supercritical fluid extraction).[15,37,38] Figures 1 and 2 present a comprehensive overview of the drug nanocrystal production process.

Top-down method

The application of "top-down" technology involves the utilization of various grinding and homogenization techniques as dispersion methods. This mechanism is responsible for the fragmentation of large crystal particles into smaller fragments. This technology can be achieved through the processes of media milling (NanoCrystals) or high-pressure homogenization (Nanopure, IDD-P, DissoCubes).[15,40] The main advantage of utilizing this approach is its versatility in terms of production scale and its ability to generate crystalline nanoparticles, making it a widely applicable technique. Consequently, this technique has been frequently used for the production of commercial products. Apart from Triglide which is manufactured by IDD-P, most commercial drug nanocrystals were manufactured by NanoCrystals. The disadvantages of this method include its time requirements and high energy consumption, along with the potential for contamination from the grinding media.[25]

Bottom-up method

The conventional term used to describe bottom-up approaches for drug nanocrystal preparation is precipitation methods. As a result, solid dispersions are typically not regarded as the primary product that contains them. Nevertheless, particular forms of solid dispersions comprise drug particles that are naturally nanocrystalline and are incorporated within a matrix.[41] The very first nanocrystal drugs generated through the precipitation approach were hydrosols, a product of List and Sucker, which was formerly under the ownership of Sandoz but is now a part of Novartis.[42] The underlying principle behind this procedure is that the drug is solubilized in a solvent, followed by the addition of an antisolvent

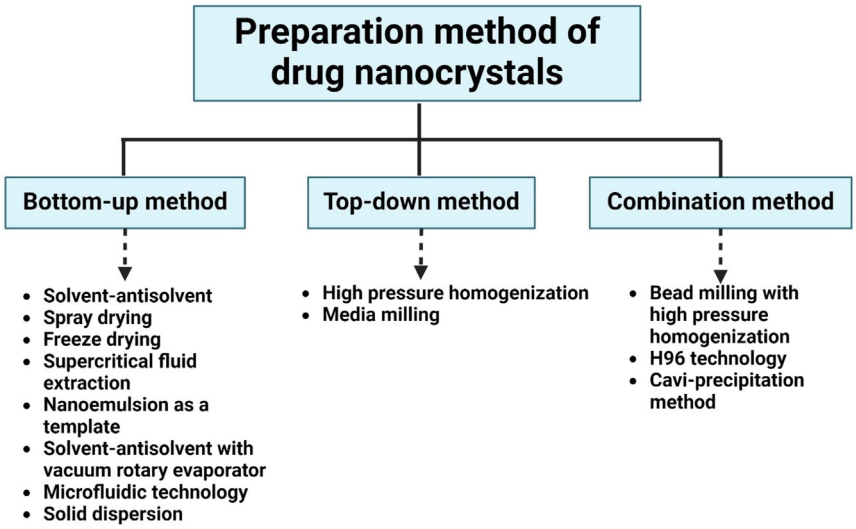

Figure 2. A summary of the drug nanocrystal manufacturing process
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that is miscible with the solvent. When stabilizers are present, nanocrystals precipitate. A primary benefit of this approach is that it's fast and inexpensive. Scalability is also straightforward in this procedure. Several parameters, including agitation speed, drug concentration, temperature, stabilizer, type of solvent, viscosity, and the ratio of solvent/antisolvent, need to be investigated as ways to get a homogeneous nanosuspension.[15,43] An additional important consideration is the requirement for the solvents applied in the processes to be diminished to an acceptable level in the final product.[44] The manufacturing process needs strict control and prevention of recrystallization within the micrometer range.[41]

Apart from the preparation by solvent-antisolvent method, several techniques for the bottom-up method have been developed, including spray drying, freeze drying, supercritical fluid extraction, and nano-emulsion as a template.[45–52] Additionally, applying a vacuum rotary evaporator to the preparation process can decrease the amount of stabilizer in the formulation.[53]

Recently, the preparation of nanocrystal particles was conducted using the solvent-antisolvent method, as well as vacuum rotary evaporation for drying, and ultimately hydrated with a stabilizer solution, in accordance with the findings of Brice Martin and Panpan Ma's research. The study revealed that the particle size measured less than 250 nm and exhibited favorable physical stability along with an improvement in antitumoral properties.[53,54]

The generation of drug nanocrystals typically involves the utilization of conventional methods, which ultimately result in the formation of a nanosuspension. The production of an oral solid dosage form necessitates substantial downstream processing, including the appropriate drying of the liquid and subsequent granulation. Sanika Jadhav established a spray drying technique to produce nanocrystalline solid dispersion (NCSD) consisting of drug nanocrystals. The application of the spray drying technique results in nanocrystals being obtained in powder form, thereby minimizing downstream processing requirements. This renders the nanocrystals suitable for immediate use in oral solid dosage form. The downstream processing expenses for NCSD were significantly reduced by up to 80% in comparison to traditional methods. The study has shown that the utilization of the one-step method proved to be highly effective and required less post-processing in comparison to the multi-step procedure, including wet milling combined with drying techniques for the conversion of nanocrystals into oral solid dispersion.[46]

Comparable to the technique of spray drying, the process of lyophilization has also been used for the preparation of drug nanocrystals. Hans de Waard regulated drug crystallization during lyophilization process, then used a 3-way nozzle spray technique to produce drug nanocrystals. Interestingly, this semi-continuous process can apply to the production scale. Drug nanocrystals prepared by this method show an increase in dissolution rate in comparison to the use of freeze-drying alone. This is because the high freezing rate can increase the nucleation rate and decrease the size of drug particles.[55]

Nanoemulsion as a template technique was used to produce drug nanocrystals. For example, some researchers have further developed another fascinating method. They use this technique to prepare drug nanocrystals and then incorporate these drug particles into the hydrogel in order to control the drug release. The result indicated that the release kinetics of this nanoformulation can be controlled within a range of 10–200 min. This finding will be beneficial for the controlled release of drugs in order to optimize their therapeutic efficacy.[52]

Microfluidic technology is the latest production method and was used in the manufacturing process.[56] This method was developed in the 1970s, involving a technology that can accurately control the small fluids within channels around 10 to 100 microns in diameter. The use of a micro-reaction chip with a reaction scale equal to or smaller than the carrier allows for precise preparation of micro- and nano-scale drug carriers. This technique provides a controllable method for preparing nano-drug preparations.[56]

Microfluidic technology offers precise controllability and easy production, making it advantageous for the preparation of nano-drug preparations.[22] By enabling rapid mixing and laminar flow properties in microchannels, these may effectively manipulate the physicochemical

characteristics of nanodrug delivery systems, such as particle size, size distribution, and morphology. This leads to a narrow particle size distribution and a high drug-loading capacity.[56] Yohann Corvis and his colleagues apply a systematic step-by-step strategy using Quality-by-Design (QbD) and Design of Experiments (DoE) to generate curcumin nanocrystals. They utilize a semi-automated nanoprecipitation technique for this purpose. The optimized process results in a consistent, robust, and reliable production of 316 nm curcumin nanocrystals with a PdI of 0.217.[57] Although it has demonstrated promising results, there are certain challenges, such as purification and yield, which hinder its scale-up production. As a result, microfluidic technology has primarily remained in the laboratory research stage and has experienced slower progress in industrial production. The development and application of microfluidic technology in nano-drug preparation were greatly stimulated by Pfizer and Moderna's successful preparation of the mRNA LNP vaccine using microfluidic technology for the first time.[56]

For the bottom-up strategy, drug nanocrystals are frequently generated using microfluidic technology.[58–62] For example, the two-step microfluidic method was used to prepare drug nanocrystals that were coated with an acidic-responsive polymer. Computational fluid dynamics is utilized to calculate the velocity and concentration fields within the production chamber. Additionally, it determines the time of mixing for the production of primary nanoparticles. These nanovectors inherit the favorable features of both drug nanocrystals and polymeric nanoparticles. It exhibits biodegradability, high drug loading, good stability, pH-responsive fast dissolution, and is easy for surface engineering.[22]

Combination method

Combination technology, which combines wet bead milling with HPH, has also recently been developed.[63] These innovative methods can enhance the reduction of particle size while overcoming the drawbacks of the conventional technique, especially extended grinding times and too much grinding speed, which can result in contamination, undesired degradation or the formation of amorphous drug particles that can influence the drug nanocrystal's stability.[64] According to Rita Ambrus's study, meloxicam nanocrystals were produced using a mixture of HPH and wet grinding methods. Drugs were pre-milled first, followed by passing through a high-pressure homogenizer. The findings indicate that the aforementioned technique proved to be a viable and efficacious means of generating nanocrystals with enhanced solubility for drugs that exhibit poor water solubility. The absence of meloxicam amorphization was confirmed through the use of DSC and XRPD.[65]

The integration of both bottom-up and top-down methods is a common practice in this technology. The hybrid approach known as Nanoedge technology involves the drugs being micro-precipitated, then being subjected to high shear or thermal annealing. An appropriate solvent-antisolvent system is used to precipitate amorphous or crystalline drug nanocrystals in the first stage. The drug particles might be entirely amorphous, partially amorphous, or entirely crystalline at this point. Annealing is a process that involves the application of energy, such as mechanical stress or direct heat, followed by thermal relaxation. This process aims to transform particles into a more stable form by transitioning from a less ordered lattice to a more ordered structure, resulting in a reduction of energy.[66]

Combining particle size reduction strategies can effectively manufacture ultra-small drug nanosuspensions. Both cavi-precipitation and H96 technology procedures may be good options for producing smaller drug nanosuspensions.[67] The H96 technique involves freeze-drying to generate a brittle API powder. As a result, when the suspension is formulated with these modified drug particles and subsequently passed through HPH, very small drug nanocrystals are obtained.[68] For the cavi-precipitation method, both high-pressure and anti-solvent precipitation is applied to achieve better control over the preparation process. A recent study revealed that the cavi-precipitation process produced particles approximately 50 nm in size.[68]

Innovative preparation techniques improve the understanding of drug nanocrystals, encourage scientific research on drug surface properties, and lead to various valuable surface engineering strategies. For example, fluid dynamics simulation has been utilized to examine the velocity and concentration distribution inside the production chamber of a microfluidic machine, with a focus on the Reynolds number (Re). The solvent-to-nonsolvent flow ratios (FR) were altered during the drug nanocrystal production process. The findings indicated that Re had an impact on both the particle size and polydispersity index (PDI) of polymeric nanoparticles and sorafenib (SFN) nanocrystals. Moreover, FR has a greater influence on the PDI and particle size of SFN nanocrystals compared to polymeric nanoparticles. By utilizing this knowledge, they develop double microfluidic processes to produce SFN nanocrystals as the core and subsequently modify the surface of the drug core with ligand-modified polymeric nanoparticles.[22] The solvent-to-antisolvent volume ratio (S/A) has significant effects on both particle size and PdI. A decrease in the activation energy for drug nanocrystal precipitation occurs at low ratios, which is associated with an increase in supersaturation. Moreover, the observed behavior of the S/A in the optimization process indicates that the cutoff value might exceed the 0.15 ratio slightly. An increased amount of ethanol can provide controlled supersaturation throughout the procedure. The impact of flow rate on particle size is mostly determined by the rate of mixing and degree of agitation in the system. At low flow rates of the organic solvent, there will be a decrease in the mixing efficacy. This will lead to a limited number of nucleation sites and extended growth process, resulting in the formation of bigger drug nanocrystals.[57] Interestingly, modifying the facets of nanocrystals can increase their ability to bind to transferrin and boost the effectiveness of these nanocrystals for clinical purposes. Different conditions were used to synthesize inorganic nanocrystals, resulting in the generation of various facets. The results indicate that, through inner-sphere thiol complexation, the greenockite (002) facet and the cadmoselite (100) facet have a preference for binding with transferrin. According to computer simulation, the binding of transferrin is believed to be regulated by the affinity of water molecules to the crystal facet in the initial hydration layer.[69]

SURFACE MODIFICATION OF DRUG NANOCRYSTALS

The surface modification of drug nanocrystals has been developed to solve problems such as the uncontrolled release of pure drug nanocrystals, MPS clearance, and lack of active targeting. This part of the review article will summarize the basic principles for surface modification and ligand design in the surface engineering of drug nanocrystals.

Basic principle for surface modification

Core drug nanocrystals exhibit poor physical stability due to their small size, which results in high surface energy. This high surface energy causes drug aggregation to minimize surface energy, causing an increase in particle size. Hence, colloidal stabilization is crucial in the development of nanocrystal formulations. To enhance the physical and chemical stability of drug nanocrystals, the use of stabilizers is essential. However, identifying a suitable stabilizer to enhance the stability of drug crystals is a challenge due to the absence of a universal stabilizer that can be applied to all drugs, which possess unique physicochemical properties. When drug nanocrystals with excellent stability are obtained, the next step involves the fabrication of drug nanocrystal interfaces to facilitate the delivery of drugs to specific organs or cells, thereby enhancing treatment efficacy. In summary, nanocrystals possess unique characteristics that enhance the solubility of hydrophobic drugs and boost their bioavailability. Modifying the surface of drug nanocrystals can further enhance their chemical and physical stability, as well as facilitate targeted delivery to organs or cells.

The presence of surface ligands allows for the manipulation of nanocrystal aggregate and nanosuspension stability. Once NC comes into contact with a suitable solvent containing a specific ligand, they are exposed to a suitable solvent for a specific type of surface ligand and disperse in the medium. Flocculation of NCs can be induced by reducing solvent quality, such as by altering its polarity.[18] Colloidal stabilization may be achieved through two primary mechanisms: electrostatic and steric. These mechanisms work together to stabilize the dispersions of nanoparticles in different types of solvent media. Figure 3 provides a thorough summary of the stability concerns related to nanosuspension, along with the principal mechanisms used for stabilization.[70]

In the case of steric stabilization, including nanoparticles that have been covered with tethered hydrocarbon molecules, a good solvent is characterized by a negative free energy of chain-solvent mixing. This phenomenon leads to the repulsion of these stabilizer chains, which in turn discourages the overlap of ligand coronas and helps stabilize the dispersions of NC. Alternatively, when placed in a medium environment with positive chain-solvent mixing energy, the tendency to reduce interaction with the liquid that surrounds prompts the ligand chains to compress, leading to the aggregation of dispersed NC.[70] Typically, nonpolar liquids such as hexane, toluene, and chloroform are considered good solvents for hydrocarbon-capped NC. On the other hand, polar substances like ethanol, acetone, and acetonitrile are classified as non-solvents in this context.[18] Molecular dynamics studies and X-ray scattering experiments were performed to explore the behavior of stabilized NC under different solvent conditions and capping ligand lengths.[71,72] In general, the pair potentials for nanoparticles in a good solvent are primarily repulsive. However, in vacuum or non-solvent conditions, an attractive force becomes dominant.[18]

Colloidal nanocrystals can also be electrostatically stabilized by adsorbing charged substances onto the drug surface. In such a scenario, the electric charge of the NC is counterbalanced by counterions that have opposite charges. These counterions are dispersed around the drug particle. Effective solvents for electrostatic stabilization are typically solvents with a high dielectric constant, ε. This is important because a high dielectric constant facilitates efficient shielding of the electrostatic attraction between ions of opposing charges. For instance, formamide, with a dielectric constant of approximately 110, is an example of a good solvent for this purpose. The close proximity of electrostatically stabilized nanoparticles is limited in these solvents due to the increase in entropy associated with the aggregation of opposing ions in the diffuse double layer. The introduction of solvents with poor properties, such as toluene ($\varepsilon \approx 2$), causes the dispersed counterion cloud to collapse and the flocculation of NC to occur.[18] The Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory may be used to examine the possible association between a pair of charge-stabilized nanoparticles. This theory considers both the repulsion induced by the double layer and the attraction owing to van der Waals interactions between their cores.[73,74] Apart from the formulation perspective, the charge stabilizer may be utilized to specifically target telomere DNA. The atomic-level interaction between anionic 3,4′,4″,4‴-tetrasulfonic acid (APC) and human hybrid (3 + 1) G-quadruplex (G4) was examined for the first time. This anionic stabilizer suppresses telomere lengthening by telomerase in cancer by stabilizing telomere DNA. These findings contribute novel insights to the development of specific stabilizers that target telomere G4 in cancer.[75]

Ligand design in surface engineering

During the initial stages of drug nanocrystal development, both polymers and surfactants are frequently used to modify the surface of drug nanocrystals. The main purpose of this surface modification is to improve the stability of the drug nanocrystals. However, these drug nanocrystals still lack specific drug delivery abilities. Therefore, the development of functional targeting ligands in recent years has been used to engineer the surface of drug particles, thereby increasing the effectiveness of targeted delivery. In the field of biomedical applications, ligand design is based on three key principles: These principles include ensuring a strong attachment of hydrophilic molecules to the surface of the NC, effectively repelling proteins in both the cytosol and blood, and enabling controlled attachment of functional moieties that allow for sensing, targeting, or therapy.[18] As a way to improve the efficacy of drug nanocrystals in tumor therapy, a number of scientists have developed a significant amount of drug nanocrystals that have been modified with ligands. These enhanced drug nanocrystals have subsequently been assessed to determine the effectiveness of this drug delivery platform. Research findings indicate that the ligand present on the surface of drug nanocrystals has the potential to selectively bind to receptors that are excessively present on the surface of cancer cells. Consequently, this targeted interaction leads to an augmentation of anti-tumor efficacy. Various forms of ligands, including antibodies, proteins polypeptides, small molecules, polysaccharides, and cell membranes, have been used so far for the purpose of specific drug delivery to tumors.[22,76–80] Figure 4 illustrates a full overview of the recently functionalized target unit applied with drug nanocrystals.

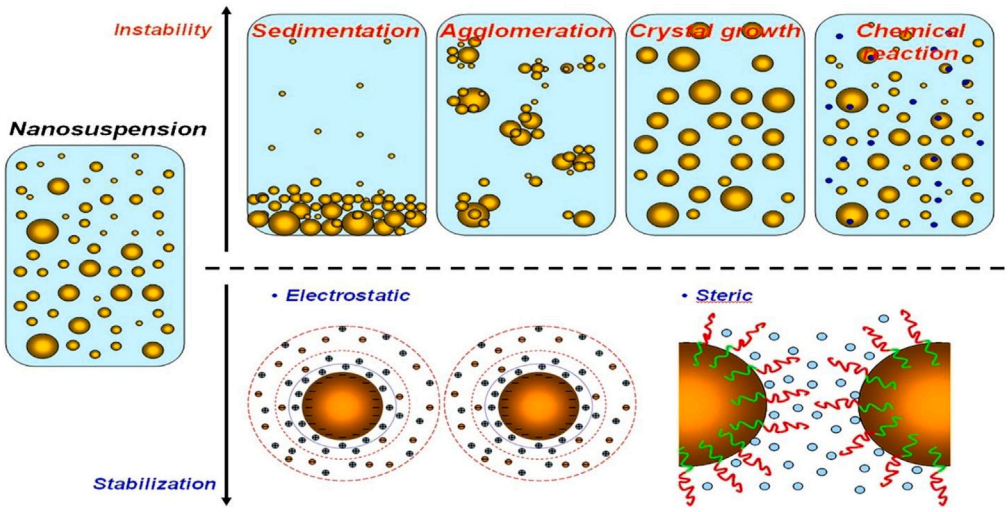

Figure 3. The summary of stability issues associated with nanosuspension and the main stabilization mechanisms
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Folic acid

Folate receptors (FR) have been identified as promising sites for the precise transport of therapeutic drugs and diagnostics utilizing folic acid (FA). This receptor is a membrane protein that is anchored by glycosylphosphatidylinositol (GPI). It is known to have high levels of expression in various kinds of cancer, including ovary, breast, liver, kidney, uterine, testis, brain, colon, and lung cancers. The presence of the folate receptor in normal tissues is somewhat limited, which makes it a valuable indicator for the accurate delivery of drugs to cancers.[81,82] Drug nanocrystals can additionally be encapsulated into liposomes that have a functionalized surface, including FA (Figure 5A). The morphology of paclitaxel nanocrystals modified with polyethylene glycol and FA for enhanced stability and target breast cancer cells is shown in Figure 5B. Liu et al. have invented a unique drug delivery platform by integrating the advantages of drug nanocrystals and polymeric nanoparticles. The study included the preparation of sorafenib core nanocrystals, which were then coated with a pH-responsive polymer that had been modified with FA. The artificially created nanovector effectively prevented the untimely release of therapeutic substances into the surrounding extracellular environment. Instead, it facilitated rapid dissolution, specifically in response to the intracellular acidification that occurs during internalization mediated by the folate receptor (Figure 5C).[22] The observed strategy demonstrated increased tumor accumulation and significantly improved *in vivo* antitumor activity, resulting in a high rate of tumor growth inhibition.[83] The researchers also used the SMMC-7721 liver cancer

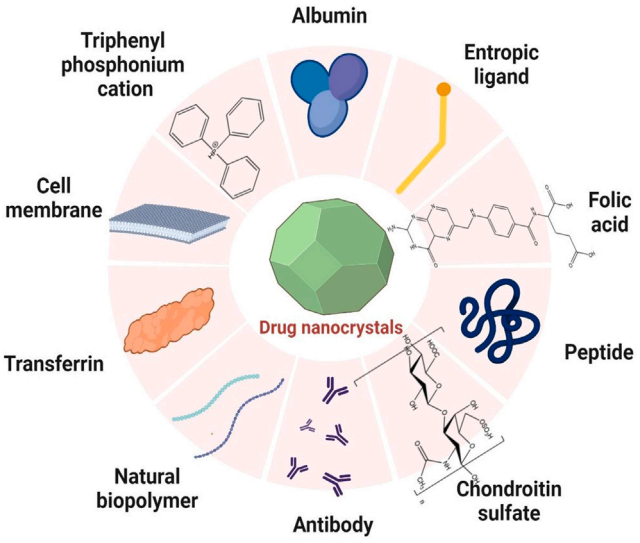

Figure 4. A summary of a recently applied functionalized target unit applied with drug nanocrystals
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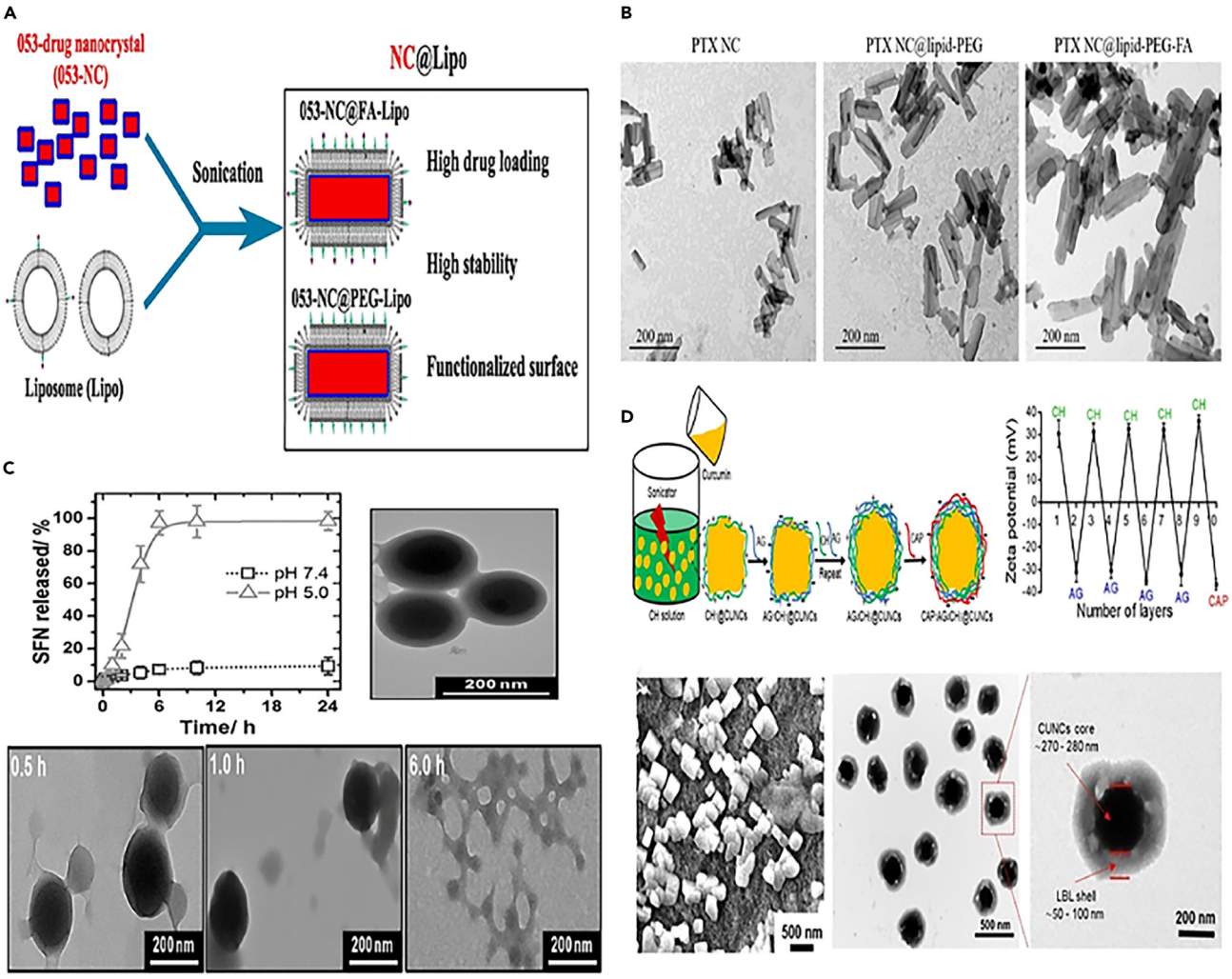

Figure 5. Ligand design focuses on targeted and controlled drug delivery

(A) Illustration presenting the engineering of drug nanocrystal@liposome (NC@Lipo) with folic acid to specifically target tumor cells. Reproduced with permission.[83] Copyright 2021, Elsevier.

(B) TEM images of paclitaxel nanocrystals modified with polyethylene glycol and folic acid for enhanced stability and target breast cancer cell. Scale bars: 200 nm. Reproduced with permission.[84] Copyright 2021, American Chemical Society.

(C) The drug release of sorafenib nanocrystals coated with folic acid conjugated spermine-functionalized acetalated dextran was studied at pH 7.4 and 5.0, and TEM image of these nanoparticles was taken after incubating at pH 7.4 (10% FBS, v/v) for 6 h. The morphology change of these particles was observed at pH 5.0 (10% FBS, v/v) during different incubation times (0.5, 1.0, and 6.0 h). Error bars represent the mean ± SD (n = 3). Scale bars: 200 nm. Reproduced with permission.[22] Copyright 2017, Wiley.

(D) Fabrication of core-shell curcumin nanocrystals, measurement of their zeta potential at various stages, and SEM and TEM images of these multilayer core-shell curcumin nanocrystals. Scale bars: 200 nm and 500 nm. Reproduced with permission.[85] Copyright 2020, American Chemical Society.

cell membrane, which had been functionalized with FA, to modify paclitaxel nanocrystals for the purpose of treating hepatoma. The results of the cell studies indicated that the drug delivery system exhibited effective targeting capabilities, as it was successfully internalized by SMMC-7721 cells and led to a considerable inhibition of their growth.[86] Interestingly, the folate receptor-β is also seen to be present on the surface of activated macrophages.[87] By using this particular concept, macrophages have the ability to transport drugs to endosomal cell compartments. The gradual release of a drug from macrophages enables the maintenance of sustained drug concentrations, hence influencing the long-term suppression of antiviral activities.[88]

Macrophages, which possess a high degree of mobility, are potentially recruited to locations of inflammation and infection, thereby facilitating the transportation of cellular payloads over physiological barriers. The stabilization of cabotegravir nanocrystals was achieved with the use of poloxamer 407 and FA. The observed particles exhibited a higher level of absorption by macrophages in comparison to the non-targeted particles. The pharmacokinetic analysis demonstrates an improvement in the duration of action for these specific antiviral drugs.[89]

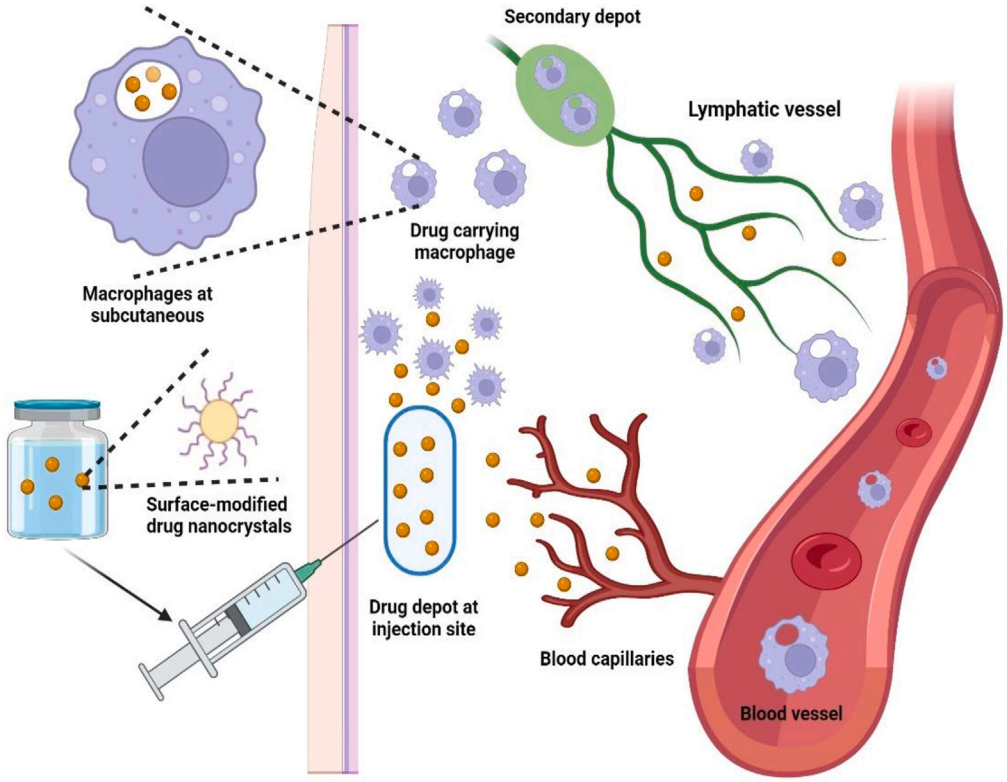

Figure 6. A mechanism of long-acting surface-modified drug nanocrystals
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Figure 6 illustrates the long-acting drug nanocrystal's mechanism. When modified drug nanosuspensions are injected intramuscularly or subcutaneously, the primary drug depot is formed at the injection site. Following this, some of the drug nanocrystals start to dissolve and completely absorb through the blood capillaries. Hence, the plasma drug concentration will rise and reach the therapeutic concentration. However, this mechanism alone cannot prolong the drug release. The immune response is the main mechanism for controlling drug release. The surface-modified drug nanocrystal will stimulate the immune response. The macrophages will move to the injection site. Then, these drug nanocrystals were uptaken by macrophages. Finally, these drug-carrying macrophages will move to the lymphatic system, leading to the creation of a secondary depot at the lymph node and gradually releasing the drug into the blood circulation. This mechanism will help to maintain the plasma drug concentration above the minimum therapeutic concentration for an extended period of time.[90]

Triphenylphosphonium

Mitochondria have become interesting targets for malignancy treatment, particularly in the case of tumors that exhibit multidrug resistance. Agents or delivery methods that specifically target mitochondria have the potential to restrict the pumping function of efflux transporters in cancer cells. This may be achieved by reducing the level of ATP inside the cell and diminishing the membrane potential of mitochondria.[91] One of the mitochondrial homing moieties that have been utilized for modifying drug nanocrystals is the triphenylphosphonium cations. The development of paclitaxel nanocrystals, which were modified with triphenylphosphonium cations conjugated with brij98, was successful. These modified nanoparticles showed a preference for accumulating in mitochondria, which resulted in an enhanced cytotoxic effect and proliferation suppression in MCF-7/ADR and MCF-7 cell lines.[76]

Entropic ligand

Another interesting ligand that has not yet been investigated for drug nanocrystals is the entropic ligand. Based on macroscopic measurements, it is suggested that entropic ligands can enhance the water solubility of the core nanocrystal. This is achieved by significantly decreasing the dissolution enthalpy and enabling the dominance of entropy in Gibbs free energy.[92,93] CdSe nanocrystals coated with various types of n-alkanoate ligands were studied. The findings indicated that CdSe-myristate complexes, measuring 4.7 nm in size, were found to be practically insoluble at room temperature. The solubility of these complexes is less than 0.001 mg/mL. When myristate is mixed with 25–75% of docosanoate, this complex's solubility increases by approximately 10^2–10^6.[92] Applying solid-state nuclear magnetic resonance (NMR) techniques can accurately analyze the atomic arrangement at the surface of nanocrystals and the interaction between ligands. According to the

investigations, the CdSe nanocrystal's mixed ligands of n-alkanoates separate in many areas, and their rotational flexibility is unlocked by a special arrangement. The mathematical approach, which relies on NMR-derived ligand partition and dynamics, accurately forecasts the greatly increased solubility of nanocrystal mixed ligand complexes compared to those with pure ligands.[94]

Hyaluronic acid

The scientific literature extensively reports the association between hyaluronic acid (HA) and the hyaluronan receptor, CD44. The binding of HA to CD44 receptors initiates intracellular signaling pathways that have an impact on cellular processes, including differentiation, migration, and proliferation. CD44 overexpression has been shown to be correlated with malignant angiogenesis and other forms of tumor growth. Consequently, they have been used as a specific functional unit for drug delivery in several nanomedicine applications, such as drug nanocrystals.[95–97] Lapatinib nanocrystals were synthesized using high-pressure homogenization, followed by a straightforward coating process using HA. The effectiveness of this intervention was assessed in an animal study. Results from this research indicate that the use of modified drug nanocrystals led to a notable retardation in tumor development and an overall improvement in the likelihood of animal survival. This can be attributed to the improved, precise delivery of the nanocrystals to the cancer cell and their prolonged presence in the body.[98] A coating process termed layer-by-layer coating, using the principle of charged interactions, has been used to create a multiple-layer structure consisting of an anticancer drug, cellulose nanocrystals (CNC), and targeting ligands. This drug design has been developed, particularly for the targeted delivery of drugs to tumor sites. At first, the CNC with a negative charge was coated with cationic doxorubicin and afterward enveloped with an anionic charge of HA. The animal study confirmed the observed high anti-proliferation effect and high cancer cell internalization in CD44 receptor-positive lung cancer of these particles.[99] It is noteworthy that ocular tissue likewise exhibited a significant presence of CD44 receptors. Consequently, the use of HA-coated drug nanocrystals may enhance the specificity of ocular targeting and improve cellular absorption.[100] The nanocrystals of acetazolamide (ACZ) were modified by incorporating HA. The researchers found that the nanosuspension was safe and well tolerated in the eyes of rabbits. The ocular delivery of spray-dried ACZ nanosuspension achieved a significantly higher reduction of intraocular pressure compared to ACZ solution.[101]

Chondroitin sulfate

Chondroitin sulfate is an anionic mucopolysaccharide known for its outstanding biocompatibility and minimal immunogenicity.[102] Chondroitin sulfate has the potential to undergo internalization inside cells through CD44 receptor-mediated endocytosis in a manner comparable to HA.[103] CD44 is often upregulated in several types of cancer, hence conferring them with a notable ability to selectively migrate toward and accumulate inside tumors.[104] A successful development was achieved by coating doxorubicin nanocrystals with chondroitin sulfate using a mix of hydrophobic and electrostatic interactions. When incubated with cancer cells or dispersed in hyaluronidase-containing media, these nanoparticles displayed enhanced drug-release behavior. They were efficiently internalized through malignant cells via CD44 receptor-mediated endocytosis; however, their internalization into normal cells was rare.[104] The study of paclitaxel nanocrystals coated with modified chondroitin sulfate also yielded similar results.[105]

Natural biopolymer

Researchers in the pharmaceutical and biotechnological fields have been studying and utilizing biopolymers derived from natural sources. Alginate-based microcapsules and microspheres are increasingly popular for the development of controlled drug release strategies due to their beneficial features, such as biodegradability, non-toxicity, and biocompatibility when administered *in vivo*.[106] In addition, alginate microspheres demonstrate pH-dependent swelling, which controls the release of drugs and is influenced by the pH sensitivity of the alginate component (Figure 5D).[107] The nanocrystals-in-microparticles technology was developed to enhance dissolution and oral hydrophobic drug absorption. Hydroxycamptothecin (HCPT) nanocrystals were encapsulated within a matrix of chitosan and alginate microparticles. X-ray diffraction analysis revealed that the crystallinity of these drugs in microparticles was identical to that of nanocrystals, indicating that the microparticle preparation did not cause any damage to the nanocrystal structure. The *in vitro* release studies demonstrated that the microparticles effectively shielded the nanocrystals from gastric acid, facilitating their release into the intestinal tract. In a rat model, the oral bioavailability of these particles showed a substantial improvement compared to bulk HCPT.[78] Furthermore, it has been demonstrated that glycol chitosan nanoparticles aggregate in tumors, indicating that this type of nanoparticle could serve as a useful starting point for further modifying drug nanocrystals.[108]

Transferrin

Transferrin (Tf), the glycoprotein found in blood plasma, plays a crucial role in transporting ferric ions (Fe^{3+}) via transferrin receptors (TfRs) located on the surface of the plasma membrane. Fe^{3+} is delivered by clathrin-mediated endocytosis, and these receptors are subsequently returned to the cell surface through recycling. They are expressed at higher levels in malignant cells as a result of the high demand for iron, especially for cancer cell activity. Additionally, it shows a direct relationship between the level of these receptors and the tumor proliferation rate.[109] Furthermore, it should be noted that the apical membranes of the small intestine epithelial cells show an increased expression of TfRs. This upregulation serves the purpose of facilitating the active transportation of various nutrients, substrates, and ligands.[110,111] Interestingly, Tf has the ability to adsorb onto hydrophobic surfaces in order to stabilize nanocrystals. Furthermore, it exerts steric stabilization on the clustering and enlargement of drug nanocrystals, thereby preventing crystal growth and maintaining their stability for prolonged circulation in the

bloodstream.[112] Ying Lu et al. have prepared paclitaxel nanocrystals that are stabilized by the serum protein Tf. The results showed that the formula consisted of roughly 55–60% of the medicine and maintained its stability for a minimum of 3 months when stored at a temperature of 4°C. The antitumor activity conducted on mice injected with KB cells showed that the paclitaxel-transferrin formulation had a considerably higher rate of tumor suppression, reaching 45.1%, compared to the 28.8% rate observed with paclitaxel nanosuspension treatment alone.[112] In a work conducted by Jin Seok Choi, docetaxel (DTX) nanocrystals were prepared and then modified using Tf. The findings indicated that Tf-DTX-NC and DTX-NC demonstrated a fast release of the drug, but DTX in its pure form displayed a slower release. The Tf-DTX-NC exhibited more cellular uptake compared to the DTX-NC, as shown by confocal microscopy and quantitative investigations. Furthermore, after applying 100 μg/mL of DTX, the cytotoxicity of Tf-DTX-NC (82.6% ± 0.8%) was found to be greater than that of DTX-NC (77.4% ± 4.1%) and pure DTX (20.1% ± 4.6%) after a treatment period of 72 h.[113] Overexpressing the Tf receptor on the epithelium of the intestine is believed to provide a beneficial approach to enhancing the delivery of drugs that have a low absorption rate via the intestinal membrane. This concept was investigated through an *in situ* intestinal perfusion study conducted by Shidi Han et al. The study demonstrated that the absorption rate of paclitaxel nanocrystals coated with transferrin in the intestines was significantly higher in comparison with Taxol and unmodified paclitaxel nanocrystals.[111]

Albumin

Albumin is receiving significant attention as a prospective surface modification owing to its biocompatibility, low toxicity, enhanced physical stability, and capacity to mitigate phagocytosis activity in the reticuloendothelial system.[114–116] Albumin, the protein with the highest abundance in plasma, plays a vital function in enhancing the transendothelial movement of nutrients and medicines.[117] Albumin, being an endogenous protein with a prolonged half-life in circulation, has the ability to hinder the opsonization and phagocytosis of hydrophobic nanoparticles when attached to them.[118] Furthermore, a significant number of tumors exhibit a heightened reliance on them for the primary supply of nutrients and energy, resulting in an augmented ability to uptake them.[119] They have the ability to extravasate by both the paracellular route and the transcellular pathway, which is facilitated by the presence of gp60 (also known as albondin).[117] In regard to cancer, it has been shown that albumin has the ability to interact with SPARC (a secreted protein that is acidic and rich in cysteine), which is present in different malignant cells and the surrounding cancer interstitium.[120,121] Depending on these characteristics, albumin modification will assist NC in avoiding MPS absorption, translocating through the cancer cell membrane, and remaining in cancer. The development of albumin-coated paclitaxel nanocrystals was undertaken by Joonyoung Park. The findings indicate that the drug delivery platform has the potential to inhibit phagocytosis by decreasing uptake by J774A.1 macrophages and increasing uptake by SPARC-positive B16F10 melanoma cells. Additionally, the experimental formulation demonstrated superior performance compared to Abraxane, a commercially available nanoparticle formulation of paclitaxel that utilizes albumin as a carrier, in a B16-F10 melanoma model.[122] The research conducted by Ji Eun Park also yielded a similar outcome. Carfilzomib (CFZ) is used as an anticancer drug and then coated with albumin. The findings indicate that there was an observed enhancement in metabolic stability as well as better cytotoxic effects, especially in breast carcinoma.[115] However, its effectiveness in treating tumors remained less than optimal. One possible approach to addressing this issue is to decrease the size of particles. By reducing the drug nanocrystal's size, their distribution in the reticuloendothelial system (RES) can be decreased while selectively increasing tumor cell uptake. The CFZ nanocrystals coated with albumin, specifically those with a size of 168 nm (NC168), demonstrated enhanced uptake by carcinoma cells, reduced phargocytosis by macrophages, and reduced immune cell toxicity compared to the CFZ nanocrystals coated with albumin with a size of 325 nm (NC325). The size of CFZ nanocrystals in mice influenced both their biodistribution and chemotherapy efficacy. In the breast tumor model, NC168 demonstrated higher antitumor efficacy and tumor accumulation compared to NC325. However, NC168 exhibited lower RES accumulation. The findings of this research offer evidence supporting the idea that formulating drug nanocrystals with an appropriate size may enhance the curative effectiveness of CFZ in treating cancer.[123] The mouse model of B16F10 melanoma revealed the observed advantage of the biodistribution and pharmacokinetic profiles of paclitaxel-coated albumin (Cim-F-alb). The results indicate that Cim-F-alb has a greater accumulation of PTX in cancer cells and provides a longer plasma drug half-life compared to Abraxane, with approximately 1.5-fold and 4.6-fold increases as well. The analysis using biolayer interferometry shows that it has a lower level of serum protein interaction compared to those without further surface modification. This suggests that the albumin coating on the surface provides protection against protein opsonization during the early deposition phase.[118]

Peptides

Peptides have shown efficacy as targeted ligands. The effective design of this drug delivery approach relies on the binding affinity of the proteins that are overexpressed in the targeted areas. An example of this is the RGD and VAP peptides. For RGD peptide, the overexpression of the integrin adhesion molecule αvβ3 in cancer angiogenesis and cancer cells is of paramount importance in facilitating tumor progression.[124] Tumor cell efficiency may be enhanced by the internalization of drug nanocrystals that are modified with RGD peptides, facilitated by the process of receptor-mediated endocytosis. To prepare paclitaxel nanocrystals for PEGylation and RGD peptide conjugation, polydopamine was applied to their surfaces. These modified nanocrystals outperformed those without RGD modification in lung cancer cell line A549 growth inhibition and cellular uptake studies. In the tumor-bearing mice, these nanocrystals increased intratumor accumulation and slowed tumor development.[77] The stable peptide VAP, which is made from 7 D-amino acids, has a high affinity for the overexpressed GRP78 protein seen in tumors, including glioma cells, neovasculature, and the blood-brain tumor barrier (BBTB), but not in normal cells.[125] A novel formulation consisting of cabazitaxel nanocrystals encapsulated inside liposomes, which have been modified with VAP, has been successfully developed. The nanoparticles have shown exceptional capacity to target gliomas, pass through barriers, and penetrate tumor spheroids.[126]

Interestingly, a recent study has shown that drug nanocrystals coated with polycatecholamine exhibit increased liver uptake and rapid clearance. Despite achieving high grafting densities of PEG, the fast clearance was linked to a higher adsorption of coagulation-related proteins to these modified drug surfaces, with fibrinogen becoming the predominant protein. The interaction of coagulation proteins with them brings attention to one of the main issues associated with using these peptides for drug delivery. However, it could offer valuable information for the use of those substances for hemostatic purposes.[127]

Antibodies

Extensive research and development efforts have been dedicated to the investigation of nanoparticles coupled with antibodies.[128] This platform exhibits distinctive characteristics, including precise targeting ability, extended retention inside tumors, higher drug bioavailability, improved solubilization of drugs in tumors, and fewer non-specific adverse effects associated with chemotherapy.[129] Drug nanocrystals were successfully modified with humanized monoclonal antibodies, including herceptin.[79,130–132] Herceptin selectively binds to the HER2 receptor's extracellular p185 glycoprotein domain, which is overexpressed in HER2-positive breast cancer.[133] Paclitaxel nanocrystals, when coated with herceptin, demonstrate an increased propensity for binding and internalization of HER2-positive breast cancer cells. Then, increase cell growth inhibition.[79] The *in vitro* study also yielded comparable results for paclitaxel nanocrystals wrapped in cell membranes functionalized with herceptin. The efficacy of these particles in suppressing the growth of HER2-positive breast tumors and inducing apoptosis was shown by an *in vivo* study.[132]

Cell membrane

Recently, there has been outstanding progress in biomimetic cell membrane coating technologies. These advancements have mostly focused on the capacity of these coatings to effectively disguise nanoparticles as intrinsic components. As a result, the pharmacokinetic characteristics of nanoparticles in blood circulation have been greatly enhanced.[134] The use of this technique was also implemented in drug nanocrystals. Numerous researchers have used diverse biomimetic cell membranes to modify drug nanocrystals, including the erythrocyte membrane, cancer membrane, platelet membrane, and lipid membrane.[86,126,135–138] Due to the nature of the surface glycosyl groups and membrane proteins, red blood cell membrane coating could minimize nanoparticle immunorecognition, enhance drug circulation, and prevent RES uptake.[139] The modification of paclitaxel nanocrystals by the erythrocyte membrane was satisfactorily achieved. These particles exhibited enhanced uptake by tumor cells, increased cytotoxicity, and greater drug accumulation in cancer cells as compared to unmodified paclitaxel nanocrystals. In tumor-bearing mouse models, it was shown that they had the most efficacy in inhibiting tumor development while demonstrating lower levels of toxicity.[135] Coating drug nanocrystals with a cancer cell membrane has many benefits, including immune evasion and specific binding, which are closely linked to the presence of plasma membrane proteins.[140] Enhancing the delivery efficiency might potentially be achieved by incorporating additional cancer-targeting ligands onto cancer cell membranes, thereby facilitating binding to tumor locations.[86,137] HepG2 malignant cell membrane-coated hypocrellin B nanocrystals, tailored with transferrin, were effectively developed and shown to have the ability to selectively transport a substantial quantity of poorly water-soluble medication into cancer regions with good safety performance. These nanocrystals outperform hypocrellin B nanoparticles and free hypocrellin B in terms of bioactivity and targeting of hepatocellular cancer.[137] Due to platelet-mimicking features, including preferential attachment to injured vasculatures and improved binding to platelet-adhering pathogens, platelet membrane has been extensively utilized in the design of several high-efficiency delivery systems.[141] A successful development was achieved in the fabrication of paclitaxel nanocrystals coated with a bio-mimetic platelet membrane, exhibiting great drug loading capacity, and targeted efficiency. In a model simulating post-surgery treatment, these particles demonstrated the ability to selectively transport substantial amounts of a hydrophobic drug to tumor locations, resulting in an increased animal survival rate. This improvement may be attributed to the particles targeting capability toward the coagulation cascade. By creating an artificial coagulation environment inside breast tumors in mice, this nanosystem may boost drug accumulation via a coagulation cascade mechanism.[138] The aim of surface-modifying drug nanocrystals with a lipid membrane was to reduce drug agglomeration and make them flexible for further engineering with an active targeting unit. This concept has been shown to be a promising method for enhancing stability and prolonging circulation time. The successful establishment of targeting liposome-encapsulated cabazitaxel nanocrystal has resulted in better anti-glioma efficacy, enhanced cancer penetration, improved transport across the barrier, and ultimately higher drug accumulation.[126]

APPLICATIONS IN TARGETED DELIVERY

Nanocrystals were developed for various *in vivo* applications, including targeted drug delivery, diagnosing disease's progression, and enhancing previously established medication efficacy. Surface-engineered nanocrystals have emerged as a highly promising class of materials for both therapeutic and diagnostic purposes.[18] For example, there has been a growing interest in the use of multimodal imaging-guided synergistic nanocrystals for the effective detection and treatment of cancer. Baochan Yang and his colleagues have developed multifunctional FePtMn nanocrystals. The nanocrystals are formed by chemically attaching the photosensitizer chlorin e6 (Ce6) and the targeted molecule FA to the surface of the nanocrystals. They exhibit high photothermal conversion efficiency when exposed to an 808 nm laser, which helps in the dual-ROS oxidation process to inhibit solid tumor growth. In addition, the release of Mn^{2+} from nanocrystal can improve the longitudinal relaxivity for T1-weighted magnetic resonance (MR) imaging and enhance the transverse relaxivity in synergy with Fe for T2-weighted MR imaging. This feature is particularly useful for diagnosing solid tumors. Fascinatingly, their fluorescent/photothermal (FL/PT) imaging ability can also precisely monitor tumor position.[142] This example highlights the potential for incorporating additional types of synergistic compounds,

such as targeting ligands, into nanocrystals. This expansion of nanocrystal-based drug delivery systems might enhance their applicability beyond just bioimaging agents.[143] In addition, nanocrystals have the potential to serve as a diagnostic tool for detecting viral infections. Researchers have chemically linked nanocrystals with certain antibodies or nucleic acid sequences that can recognize viral particles or genetic material. Upon encountering their target, these modified nanocrystals release distinctive signals, such as fluorescence or luminescence, which may be readily identified and measured.[36] For example, Huaibin Shen and colleagues invented photoluminescent (PL) reverse type-I ZnSe/CdSe nanocrystals and ZnSe/CdSe/CdS/CdxZn1—xS/ZnS multishell nanocrystals. These nanocrystals show promise in detecting hepatitis B surface antigen with enhanced sensitivity to the specific antigen.[144]

Active drug targeting, achieved through the attachment of specific targeting ligands onto the surface of drug nanocrystals, has been extensively validated as a promising strategy to improve therapeutic effectiveness and minimize toxicity. This is accomplished by precisely controlling drug delivery through specific binding to receptors that are highly expressed on the targeted site, followed by receptor-mediated endocytosis.

Surface-engineered drug nanocrystals has been applied to various clinical applications due to its unique properties, which can help drugs overcome the challenges of conventional drug formulations. Among the routes of administration, the oral route is the most prominent, as it is considered the safest and most convenient. By reducing the size of the drug to the nanoscale, nanocrystals primarily enhance bioavailability by increasing solubility and improving drug adhesion to the intestinal wall.[30] In addition to oral administration, other routes that may benefit from this technology include ophthalmic, intranasal, inhalation, transdermal, and parenteral routes.[145] The example of recent modified drug nanocrystals used in various routes of administration and preclinical evaluation is shown in Table 1.

Based on the findings analyzed in this review, drug nanocrystals have clearly contributed to improving the hydrophobic drug's formulation by enhancing their bioavailability. Moreover, drug nanocrystals have seen significant improvements through the use of surface modification techniques. The application of functionalized targeting ligands allows for the specific delivery of modified drug nanocrystals to various organs. Therefore, this study will provide a summary of the recent applications of modified drug nanocrystals for various targeted drug delivery systems. Figure 7 summarizes the latest applications of surface-modified drug nanocrystals in variety of drug delivery systems.

Surface-engineered drug nanocrystals for targeted cancer cells

The use of different ligands that specifically bind to receptors that are overexpressed by malignant cells is an attractive strategy to enhance the targeting of drug nanocrystals to cancer cells. The attachment of ligands to the surface of nanocrystals enables the targeted delivery of drugs to cancer cells through receptor-mediated endocytosis while minimizing accumulation at non-specific sites.

Brain cancer

Delivering drugs to the brain for treating brain cancer is particularly challenging due to the presence of the blood-brain barrier (BBB) and the BBTB. These barriers are specialized endothelial cell membranes located in the brain's microvessels.[150] The tight junctions among brain endothelial cells form a selective barrier, preventing a large number of blood-borne substances from entering the brain. The BBB, as a physical barrier, restricts the passage of pathogens or toxins, thereby complicating drug delivery.[27] Effective treatment of brain cancer requires that drugs penetrate the BBB to reach the brain in sufficient concentrations.[151] An innovative formulation of lipid membrane-coated cabazitazel nanocrystal with targeting ligands, including *p*-hydroxybenzoic acid (pHA) and VAP peptide, has been successfully developed. These modified drug nanocrystals may effectively traverse the BBB by utilizing a combination of VAP peptide, which exhibits a strong affinity for GRP78 protein overexpression on glioma cells, and pHA, which serves as a brain-targeting ligand, respectively. The findings suggested that these particles have the ability to pass through the BBB and BBTB, disrupt vasculogenic mimicry, and ultimately eliminate glioma cells, offering a comprehensive approach to glioma treatment, as shown in Figures 8A, 9A, and 9C.[126]

Skin cancer

Melanoma is a malignant skin cancer with a poor prognosis and a high risk of spreading in its early stages.[153] In a B16F10 melanoma mouse model, drug nanocrystals modified with albumin exhibited increased antitumor efficacy.[118,122] Albumin has the ability to bind to SPARC, which is expressed in different cancer cells, including skin cancer.[120] Therefore, albumin modification is expected to facilitate drug nanocrystal translocation across tumor endothelial cells and retention in cancer cells. The paclitaxel nanocrystal with albumin coating demonstrates a prolong half-life in plasma and higher drug deposition in tumors compared to Abraxane, a commercial albumin-based PTX nanoparticle product, by approximately 1.5 and 4.6 times, respectively. Biolayer interferometry analysis demonstrates that these modified drug nanocrystals have less interaction with serum proteins compared to naked nanocrystals suggesting that the albumin coating on the surface provides a protective effect against opsonization during the first deposition phase.[118] A similar result was observed in a study conducted by Joonyoung Park.[122]

Liver cancer

Liver cancer is a prevalent malignant tumor affecting people all over the world, and unfortunately, the 5-year survival rate is less than 10%.[154] Chemotherapy is a widely used approach for treating liver cancer.[155] Unfortunately, commonly used chemotherapy drugs such as paclitaxel are almost insoluble in water. This will reduce bioavailability and hinder clinical application. Therefore, improving solubility and targeting ability are crucial for optimizing therapeutic efficacy.[86] Paclitaxel nanocrystals modified with FA-functionalized SMMC-7721 liver cancer cells have been successfully developed. The cell and animal studies confirmed the excellent targeting capability of the modified drug nanocrystals. The

Table 1. The overview of recent modified drug nanocrystals used in various routes of administration and preclinical evaluation

Route of Administration	Drug	Preparation Method	Particle Size	Surface Modification	Preclinical Evaluation	Reference
Oral	Curcumin	Ultrasound-assisted antisolvent crystallization and layer-by-layer coating techniques	421 nm	Chitosan, sodium alginate, cellulose acetate phthalate	*In vivo* anti-inflammatory studies revealed that modified curcumin nanocrystals exhibit enhanced efficacy in alleviating inflammation-related symptoms in a mouse colitis model.	Oshi et al.[85]
Ophthalmic	Itraconazole	Media milling	378 nm	Pluronic F127	The *ex vivo* antifungal activity of modified itraconazole nanocrystals was significantly increased, as demonstrated by a reduction in the C. albicans population observed 48 h post-infection in a porcine eye model.	Permana et al.[146]
Intranasal	Harmine	High pressure homogenization and spray drying	179 nm	Vitamin E polyethylene glycol succinate, deacetylated gellan gum	The bioavailability of intranasal harmine nanocrystals in the brain was 25 fold higher than that of oral harmine nanocrystals.	Huang et al.[147]
Inhalation	Genistein	Media milling and thin film hydration	206 nm	Lecithin, cholesterol, stearamine, D-α-tocopherol polyethylene glycol 1000 succinate	*In vivo* study of lung retention and distribution revealed that more genistein was retained in the lung after administration of lipid-coated genistein nanocrystals.	He et al.[148]
Transdermal	Glibenclamide	Solvent -antisolvent precipitation	352 nm	Kolliphor HS15, collagen, chitosan	*In vivo* studies using a rat model of full-thickness wounds resulted in rapid closure, reduced inflammation, and enhanced wound healing, without causing scar formation.	Youssef et al.[149]
Parenteral	Cabazitaxel	Thin film hydration	140 nm	D-α-tocopherol polyethylene glycol 1000 succinate, lipid membrane, *p*-hydroxybenzoic acid, VAP peptides	*In vivo* studies indicated that surface-engineered cabazitaxel nanocrystals could cross both the blood-brain barrier and blood-brain tumor barrier, disrupt vasculogenic mimicry, and eventually eliminate glioma cells.	Wu et al.[126]

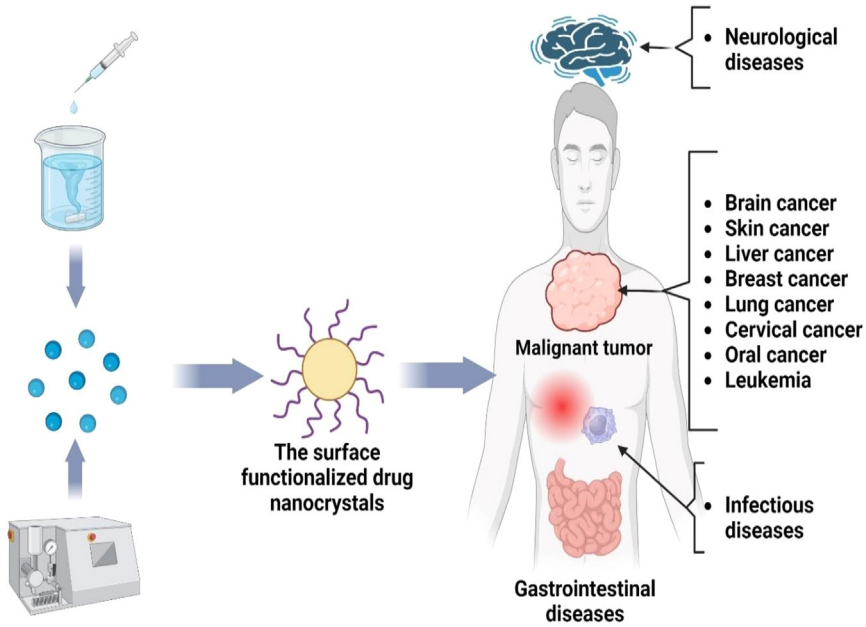

Figure 7. Application of the surface-modified drug nanocrystals in various drug delivery systems
Created by BioRender.com/Mahidol University.

internalized drug can suppress the growth of these cancer cells, as shown in Figures 8C, 9D, and 9E.[86] Comparable outcomes were observed when the drug was coated with chondroitin sulfate. The efficacy of paclitaxel coated with chondroitin sulfate in terms of internalization efficiency has been examined by cell and animal studies, indicating superior performance compared to unmodified paclitaxel nanocrystals.[105] Yan Liang successfully developed a nanocrystal formulation of doxorubicin modified with chondroitin sulfate. This targeted drug delivery offered stronger inhibition of cancer cells. The drugs demonstrated a high uptake capacity by cancer cells through CD44 receptor-mediated endocytosis, while normal fibroblasts showed little internalization.[104] Furthermore, the use of transferrin-modified cancer cell membrane to coat hypocrellin B nanocrystals exhibited exceptional targeting ability and treatment effectiveness in BALB/c nude mice with HepG2 tumors.[137]

Breast cancer

Breast cancer ranks second in terms of cancer-related mortality among women globally.[115] Throughout their lives, every eight to ten female has the possibility to develop breast cancer.[156] Chemotherapy remains a widely used treatment in clinical management for breast cancer.[157] Nevertheless, chemotherapy may not fully meet people's expectation due to its potential side effects. These can include non-specific toxicity, limited solubility, and challenges in targeted drug delivery.[158] Interestingly, drug nanocrystals modified with a targeting ligand have the potential to tackle these problems. For example, a new approach for breast cancer with HER2 overexpression has been developed using paclitaxel nanocrystals that are modified with herceptin-functionalized SK-BR-3 cell membrane. The anticancer studies conducted both *in vitro* and *in vivo* have revealed the potential to elevate apoptosis and inhibit proliferation of the HER2 breast cancer.[132] Furthermore, herceptin-functionalized paclitaxel nanocrystals exhibit increased binding affinity along with cell-specific internalization for HER2-positive breast cancer compared to unmodified nanocrystals, leading to improved cell growth inhibition.[79] Similar results were seen in the development of docetaxel nanocrystals surface-modified with herceptin for the treatment of breast cancer.[130] Drug nanocrystals functionalized with FA provide satisfactory results. Paclitaxel nanocrystals, decorated with FA, demonstrate enhanced ability to target breast cancer and effectively suppress tumor development.[84] A formulation of nanocrystals coated with albumin has been developed for the treatment of breast cancer. Albumin-coated carfilzomib nanocrystals exhibit significantly greater cytotoxicity compared to the commercial formulation when tested against breast cancer cells.[123] Ji Eun Park also reported a similar result when they developed albumin-coated carfilzomib nanocrystals.[115] However, it is important to highlight that the biodistribution and antitumor effect of these particles in mouse model varied depending on their size. In the 4T1 mouse model, it was observed that smaller particle sizes exhibited higher therapeutic ability against tumor and has elevated intra-tumoral accumulation level while also showing lower level of RES accumulation compared to bigger particle sizes.[123] A novel approach has been successfully developed for treating breast cancer, involving the coating of paclitaxel nanocrystals with erythrocytic and platelet membranes. In the 4T1 breast cancer model, they exhibit improved tumor cell internalization, enhanced cytotoxicity, as well as increased intra-tumoral drug accumulation.[135,138] Dongfei Liu has recently developed sorafenib nanocrystals that are coated with a pH-responsive polymer and conjugated with FA. This highlights the effectiveness of fabricated particles, which could protect drugs from premature exposure in the extracellular environment. These particles facilitate rapid dissolution exclusively due to intracellular acidification, which occurs following folate receptor-mediated internalization.[22] Drug nanocrystals coated with HA also yield satisfactory outcomes. Numerous studies have been conducted on drug nanocrystals that

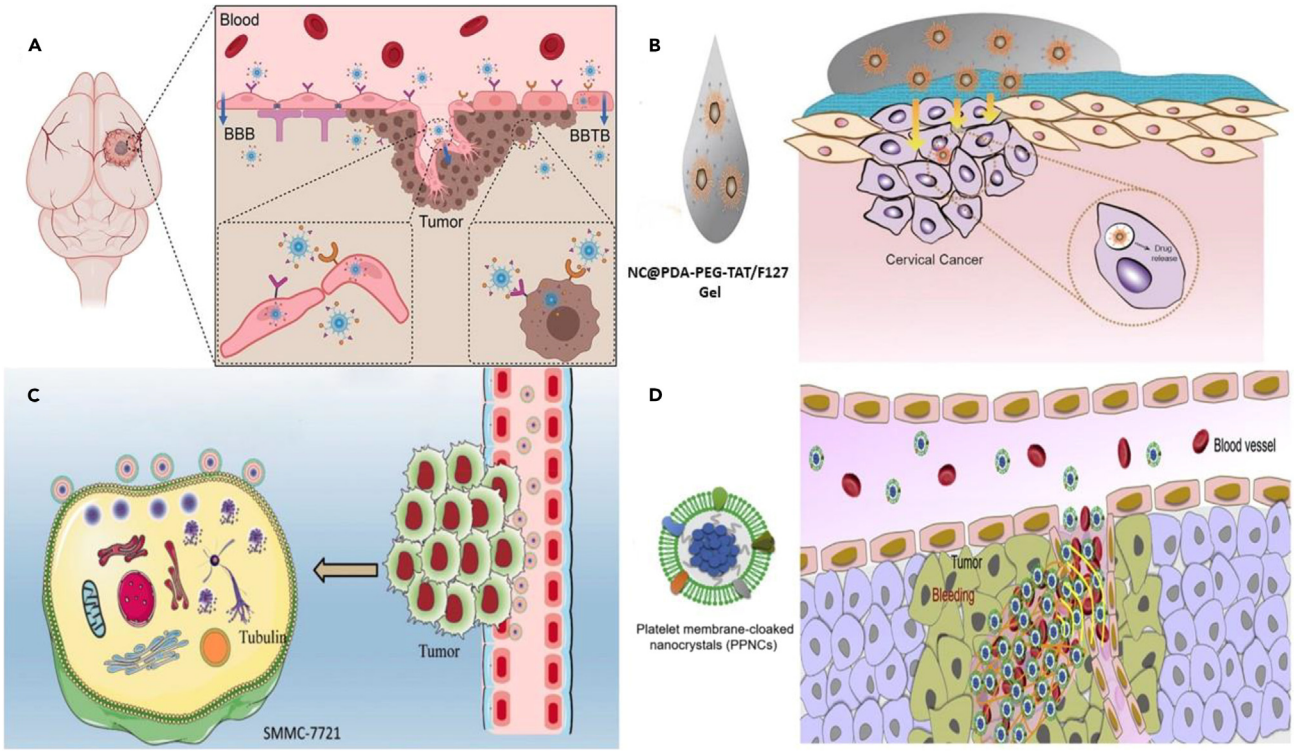

Figure 8. Updates on the latest advancements in surface engineering drug nanocrystals for targeted treatment of various malignant cancers

(A) Illustration of glioma-targeting using lipid membrane-coated cabazitazel nanocrystals with pHA and VAP peptide (pV-Lip/cNC) to cross BBB and BBTB, demolish VM, and target glioma cells. Reproduced with permission.[126] Copyright 2022, Elsevier.

(B) Schematic of paclitaxel nanocrystals modified with folic acid-functionalized SMMC-7721 liver cancer cell (FCPN) production and anticancer activity. Reproduced with permission.[152] Copyright 2021, Taylor&Francis.

(C) Preparation of functionalized docetaxel nanocrystals for the treatment of cervical cancer through intravaginal administration. Reproduced with permission.[86] Copyright 2021, Elsevier.

(D) Illustration showing the distribution of platelet membrane-cloaked paclitaxel-nanocrystals (PPNCs) to enhance the effectiveness of postoperative chemotherapy. Reproduced with permission.[138] Copyright 2020, Elsevier.

have been modified with HA. The results suggest that they have the potential to improve the effectiveness of anticancer treatment and could be a promising nanoplatform for delivering drugs to treat breast cancer.[159–162] The application of triphenylphosphonium cation-conjugated Brij 98 was used to modify paclitaxel nanocrystals. They showed the highest cytotoxic ability against MCF-7 and MCF-7/ADR cells, which was linked to reduced mitochondrial membrane potential. This technique shows potential for achieving successful clinical results in treating multidrug-resistant breast cancer.[76] Drug nanocrystals modified with proteins that target the tumor microenvironment were successfully developed for treating metastasis in lung cancer. The tumor microenvironment (TME) plays a crucial role in controlling metastasis.[163] The TME is mostly composed of non-cancer cells, extracellular matrix (ECM), blood vessels, and lymphatics, and it serves as a home for cancer cells.[164] Matrix metalloproteinases (MMPs) decompose the extracellular matrix (ECM), leading to the destruction of the TME and subsequent metastasis.[165] Thus, decreasing MMP expression and activity can prevent metastasis.[166] Paclitaxel nanocrystals stabilized with MMP-sensitive β-casein/marimastat (MATT) complexes have been successfully developed for metastatic breast cancer treatment. These drugs effectively treated metastatic cancer by inhibiting MMP expression and preventing lung metastasis and angiogenesis.[167]

Lung cancer

Lung cancer is a prevalent type of cancer worldwide, known for its significant impact on mortality rates.[168] Current therapeutic options, including surgery, radiation, immunotherapy, and chemotherapy, are insufficient.[169] Therefore, lung cancer-targeted drug nanocrystals were developed. Inhalable drug nanocrystals may tackle EPR-related challenges in lung cancer treatment through systemic nanomedicines.[168] The effectiveness of delivering drugs to the lungs by inhalation is significantly influenced by the physicochemical properties of nanocomposite formulations, including the polymer molecular weight and the surface charge.[168] A nanocomplex consisting of lactoferrin and chondroitin sulfate has been successfully developed for the dual delivery system using doxorubicin and ellagic acid nanocrystals for lung cancer treatment.[168] The nanocomplex showed remarkable cytotoxicity and was efficiently internalized by A549 cells through a mechanism involving transferrin and CD44 receptor-mediated endocytosis. This study showed that these particles improved the effectiveness of

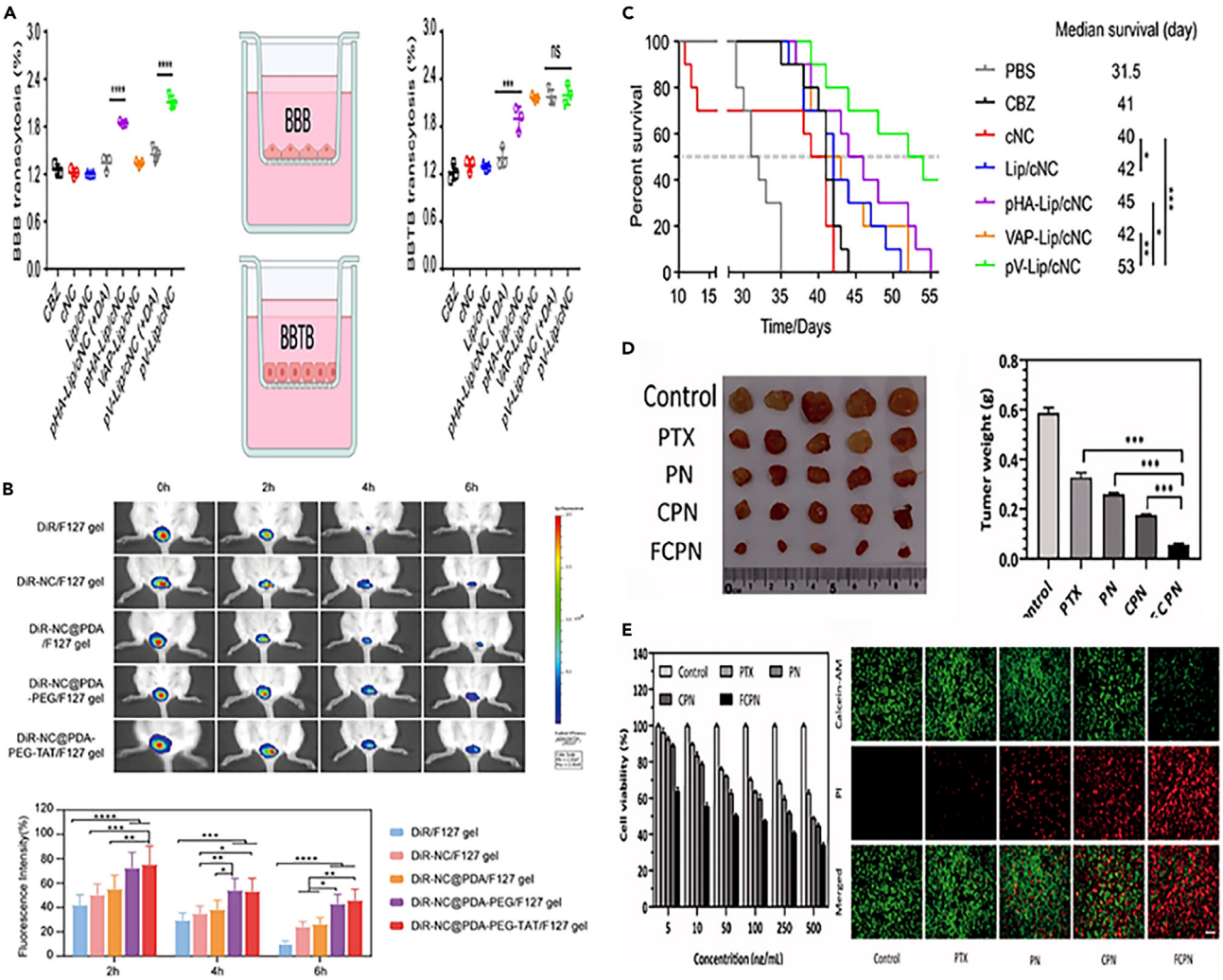

Figure 9. Enhancing the effectiveness of drug nanocrystals through surface engineering to specifically target different types of cancer cells

(A) Transcytosis efficiency of lipid membrane coated cabazitaxel nanocrystals in blood brain barrier or blood brain tumor barrier model after 2 h of incubation. Error bars represent the mean ± SD ($n = 3$). The statistically significant threshold was $p < 0.05$ (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).Reproduced with permission.[126] Copyright 2022, Elsevier.

(B) *In vivo* vaginal residence of DiR-labeled docetaxel nanocrystals modified with TAT protein in F127 gel after vaginal administration: typical fluorescent images and semi-quantitative fluorescent intensity. Error bars represent the mean ± SD ($n = 6$). The statistically significant threshold was $p < 0.05$ (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$) and $p < 0.01$ was considered highly significant. Reproduced with permission.[152] Copyright 2021, Elsevier.

(C) Anti-cancer effect of lipid membrane-coated cabazitaxel nanocrystals; Kaplan-Meier survival curves of mice bearing glioma ($n = 10$). The statistically significant threshold was $p < 0.05$ (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$). Reproduced with permission.[126] Copyright 2022, Elsevier.

(D) Tumor images and tumor weight after treatment with paclitaxel nanocrystals modified with a folate-functionalized liver cancer cell membrane. All data represent the mean ± SD ($n = 3$) (***$p < 0.001$). Reproduced with permission.[86] Copyright 2022, Taylor&Francis.

(E) The cell viability of SMMC-7721 cells after incubation with paclitaxel nanocrystals modified with liver cancer cell membrane and fluorescence images of live and dead cells stained with Calcein-AM/PI after treatments. All data represent the mean ± SD ($n = 3$).Reproduced with permission.[86] Copyright 2022, Taylor&Francis.

antitumor treatment in animals with lung cancer when compared to combined drugs delivered via intravenous and inhalation routes.[168] The positive charge of lactoferrin allows it to attach to negatively charged glycosaminoglycans on the cell membrane, leading to increased cellular uptake through charge-based interactions.[170] Additionally, chondroitin sulfate has the ability to bind HA CD44 receptors that are overexpressed on cancer cells, resulting in an effective tumor-homing property.[171] Hence, the combination of these polymers in a single nanocarrier can provide a highly effective approach for targeting lung cancer. Investigations were conducted on transferrin-coated docetaxel nanocrystals for lung cancer targeting. The findings showed a significant enhancement in the cellular uptake and cytotoxicity of docetaxel in the lung cancer cell line A549.[113] The drug nanocrystals, modified with polydopamine and a targeting peptide, have also been utilized for the lung cancer treatment. For example, camptothecin nanocrystals coated with polydopamine and conjugated with XQ1 peptide showed

significantly stronger anti-cancer activity compared to the free drug and naked drug nanocrystals. Additionally, they displayed a high level of selectivity, which can be attributed to their tumor target specificity. In animal studies, paclitaxel nanocrystals coated with polydopamine and conjugated with RGD peptide showed greater intratumor accumulation and slower tumor development than drug nanocrystals coated with polydopamine alone or a free drug.[77]

Cervical cancer

Cervical cancer has the fourth highest incidence and fatality rate among women globally.[172] Systemic chemotherapy is often correlated with poor intratumoral drug distribution and severe adverse effects.[173] There are significant challenges when it comes to administering formulations intravaginally, including inadequate intravaginal accumulation, transmucosal drug penetration, and targeted delivery. To tackle this problem, functionalized docetaxel nanocrystals were developed. Docetaxel nanocrystals were coated with polydopamine, which allowed TAT (trans activator of transcription) peptide modification and PEGylation. PEGylation will reduce mucus entrapment. Next, the functionalized docetaxel nanocrystals were subsequently dispersed in a thermosensitive gel containing poloxamer 407. This nanoformulation demonstrated extended intravaginal retention, improved mucosal penetration, and more potent inhibition to murine orthotopic cervical cancer growth compared to unmodified drug nanocrystals (Figure 9B).[152] Figure 8B illustrates the design of PEGylated docetaxel nanocrystals that have been modified with TAT through polydopamine coating, specifically for intravaginal administration.

Oral cancer

Oral squamous cell carcinoma is a frequently occurring cancer in the oral and maxillofacial cavity, with a 5-year survival rate of approximately 50%.[174] Aloe-emodin (AE) has the ability to potentially promote ferroptosis and function as a photosensitizer for particular surface diseases. However, it still has undesirable pharmacokinetic characteristics. Therefore, AE nanocrystals were developed by Mingbo Wu and then modified with ferritin and red blood cell membranes to treat oral squamous cell cancer. The findings demonstrated that they enhanced both the extended circulation and cancer-targeting capabilities. Both *in vitro* and *in vivo* results, clearly demonstrate the remarkable therapeutic effects achieved through the combination of photodynamic therapy and ferroptosis.[136] Furthermore, a novel treatment for oral cancer has been developed using paclitaxel nanocrystal stabilized with transferrin. The tumor inhibition rate of functionalized paclitaxel nanocrystals is significantly higher when compared to treatment with unmodified paclitaxel nanosuspension alone.[112] In both animal models and oral cancer patients, topical transmucosal cisplatin nanoparticles modified with chitosan can enhance an anti-tumor response.[175]

Leukemia

Chronic myeloid leukemia (CML) is a malignancy that occurs in the hematopoietic system and is characterized by clonal proliferation caused by a chromosomal translocation, resulting in the formation of the BCR-ABL fusion gene. The BCR-ABL1 protein has abnormally increased protein tyrosine kinase (PTK) activity, which phosphorylates downstream signal molecules and triggers biological events that contribute to CML pathogenesis.[176,177] A successful development has been made in treating CML by co-delivering realgar (As_4S_4) nanocrystals and imatinib (IMA). Realgar nanocrystals were coated with albumin-conjugated FA. Then, IMA (tyrosine kinase inhibitor) was loaded into the hydrophobic domains of albumin for co-delivery. After internalization into tumor cells, they can suppress PTK activity through degradation and inhibition of BCR-ABL, leading to a strong anti-tumor effect via cell apoptosis. *In vivo*, this nanosystem effectively extended the circulatory half-life of IMA, increased tumor accumulation, and outperformed individual monotherapy of As_4S_4 and IMA.[178] Furthermore, a novel treatment for leukemia has been developed using CHMFL-ABL-053 (a hydrophobic drug candidate) nanocrystal coated with a FA-functionalized liposome. They showed a significant increase in tumor accumulation and demonstrated highly effective antitumor effects in K562 xenograft mice, remarkably resulting in an inhibition rate against tumor growth up to 98%.[83]

Application of surface-engineered drug nanocrystals for other diseases

Although almost functionalized targeting ligands are applied to the surface of drug nanocrystals for drug delivery to cancer cells, certain targeting ligands can be used to specifically target organs affected by other diseases or normal cells, based on the unique pathogenesis environment and specific receptors on the cell surface.[21]

HIV infection

The development of long-acting parenteral formulations, designed to improve drug pharmacokinetics and therapeutic efficacy, has been driven by the need for sustained treatment options for chronic diseases.[179] The application of the prodrug concept, combined with or independent of particle size reduction, has led to the successful preparation of long-acting drug nanocrystals.[8,180–183] Enhancing the surface of drug nanocrystals with a targeting ligand can further increase the long-term efficacy of these formulations. Ligand-targeted nanoparticles can achieve macrophage targeting by binding to specific cell receptors, enabling more effective drug delivery, and releasing drugs at the site of action. For instance, FA can be utilized as a ligand to specifically target folate receptor-β found on the surface of activated macrophages.[87] This approach facilitates the gradual release of the drug from macrophages, maintaining sustained drug levels that are beneficial for long-term medication treatment.[184] As an example, the cabotegravir nanocrystals modified with FA showed a higher uptake by macrophages compared to unmodified particles. The pharmacokinetic analysis revealed a substantial increase in the drug's half-life (Figure 10A).[89]

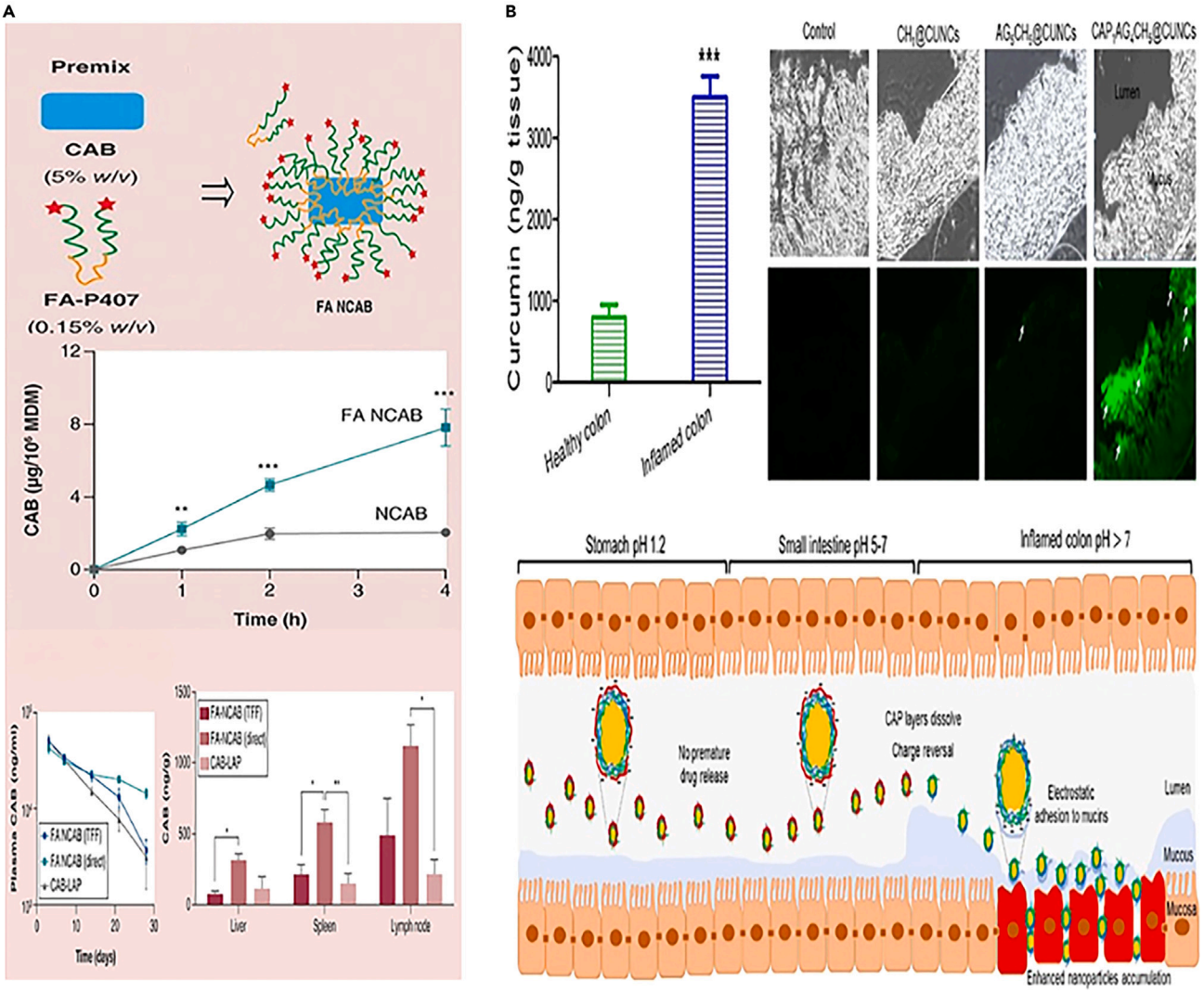

Figure 10. The surface modification of drug nanocrystals focuses on targeted drug delivery to organs or cells affected by disease.

(A) Monocyte-derived macrophage (MDM) uptake of cabotegravir decorated with folic acid-conjugated poloxamer 407 and pharmacokinetic and biodistribution profiles. Error bars represent the mean ± SD (n = 3). The statistically significant threshold was $p < 0.05$ (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$). Reproduced with permission.[89] Copyright 2018, Taylor&Francis.

(B) Illustrates the adhesion and accumulation of curcumin nanocrystals coated with pH-responsive polyelectrolyte in the inflamed colons of colitis mice following oral treatment. Data are represented as means ± SD (n = 6, ***$p < 0.001$) vs healthy colon. Reproduced with permission.[85] Copyright 2020, American Chemical Society.

Neurological diseases

Although there are efficient antipsychotic medications available for treating schizophrenia, more than 80% of patients who take these medications orally still have relapses. Miserably, the higher recurrence rate significantly leads to a 10% mortality rate by suicide among them.[185] The primary factor contributing to recurrence is the lack of adherence to oral treatment, mostly owing to its frequent dosing regimen. Hence, it is needed to find a novel and advanced drug delivery system to deal with this problem. Drug nanocrystal technology has been used to create extended-release nanoformulations by modifying the core drug with some stabilizers.[184] An investigation was conducted to examine the influence of commonly used additives for stabilizing nanosuspensions on the characteristics and progression of histopathological alterations in intramuscular tissue. The histopathology and immune histochemical analysis showed that there were signs of inflammation occurring at the intramuscular injection sites and the draining lymph nodes. These alterations varied depending on the kind of stabilizer used. While the general progression of inflammatory responses was comparable among each group, variations in the severity, and timing of the inflammatory response were found between animals injected with nanosuspensions containing poloxamer, TPGS, and polysorbate 80.[186] Paliperidone palmitate nanocrystals are an effective drug for schizophrenia management. For the formulation aspect, stabilizers, including polysorbate

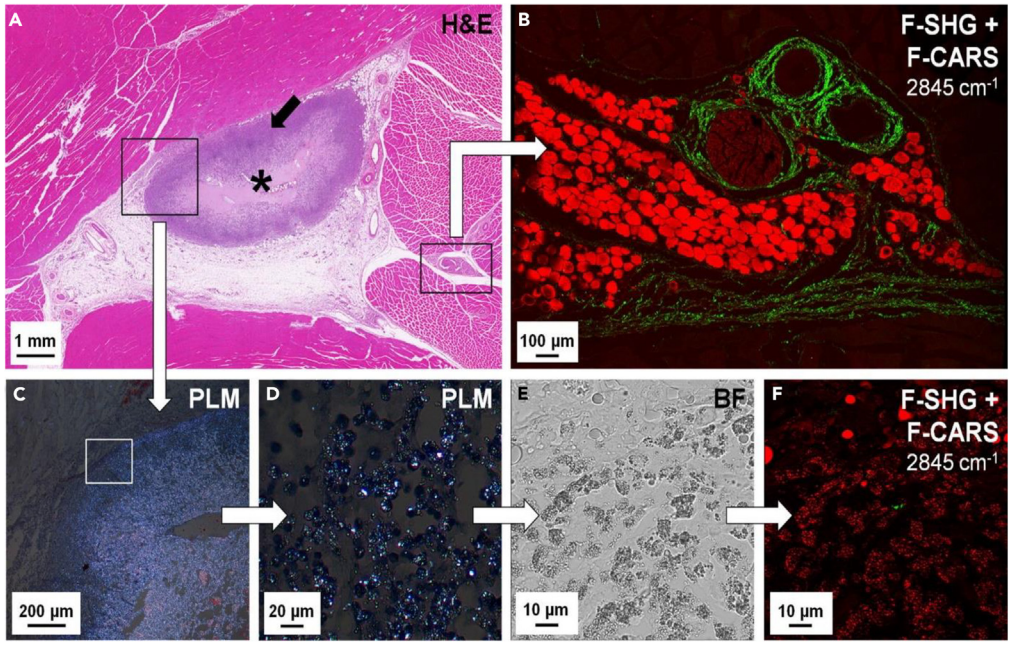

Figure 11. Linear vs. non-linear imaging of paliperlidone nanocrystals in a histological section

(A) BF micrograph of an H&E-stained cross section of the PP-NC administration site 7 days after i.m. injection in rats. The depot formed by the PP-NC is indicated with (*). The black arrow indicates macrophage infiltration (i.e., iatrogenic foreign body granuloma). Scale bars: 1 mm.

(B) F-CARS (2845 cm^{-1}; red) and F-SHG (green) merged micrographs of a detail of the adjacent unstained tissue section. Scale bars: 100 µm.

(C) Low and (D) high magnification PLM images of a detail of the adjacent unfixed cryosection showing birefringent PP-NC nano-/microcrystals. Scale bars: 20 µm and 200 µm.

(E) BF detailed micrograph of the infiltrating macrophages. Scale bars: 10 µm.

(F) Detail of F-CARS (2845 cm^{-1}; red) and F-SHG (green) merged micrograph of the similar section showing intracellular PP-NC nano-/microcrystals. Scale bars: 10 µm. Reproduced with permission.[188] Copyright 2015, Elsevier.

20 and polyethylene glycol 4000, were used in drug formulation to improve the physical stability of these drug particles.[187] However, these stabilizers can enhance the immune response when injected intramuscularly. This function of the stabilizer is very important for long-acting injectable drug development. Darville et al. investigate the relationship between paliperidone palmitate nanocrystals and macrophage cell cultures and histological sections using CARS microscopy.[188] The macrophages move to the site of injection and uptake these drug particles. The picture of the paliperidone palmitate nanocrystal disposition in unstained histological sections is shown in Figure 11.

Gastrointestinal diseases

Delivering drugs to the colon can be quite challenging due to the various barriers presented by the gastrointestinal tract. These barriers include the degradation of the drug in acidic pH conditions found in the lumen, the presence of digestive enzymes, and the physical adsorption by the mucosa.[189,190] To address this issue, nanocrystals modified with biopolymers have been developed. The alteration of the surface charge of the biopolymer can be utilized for colon-targeted therapy for ulcerative colitis. Curcumin nanocrystals were coated with pH-responsive polyelectrolyte shells made of chitosan, sodium alginate, and cellulose acetate phthalate. Confocal imaging of the colons showed that they tended to accumulate more in inflamed tissues compared to healthy tissues. Studies conducted on mice with colitis have shown that they have a greater ability to reduce inflammation and alleviate related symptoms.[85] As illustrated in Figure 10B, the fabrication of a shell for curcumin nanocrystals using polyelectrolytes may alter the surface charge. The surface charge changes from negative charge in the upper gastrointestinal tract (GIT) to positive charge in the colon, corresponding to changing pH depending on variations in pH.[85] During ulcerative colitis treatment, pH-triggered surface charge reversal of nanoparticles promotes adherence and accumulation in colon tissues with inflammation by interacting with negatively charged mucins in the mucosa.[191]

The application of drug nanocrystal technology to poorly soluble drugs significantly enhances drug delivery capacity, leading to various prominent clinical benefits, with the primary advantage being improved solubility and bioavailability. Apart from the reduced particle size, nanocrystals can be engineered to increase drug specificity to target sites, potentially reducing off-target effects. Furthermore, this technology allows for better flexibility in drug administration, as the physical and chemical properties of the drug are modified, enabling delivery through various routes. Currently, nanocrystals are being used to address various health issues, including multiple types of cancer, as well as diseases such as HIV infection, neurological disorders, and gastrointestinal diseases. Although surface-engineered drug nanocrystals have demonstrated significant promise in preclinical studies, additional evidence and application in clinical settings are still required due

to the limited amounts of studies involving modified drug nanocrystals in clinical trials. When it comes to translating drug nanocrystal-based formulations for patients, oral medications were the first to receive FDA approval in the early 2000s. Nevertheless, the next generation of surface-engineered drug nanocrystals will encounter several obstacles that restrict their application in clinical research. For example, (1) the industrial manufacture of drug nanocrystals with specific surface ligands, or their incorporation into complex dosage forms in large volumes may present certain challenges. (2) When considering stability, it is important to differentiate between dosage forms that contain water, like ophthalmic suspensions or topical gels, and solid formulations such as tablets with low moisture content. Due to certain factors, the latter are less likely to experience instability problems. (3) The inappropriate sterilization technique may lead to a potential problem with the physical and chemical stability. (4) Functional ligands are often modified onto the drug nanocrystal surface by physical adsorption, such as chitosan, albumin, and transferrin. Physical adsorption is a reversible process and susceptible to detachment, leading to the instability of drug nanocrystals in the blood circulation. (5) The inert surface of drug nanocrystals restricts the absorption of functional ligands, complicating the process of modifying multi-target ligands on drug nanocrystals and potentially hindering their clinical application.[10,17,25] Therefore, the preparation of drug nanocrystals with ligand stability and multifunctional surfaces through optimized formulation design should be more investigated. Further research of surface-engineered drug nanocrystals in clinical trials is necessary to validate the extrapolation of favorable outcomes and to establish the correlation between preclinical and clinical results.

CONCLUSION

There is much progress in the development of surface-engineered drug nanocrystals, which will accelerate the clinical translation of surface-engineered drug nanocrystals for targeted delivery. These progressions, including (1) the current methods used for preparing drug nanocrystals, including high-pressure homogenization, media milling, and microfluidic technology, provide precise control and simple production. This makes them suitable for large-scale drug nanocrystal manufacturing. Furthermore, innovative preparation methods such as the double microfluidic process can successfully prepare core drug nanocrystals modified with targeting ligands. (2) Applying targeting ligands, including antibodies, proteins, polypeptides, small molecules, polysaccharides, and cell membranes, to the drug nanocrystal surface was successfully developed. These engineer drug nanocrystals could precisely deliver drugs to the target site. (3) *In vivo* studies demonstrated that functionalized drug nanocrystals were successfully used for the treatment of various types of cancer and other diseases. (4) Surface-engineered drug nanocrystals that can target normal cells, including macrophages, can be applied in long-acting drug delivery systems. This highlights the potential of modified drug nanocrystals to effectively manage chronic infection diseases. Future development of drug nanocrystals with immunotherapy agents will help to overcome multidrug resistance in cancer therapy.

To date, the understanding of drug nanocrystals provides valuable tools for drug surface modification. Along with innovative experimental methods and advanced computational techniques, significant insights have been revealed for the investigation of their surface properties. Incorporating functional units for sensing, targeting, and drug delivery has marked the emergence of nanomedicines. Functionalized ligands on nanocrystals have opened up exciting possibilities, enabling more effective targeting of specific organs. Drug nanocrystals offer cost-effective choices for patients. They also expand the indication of conventional drugs that may be limited by their physiological properties. Therefore, the use of a drug nanocrystal platform can accelerate the process of discovering new drugs. Their flexible systems can be modified for various routes of administration. The high loading capacity is particularly essential for administration routes where only small amounts of formulations can be used, such as the intranasal cavity and IM injections. Ensuring the long-term stability of drug nanosuspensions with suitable stabilizers is essential for the development of nanocrystals. The surface modification of drug nanocrystals offers effective solutions for regulated drug release, increased retention, targeted drug delivery systems, enhanced cancer accumulation, and reduced toxicity.

Generally, the targeting ligands on drug nanocrystal surfaces have been widely used for cancer treatment. Specific functionalized drug nanocrystals can target macrophages, making them ideal for long-acting drug delivery systems. The development of long-acting drug nanocrystals brings significant challenges. Successful implementation holds great potential for effectively managing chronic diseases, including both infectious diseases and non-communicable diseases (NCDs). Immunotherapy has also garnered robust interest in cancer treatment. The co-delivery of chemotherapy with immunotherapy, particularly with immune checkpoint inhibitors, has been demonstrated to improve treatment outcomes. Therefore, surface engineering of drug nanocrystals with immunotherapy agents offers a promising approach to overcome multi-drug resistance in cancer therapy. Future advancements in the modification of drug nanocrystals will provide new opportunities for complex and chronic disease management, thereby broadening the applications of drug delivery systems.

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AUTHOR CONTRIBUTIONS

P.L. and M.Z. wrote the manuscript, L.J. and Y.J. play a pivotal role in the literature review, B.H. and Y.H. provided valuable insights into the discussion section, X.G. and J.Z. reviewed and polished the manuscript, M.Z. and X.-J.L. supervised the project, reviewed the manuscript, and provided financial support.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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