

Natural product-based nanomedicine: recent advances and issues

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Abstract: Natural products have been used in medicine for many years. Many top-selling pharmaceuticals are natural compounds or their derivatives. These plant- or microorganism-derived compounds have shown potential as therapeutic agents against cancer, microbial infection, inflammation, and other disease conditions. However, their success in clinical trials has been less impressive, partly due to the compounds' low bioavailability. The incorporation of nanoparticles into a delivery system for natural products would be a major advance in the efforts to increase their therapeutic effects. Recently, advances have been made showing that nanoparticles can significantly increase the bioavailability of natural products both in vitro and in vivo. Nanotechnology has demonstrated its capability to manipulate particles in order to target specific areas of the body and control the release of drugs. Although there are many benefits to applying nanotechnology for better delivery of natural products, it is not without issues. Drug targeting remains a challenge and potential nanoparticle toxicity needs to be further investigated, especially if these systems are to be used to treat chronic human diseases. This review aims to summarize recent progress in several key areas relevant to natural products in nanoparticle delivery systems for biomedical applications.

Keywords: natural products, nanomedicine, drug delivery, bioavailability, targeting, controlled release

Introduction

Natural products have been used as herbal medicines throughout human history. Today, approximately one-third of the top-selling pharmaceuticals are natural products or their derivatives.¹⁻³ Although many of the drugs used today come from natural products, major pharmaceutical companies have not paid due attention to these compounds for a variety of reasons. The lack of attention could be due to the outdated idea that natural products are only useful as antibiotics: natural products had huge success in the post-World War II era as antibiotics, and the two terms have become synonymous.¹ While large pharmaceutical companies have favored screening synthetic compound libraries for drug discovery, small companies have started to explore natural products' uses against cancer, microbial infection, inflammation, and other diseases.^{4,5} The biggest issue with the use of natural products in disease treatment is their low bioavailability, which has caused problems in clinical trials.⁵ Subjects taking curcumin orally, eg, required doses of 3.6 g/day to obtain serum levels of 11.1 nmol/L. Patients who received lower doses of curcumin did not have detectable plasma levels.^{6,7} Results are similar for other common natural products, such as polyphenols and flavonoids.^{8,9}

The use of nanotechnology has shown immense success in the field of drug delivery. The definition of a nanoparticle has been highly debated, and an internationally

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accepted definition has not been reached. Many sources define nanomaterials as particles of size ranging between 1 nm and 100 nm.¹⁰ The definition of a nanomaterial, however, is more complicated than simply size. The benefits of nanotechnology are due to the range of properties and interactions that are unique to the nanoscale structure.¹⁰ Thus, particles > 100 nm can exhibit these unique properties and can be considered nanomaterials. For example, polymer nanoparticles between 10 nm and 1,000 nm in diameter can have the characteristics desired for a successful delivery system.^{5,11} The most common types of nanoparticles used for drug delivery are polymer nanoparticles, solid lipid nanoparticles (SLNs), crystal nanoparticles, liposomes, micelles, and dendrimers (Figure 1A). Each of these nanoparticles has its own advantages and disadvantages as drug delivery vehicle.

Polymeric nanoparticles have been the most tested in combination with natural products. Poly(lactic-*co*-glycolic acid) (PLGA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), poly-L-lactic acid (PLA), polycaprolactone (PCL), and chitosan are the most common polymers used due to their biocompatibility, biodegradability, and the fact that they are easy to functionalize (Figure 1B). Chitosan itself is a natural polymer that has gained attention recently in applications with natural product delivery.^{12–18} There are two types of polymeric nanoparticles: nanocapsules and nanospheres (Figure 1C). Nanocapsules contain a drug-filled core, which is surrounded by a polymer membrane. The nanospheres are porous and the drug is uniformly distributed among the pores.¹⁹

Phospholipids comprise liposomes and some micelles (Figure 1A). Liposomes are composed of phospholipid bilayers similar to cell membranes, whereas micelles composed of phospholipids only have one layer in which the head group faces the outside and the hydrophobic tails form the micelle core in a hydrophilic environment, such as the blood. The type of nanoparticles used in a given delivery application can be selected based on the physicochemical properties of the drug of interest. For example, the liposomal aqueous compartment formed by the hydrophilic head groups of the phospholipids can contain one or more hydrophilic drugs. A lipophilic drug, however, is better suited for delivery with a micelle, in which the lipophilic tails of the phospholipids form the drug-containing compartment,²⁰ although liposomes have also been used in cases in which the lipophilic drug dissolves into the liposomal bilayer.²¹ Liposomes have been formulated to show no adverse or toxic effects on healthy cells.²² Adjustments can also be made to the liposome size, surface charge, and number of lamellae.⁵

SLNs contain a solid hydrophobic core surrounded by phospholipids (Figure 1A). These nanoparticles are a good choice for hydrophobic drug delivery. SLNs are more stable than liposomes and, in some cases, are less toxic than polymeric nanoparticles.¹⁹ To overcome some limitations in the old-generation SLNs, liquid lipid has been incorporated into the solid structure, resulting in nanostructured lipid carriers (Figure 1D). Three types of lipid nanoparticles have been described: an imperfect type, an amorphous type, and a multiple type.²³ The imperfect type contains spatially different lipids and allows for increased drug-loading capacity. The amorphous type mixes solid lipids with special lipids, such as medium-chain triglycerides, to prevent crystallization and drug expulsion during storage. The multiple-type nanoparticle has added liquid lipids that increase the solubility of many drugs and decrease drug expulsion during storage.

The use of nanotechnology with natural products is a rapidly developing field. Nanotechnology brings multiple advantages to the delivery of natural compounds in the treatment of cancer and other chronic human diseases. The incorporation of nanoparticles can increase the bioavailability, targeting, and controlled-release profiles of the natural products. To our knowledge, only a few reviews with limited scopes have been published on natural product-centered nanotechnology. Past reviews have focused on the use of nanoparticles with curcumin,^{22,24,25} flavonoids,¹⁹ traditional Chinese medicine,²⁶ and the synthesis and characterization of nanoparticles with natural products.⁵ In our review, we will address key aspects of natural product-based nanomedicine, including natural compounds, bioavailability, targeting, controlled release, and related challenges.

Natural compounds

Natural compounds, which are also called natural products, are complex chemical molecules found in plants and microorganisms. Some natural compounds have pharmacological or biological activities that provide therapeutic benefits in treating human diseases. Natural compounds have been studied and used for the treatment of cancer, infectious disease, and other various disease conditions in complementary and alternative medicine. This review will discuss a representative number of the most commonly studied natural compounds. This section will focus on natural compounds that have been studied in combination with nanoparticles and used as nanomedicine. In the past 30 years, the US Food and Drug Administration (FDA) and other regulatory agencies worldwide have approved ~61% of the developed

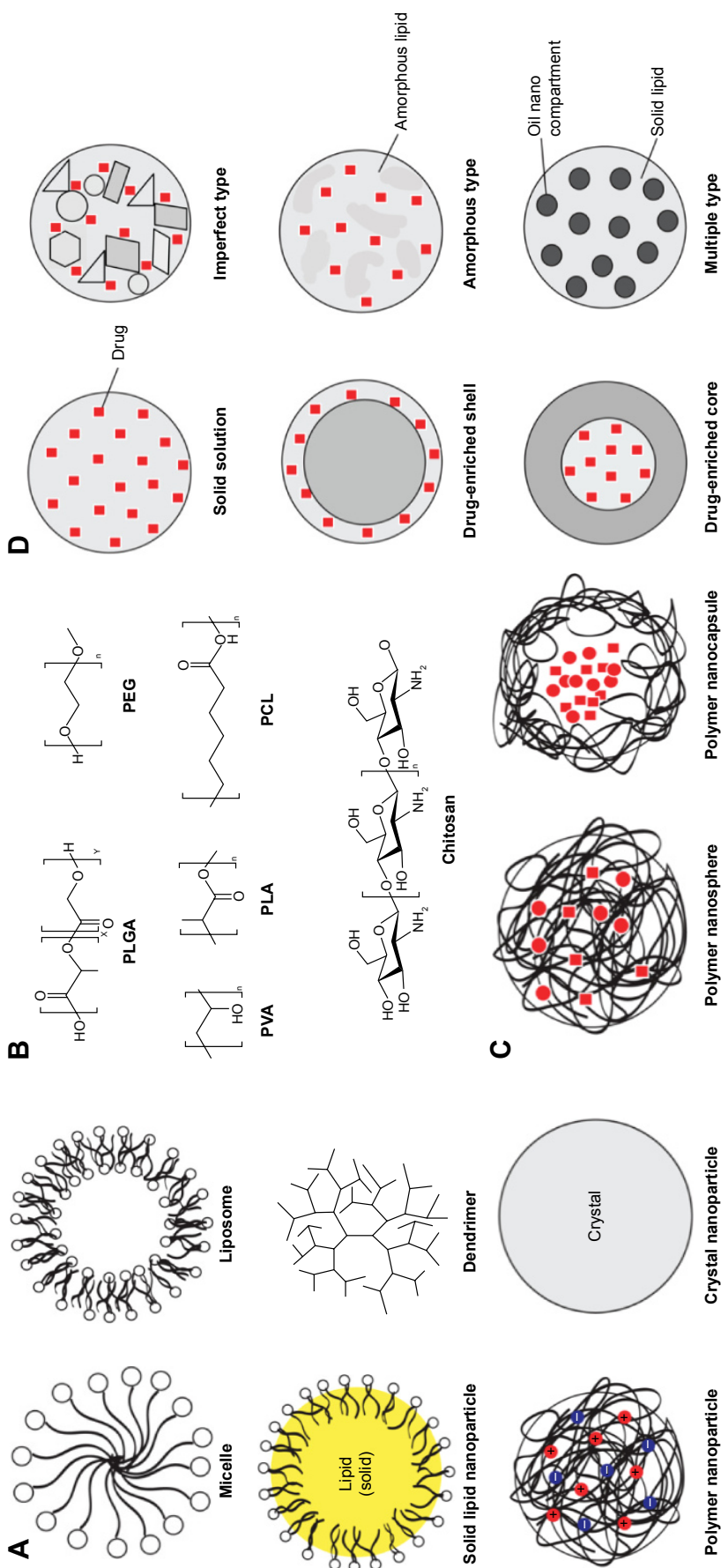


Figure 1 Schematic representations of nanoparticles.
Notes: (A) Graphical representations of nanoparticles. Charges in polymers are indicated as red and blue circles for some polymer nanoparticles. (B) Chemical structures of the most common types of polymers used in polymer nanoparticles. (C) Graphical representations of the two types of polymer nanoparticles. The drugs incorporated are shown in red. (D) Drug-incorporation models in solid lipid nanoparticles (left) and types of nanostructured carriers (right).
Abbreviations: PLGA, poly(lactic-co-glycolic acid); PEG, polyethylene glycol; PVA, polyvinyl alcohol; PLA, poly-L-lactic acid; PCL, polycaprolactone.

natural compounds to treat cancer and 49% of them to treat infections.²⁷ The mechanisms of action of these natural compounds will also be reviewed. Structures of selected natural compounds discussed in this review are shown in Figure 2. Relevant physicochemical properties of the selected compounds are listed in Table 1.

Application to cancer

The use of natural compounds for cancer treatment has been extensively studied. Some natural compounds combat cancer by the induction of tumor-suppressing autophagy. This mechanism of action has been identified for ~50 different natural compounds. The most recognizable compounds are curcumin and caffeine.^{31,32} A few studies observed that curcumin is able to induce autophagy, which is associated with cell death.^{33,34}

Xiao et al³⁵ found that curcumin induces autophagy in lung adenocarcinoma cells via the AMP-activated protein kinase signaling pathway but did not affect the healthy lung tissue. Caffeine has a different mechanism for inducing autophagy. Caffeine, with the addition of rapamycin, has been found to increase levels of autophagosomes by inhibiting the phosphoinositide 3-kinase, protein kinase B (also known as Akt), mammalian target of rapamycin (mTOR), and p70S6 kinase signaling. This is similar to the processes of several other anticancer agents, such as rapamycin, everolimus, and temsirolimus.³⁶⁻³⁸ Between 15 and 20 natural compounds have been shown to have cytoprotective autophagy characteristics. The most studied of these compounds is resveratrol, which has been shown to induce protective autophagy in both glioma and melanoma cells.³² Resveratrol has been shown to induce

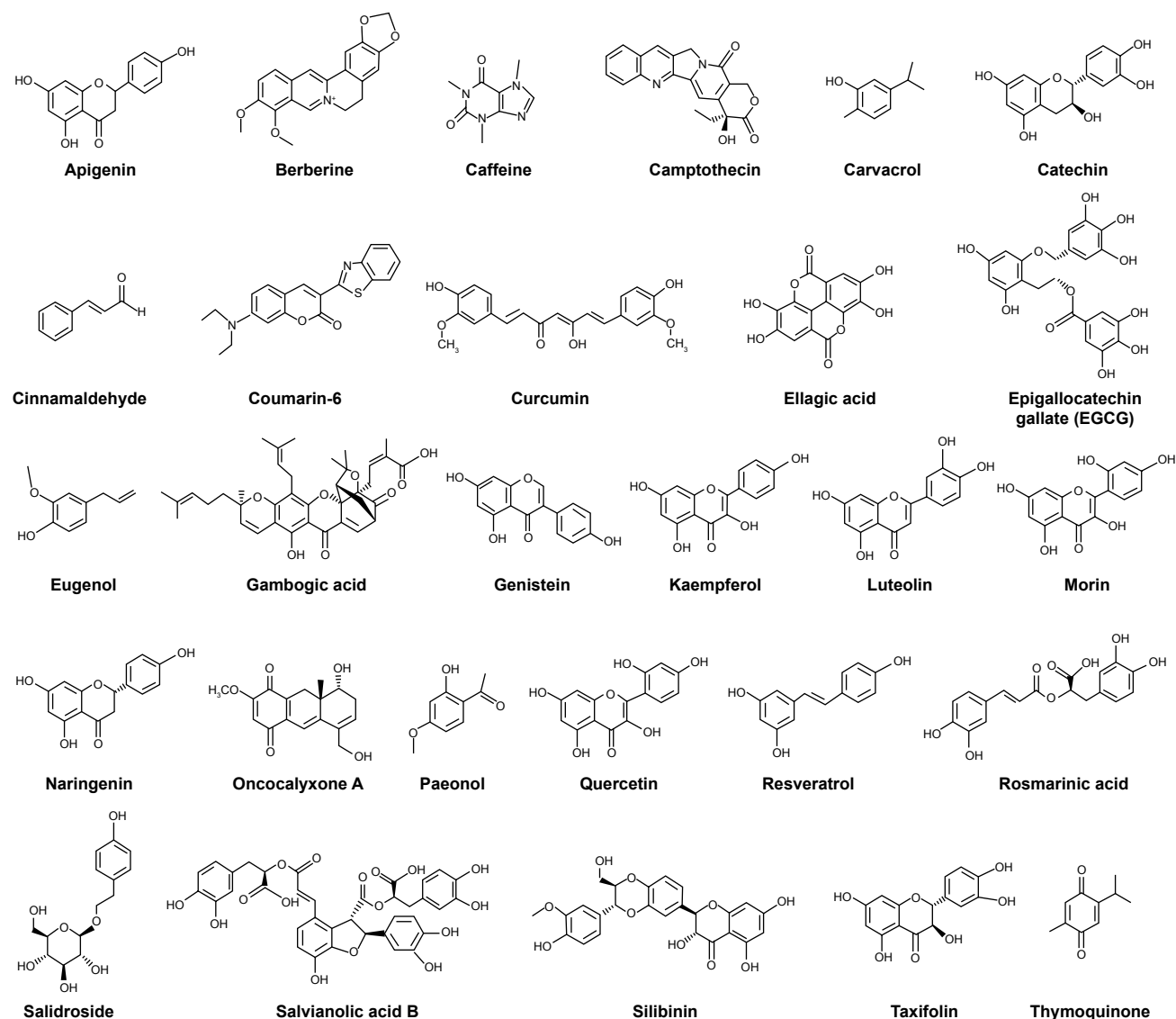


Figure 2 Chemical structures of selected natural compounds discussed in this review.

Table I Physicochemical properties of selected natural compounds^a

Natural compound	Partition coefficient (logP)	Polar surface area/molecular surface area (Å ²)
Apigenin	2.71	86.99/326.60
Baicalein	2.71	86.99/325.74
Berberine	-1.28	40.8/473.39
Caffeic acid	1.53	77.76/226.17
Caffeine	-0.55	58.44/269.15
Catechin	1.80	110.38/373.00
Cinnamaldehyde	1.98	17.07/194.07
Curcumin	4.12, 3.29 ^b	93.06/509.73
Epigallocatechin gallate	3.08	197.37/556.67
Ellagic acid	2.32	133.52/319.89
Epicatechin	1.80	110.38/373.01
Eugenol	2.61	29.46/257.78
Gambogic acid	7.78	119.36/906.97
Genistein	3.08, 3.04 ^c	86.99/325.45
6-Gingerol	3.62	66.76/507.44
Hydroxytyrosol	0.89	60.69/230.61
Kaempferol	2.46, 3.11 ^c	107.22/337.38
Luteolin	2.40	107.22/337.39
Morin	2.16	127.45/348.34
Naringenin	2.84, 2.6 ^c	86.99/351.06
Oleuropein	0.11	201.67/727.25
Paeonol	1.72	46.53/251.92
Quercetin	2.16, 1.82 ^c	127.45/348.11
Resveratrol	3.40	60.69/308.38
Rosmarinic acid	3.00	144.52/456.21
Salidroside	-0.58	119.61/426.44
Salvianolic acid B	pH dependent ^d	N/A
Silibinin	2.63	155.14/614.71
Tanshinone I	4.00	47.28/368.83
Taxifolin	1.82	127.45/367.80
Thymoquinone	2.55	34.14/245.97
Tyrosol	1.19	40.46/219.74
Ursolic acid	6.58	57.53/795.27

Notes: ^aLogP and surface area values are obtained from source <http://www.chemicalize.org> unless specified. ^bData from Grynkiewicz G, Ślifirski P. Curcumin and curcuminoids in quest for medicinal status. *Acta Biochim Pol.* 2012;59(2):201–212.²⁸ ^cData from Rothwell JA, Day AJ, Morgan MR. Experimental determination of octanol-water partition coefficients of quercetin and related flavonoids. *J Agric Food Chem.* 2005;53(11):4355–4360.²⁹ ^dData from Li J, Liu P, Liu JP, et al. Bioavailability and foam cells permeability enhancement of Salvianolic acid B pellets based on drug-phospholipids complex technique. *Eur J Pharm Biopharm.* 2013;83(1):76–86.³⁰
Abbreviation: N/A, not available.

protective autophagy through the ceramide/Akt/mTOR pathway in melanoma B16 cells.³⁹ Flavonoid extracts have also been shown to induce autophagy via caspase-dependent activation of signaling pathways by inhibiting sirtuin 1/p53-mediated mitochondrial and Akt pathways.⁴⁰

Natural compounds have also been observed to inhibit P-glycoprotein (P-gp) in cancer cells. P-gp receptors are responsible for pumping foreign materials out of cells. They are overexpressed in tumor cells, thus reducing the intracellular drug concentrations. Overexpression of P-gp in tumor cells can cause chemotherapeutic drugs to be expelled from targeted tissues, thus reducing the effectiveness of cancer treatments.⁴¹ Specific types of flavonoids,

coumarins, terpenoids, alkaloids, and saponins have been shown to have P-gp-inhibitory properties. Flavonoids and stilbenes have been shown to inhibit ATP-binding cassette transporters, such as P-gp, multidrug resistance-associated protein 1 (MRP1), and breast cancer resistance protein. Common flavonoids that have illustrated these properties are epigallocatechin gallate (EGCG), quercetin, genistein, morin, and kaempferol. EGCG has been shown to downregulate P-gp and breast cancer resistance protein, while quercetin decreases the expression of P-gp in gastric carcinoma cells.⁴² A combination of genistein, quercetin, morin, and kaempferol administered to Panc-1 cells has also resulted in a decrease in MRP1-mediated transport.⁴³

The effect of curcuminoids on the cell cycle of cancer cells has also been studied on lung cancer cell lines A549 and H460.⁴⁴ In both cell lines, the number of sub-G1 cells increased with increased amounts of curcuminoids, and the number of cells at the G0/G1 phase decreased. The addition of curcuminoids did not significantly affect the cells in the S phase. Only the highest concentration of curcuminoids caused an increase in the G2/M phase cells. The rise in G2/M phase cells could indicate cell arrest in this phase due to induction of apoptosis. Although both cell lines seem to be arrested in the G2/M phase, this could be cell line dependent. Previous studies have found that the breast cancer cell line MCF-7 is arrested in the G1 phase.

Application to bacterial infection

Most of the antibiotics we use today are natural products or their derivatives. While larger pharmaceutical companies have paid less effort to developing and screening natural antibiotics, this work has recently been conducted in smaller biotechnology companies. Three recent natural product-derived antibiotics that have been approved for use in the USA are: daptomycin, retapamulin, and fidaxomicin.⁴⁵

Other types of natural compounds, such as those discussed in the cancer section, are also being used as antimicrobial agents. Curcumin, cinnamaldehyde, eugenol, and carvacrol are compounds that have been identified as having antimicrobial characteristics.^{46,47} Cinnamaldehyde is isolated from cinnamon; eugenol is derived from cloves, oregano, cinnamon, basil, and bay leaves; and carvacrol is obtained from oregano. These compounds have not yet undergone extensive mechanistic studies. However, some studies have observed that the addition of essential oils from oregano and basil causes a disruption in the cell membrane of bacteria, which leads to cell death.⁴⁸ Other studies have found that cinnamaldehyde and eugenol are involved in the inhibition of cell wall synthesis.⁴⁹

Application to other conditions

Uses of natural compounds for other health conditions are expanding and have received growing attention. Some natural compounds used as anticancer agents also have anti-inflammatory characteristics. Compounds such as curcumin, quercetin, eugenol, rosmarinic acid, and kaempferol have anti-inflammatory properties. They suppress proinflammatory pathways, such as transcription factors NF-kappaB and AP-1, and cyclooxygenase-2, an enzyme responsible for inflammation.^{50,51} These compounds have been incorporated into nanoparticles in order to treat cancer, but they could also potentially be used to treat other inflammatory diseases, such as type 2 diabetes.

Many natural products have antioxidant properties that can be used for health benefits. Natural compounds such as quercetin, catechin, ellagic acid, *Merremia emarginata* extracts, curcumin, luteolin, and taxifolin are just some of the natural products that exhibit antioxidant properties.^{52–56} The mechanism by which these natural compounds obtain their antioxidant properties varies. Quercetin, catechin, curcumin, luteolin, and taxifolin all form phenoxyl radicals on exposure to free radicals in the body.^{57,58} Salvianolic acid B, a strong radical-scavenging compound, also has antioxidant properties.⁵⁹

Natural products have also shown promise in other disease-related applications. Berberine, a quaternary ammonium salt isolated from plants of the *Berberis* genus, has shown potential in the treatment of hepatosteatosis when incorporated into SLNs.⁶⁰ Berberine SLNs could treat hepatosteatosis by downregulating proteins important for lipogenesis, such as fatty acid synthase, stearoyl-coenzyme A desaturase, and sterol regulatory element-binding protein 1c. Thymoquinone, a compound isolated from *Nigella sativa*, has protective gastrointestinal properties. The mechanism behind these protective properties is free-radical binding. Free radicals are produced in excess after the consumption of ethanol and can lead to mucosal breakup and lesion formation. Thymoquinone's free-radical binding activity can protect the tissue and prevent the formation of ethanol-induced gastric ulcers.⁶¹ Finally, paeonol, which is found in the plant genus *Paeonia*, has protective action against ultraviolet B (UVB)-induced melanogenesis. Paeonol has been shown to inhibit tyrosinase, which is activated by UVB and produces melanin.⁶²

Bioavailability

Nanoparticles can improve the effectiveness of natural compounds in disease treatment and prevention by increasing their bioavailability. Many of the studied natural compounds, such as curcumin, resveratrol, and EGCG, are highly

lipophilic (Table 1). Highly lipophilic compounds are not ideal for drug delivery because they do not dissolve well in the bloodstream. These compounds have a low bioavailability, and therefore large quantities of the compounds must be administered in order to achieve the desired therapeutic effects. The large dose size of these compounds can lead to acute toxicity or low patient compliance.²⁰ Just encapsulating these highly lipophilic compounds can improve their water solubility and efficiency. Celia et al⁶³ have found that bergamot essential oil, which has anticancer properties, when encapsulated in liposomes, showed improved solubility of the drug and led to increased cell death in vitro. This was also true for nanoemulsified berberine. The nanoberberine was added to a phosphate buffer and in 45 minutes, 85% of the compound dissolved, compared to only 60% of the free berberine in the same time period.⁶⁴ Other classes of natural compounds, such as tannins and terpenoids, are highly hydrophilic. These compounds have low bioavailability because they cannot cross biological membranes.⁵ In both of these cases, incorporating the natural compound into a nanoparticle can improve the bioavailability and lower the dose needed to obtain a therapeutic effect.

Table 2 provides several examples of nanoparticle formulations and adjuvants that increase the bioavailability (drug concentration in plasma) of selected natural compounds. Curcumin, a diarylheptanoid derived from turmeric, has generated immense interest as a lead compound against a variety of health conditions, including cancer, inflammation, microbial infection, angiogenesis, amyloidosis, wound healing, and alleviation of morphine tolerance.^{70–73} However, poor bioavailability is a major limitation to the therapeutic utility of curcumin in clinical trials.⁷⁴ One animal study⁷⁵ found that when 1 g/kg of curcumin was orally administered, 75% of the compound was excreted through the feces. Recently, numerous animal studies have been performed with the goal of improving the bioavailability of this compound.^{53,66,67,76–79} Takahashi et al⁵³ orally administered liposome-encapsulated curcumin (LEC) nanoparticles to Sprague Dawley rats and measured their plasma curcumin levels. The levels in terms of area under the curve (AUC) values were measured for rats administered curcumin and LEC. The AUC values for the LEC were 4.96 times greater than those for curcumin.⁵³ Other liposome nanoparticles have also been shown to increase the bioavailability of curcumin.⁷⁶ Similar studies have also been done using SLNs.^{66,77} A pharmacokinetic study conducted with SLNs found that the bioavailability increases dramatically by 39-fold when 50 mg/kg of curcumin is administered in the lipid nanoparticle.⁶⁶ Polymer nanoparticles have also

Table 2 Comparison of plasma concentrations of natural compounds with the use of nanoparticles or adjuvants and in free drug form

Natural compound	Nanoparticle or adjuvant	Dose	Plasma concentration		Mixture (free drug mixed with empty nanoparticle)	References
			Encapsulated by nanoparticle (or with piperine)	Free drug		
Apigenin	Carbon nanopowder solid dispersion	60 mg/kg body weight	3.26 µg/mL	1.33 µg/mL	1.43 µg/mL	65
Curcumin	Liposome	100 mg/kg body weight	319.2 µg/L	64.6 µg/L	78.3 µg/L	53
Curcumin	Solid lipid nanoparticle	50 mg/kg body weight	14.29 µg/mL	0.292 µg/mL	N/A	66
Curcumin	PLGA nanoparticle	100 mg/kg body weight	6.75 µg/mL	1.55 µg/mL	N/A	67
Curcumin	Piperine as adjuvant in rats	2 g/kg curcumin and 20 mg/kg piperine body weight	1.8 µg/mL	1.35 µg/mL	N/A	68
Curcumin	Piperine as adjuvant in humans	2 g/kg curcumin and 20 mg/kg piperine body weight	0.006 µg/mL	0.18 µg/mL	N/A	68
EGCG	Piperine as adjuvant in mice	163.8 µmol/kg EGCG and 70.2 µmol/kg piperine body weight	0.66 µmol/L	0.32 µmol/L	N/A	69
Taxifolin	Nanoparticles by liquid antisolvent precipitation	50 mg/kg body weight	13.5 ng/mL	1.3 ng/mL	N/A	56

Abbreviations: EGCG, epigallocatechin gallate; N/A, not available; PLGA, poly(lactic-co-glycolic acid).

been shown to increase the bioavailability of curcumin.^{67,78,79} In a pharmacokinetic study,⁶⁷ curcumin-loaded PLGA nanoparticles increased the relative oral bioavailability by 563%, compared to free curcumin bioavailability. Other less-common types of nanoparticle delivery systems have also been found to increase the bioavailability of curcumin. Wet milling techniques along with crystallization have produced nanosized curcumin particles that have higher bioavailability than the powder counterparts.^{80,81}

The mechanism behind PLGA nanoparticles increasing the bioavailability of curcumin has been investigated.⁶⁷ Xie et al⁶⁷ hypothesized that the increase in bioavailability of curcumin was due to the inhibition of P-gp-mediated efflux. P-gp is abundant in the intestinal membrane. The authors tested this hypothesis by adding verapamil (VRP), a P-gp inhibitor, to curcumin or PLGA–curcumin nanoparticles. After 120 minutes of treatment, the remaining curcumin ratio (RCR) in the jejunum was measured. Significant differences in the RCR were observed for the treatments with plain curcumin versus PLGA–curcumin nanoparticles, either with or without VRP. There was no significant difference in the RCR of curcumin with VRP and the PLGA–curcumin nanoparticles without VRP. These data led the authors to conclude that the PLGA nanoparticles inhibit P-gp, which allows increased drug permeability and bioavailability. In a different study, Shen et al⁷³ compared the attenuation of morphine tolerance in mice induced by curcumin encapsulated in nanoparticles of PLGA and in nanoparticles of PEG-*b*-PLA. The mice that were given the curcumin–PLGA nanoparticles orally exhibited significantly greater attenuation of morphine tolerance than those mice given the curcumin–PEG-*b*-PLA nanoparticles, presumably due to greater absorption of the curcumin when formulated with PLGA. These studies demonstrate the significant potential of nanoparticle-mediated drug delivery. Elucidation of the mechanisms of nanoparticle-based natural compound drug delivery will be important in further development of this promising technology.

Nanoparticles have been used to increase the bioavailability of several other natural compounds as well. Apigenin, a plant-derived flavone, has low lipid and water solubility. The solubility was improved by incorporating the compound into a carbon nanopowder solid distribution. Solid dispersion (SD) is a successful technique used to increase the water solubility of certain drugs. Carbon nanopowder is an inert, large-surface-area nanomaterial that reduces the aggregation size of a compound and increases its dispersibility. The oral bioavailability of apigenin carbon SD was investigated in

Sprague Dawley rats and their plasma concentration was measured. The carbon SD increased the bioavailability of apigenin by 183%.⁶⁵ Thymoquinone, the main bioactive component in *Nigella sativa*, when encapsulated in a lipid nanocarrier, had a six-fold increase in bioavailability compared to that of free thymoquinone and it showed an increase in gastrointestinal protective properties.^{61,82} Natamycin, an antifungal agent produced by the bacterium *Streptomyces natalensis*, is commercially available for the treatment of corneal fungal infections. Bhatta et al⁸³ used lecithin mucosal adhesive nanoparticles to deliver natamycin and compared it to the commercially available natamycin ophthalmic suspension (USP Natamet). The nanoparticle increased the bioavailability of natamycin by 1.47-fold and decreased clearance by 7.4-fold.⁸³ Icaritin, a flavanol (a glycoside derivative of kaempferol) used to prevent osteoporosis, was incorporated into a nanocrystal. The incorporation caused the AUC value of icaritin to double.⁸⁴ Self-nanoemulsifying quercetin also had an increased bioavailability compared to quercetin alone, with two times higher AUC values.⁸⁵ Taxifolin, a flavanol plant derivative, also had improved bioavailability when incorporated into a nanoparticle. In this experiment, nanoparticles of pure taxifolin were formed by liquid antisolvent precipitation in which the size of these nanoparticles could be controlled by the precipitation conditions. The oral bioavailability of the nanoparticles was seven times that of normal taxifolin.⁵⁶ Polymeric nanoparticles have been utilized to increase the bioavailability of luteolin, EGCG, tea polyphenols, and silibinin, using polymers such as PEG, PVA, and PLA.⁸⁶⁻⁸⁹ As mentioned earlier, EGCG has been shown to inhibit P-gp, which is abundant in the intestinal lining. Work by Dube et al¹² has shown that EGCG encapsulated by chitosan nanoparticles significantly increased intestinal absorption compared to EGCG alone.¹² This leads to an increased bioavailability of the compound.

As discussed above, use of nanoparticles is one way to significantly increase the bioavailability of natural compounds. The improvement in their pharmacokinetic properties leads to a better therapeutic effect, without high-dose-induced acute toxicity. Nanoparticles, however, are not the only resource to increase the bioavailability of natural compounds. Researchers have found that the addition of adjuvants to curcumin can also improve its bioavailability. Piperine has been one of the most promising adjuvants. A few mechanisms may contribute to piperine's ability to increase curcumin's bioavailability. One of the possible mechanisms is inhibiting the glucuronidation of curcumin.⁷ Glucuronidation-based small-molecule metabolism involves

the addition of a glucuronic acid group to curcumin, which reduces curcumin's activity. Another contributing mechanism is that piperine has been shown to inhibit P-gp and cytochrome P450 (CYP) 3A4.⁹⁰ As discussed above, P-gp is responsible for pumping materials out of the intestinal lining and thus reduces bioavailability. This receptor is an ATP-binding cassette transporter, which utilizes ATP hydrolysis to position the drug and excrete it from the membrane. P-gp is not structurally specific, although it usually binds to hydrophobic substrates.⁹¹ CYP3A4 is an enzyme in the liver responsible for oxidizing small molecules. Oxidation, like glucuronidation, can decrease the activity of small molecules such as curcumin. Because of these mechanisms, piperine is a good adjuvant to increase the bioavailability of curcumin. The combination of piperine and curcumin in rats increased the bioavailability by 154%, whereas the combination in human volunteers increased the bioavailability by 2,000%.⁶⁸ Adjuvants alone do not have some of the other beneficial properties of nanoparticles, but piperine could be added to the natural compounds/nanoparticles to further increase their bioavailability.

Other types of nanomaterials can also be used to provide better bioavailability to natural compounds. Studies involving unique types of nanodelivery devices and their use with natural compounds have been conducted, but pharmacokinetic studies have not yet been performed. It is important to note that after the addition of these nanomaterials, the therapeutic effects of the compounds increase. For example, a silk fibroin nanoparticle was developed to treat breast cancer. The nanoparticle contained curcumin and was composed of a mixture of silk and chitosan. Although the pharmacokinetic properties were not studied, the uptake of curcumin by the breast cancer cell line (MCF-7) was significantly increased after the addition of the silk fibroin nanoparticle.⁹² In another study, quercetin-loaded β -cyclodextrin dodecylcarbonate nanoparticles were used on SH-SY5Y neuronal cells.⁹³ The quercetin nanoparticles caused a decrease in inflammatory mediators, such as cluster of differentiation 36 (CD36), β 1-integrin, interleukin-8, monocyte chemoattractant protein-1 and matrix metalloproteinase-9. Other studies have also found that incorporating natural products into nanoparticles can improve the health benefits of the drug. Traditional Chinese medicines isolated from plants and made into nanoparticles improved the compounds' hepatoprotective effects, and camptothecin encapsulated in liposomes increased the drug's ability to kill melanoma cells.^{94,95} These examples illustrate the benefits nanoparticles can have on natural compound delivery. Future studies should

be performed to determine the factors causing the increase in delivery efficiency and bioavailability.

Another approach for increasing bioavailability of poorly soluble natural compounds and drugs is to form amorphous solid dispersions (ASDs) of the therapeutic compound with a polymer. This has been demonstrated with micron-scale and larger particles, but not so far with nanoparticles.⁹⁶ Most drugs and natural compounds tend to crystallize, which is a barrier to dissolution at physiological conditions. Those natural compounds that are also highly hydrophobic exhibit especially poor bioavailability due to the combination of hydrophobicity and crystallinity. ASDs are solid solutions of the therapeutic agent in an amorphous polymer carrier in which attractive interactions prevent crystallization. It is important to choose the polymer for biocompatibility and controlled release of the drug under physiological conditions and for good storage stability. In particular, polysaccharides have recently been demonstrated to form ASDs that significantly enhance the solubility of natural compounds including ellagic acid, quercetin, curcumin, naringenin, and resveratrol.^{97–99} These studies suggest that ASDs in nanoparticle form could potentially be very useful in improving bioavailability through improved solubility and transport across physiological barriers.

The route of delivery greatly affects a drug's bioavailability. The definition of bioavailability is the proportion of a drug that enters the circulation and is able to have an active effect. For the majority of this section, the route of delivery has been oral. Oral delivery is beneficial because it has high patient compliance. Another route that is appealing is topical administration. One of the main issues with this route is extremely low bioavailability. Nonsteroidal anti-inflammatory drugs are some of the most common topical administered drugs, and their bioavailability is generally less than 5%–15%. However, nanotechnology may be able to enhance the route of topical administration.¹⁰⁰ Lycopene, an active compound in tomatoes, has also been incorporated using nanotechnology for topical administration. Lycopene has been shown to have anti-inflammatory properties without the side effects of typically used steroids. Lycopene was incorporated into either a “transfersome” or an endosome, which are artificial vesicles similar to a liposome. The effectiveness of this drug was compared to that of betamethasone, an anti-inflammatory steroid. The lycopene in the “transfersome” and endosome reduced the swelling by 97% and 87%, respectively, which was comparable to that of the betamethasone.¹⁰¹ Another study developed a topical salidroside and paeonol nanosphere hydrogel to protect against UV-induced

melanoma.⁶² These results suggested that the use of natural compounds with a nanoparticle could potentially be used in topical delivery to replace current creams.

A similar route to topical delivery is transdermal administration. This route also requires the drug to have the ability to penetrate the skin. In one study,¹⁰² the ethanolic extract of apple peel, which has been shown to have photoprotective properties, encapsulated by PLGA nanoparticles underwent transdermal administration. The nanoparticle also included oleic acid, a permeation enhancer, to further increase the particles' bioavailability. The nanoformulations released 90% of the drug, whereas only 25% of the drug was released in the cells exposed to the free drug.¹⁰²

Even though nanoparticles have great potential to considerably improve the bioavailability of natural compounds and there has recently been significant progress in the development of such formulation, very few nanoparticle/natural compound drugs are currently being tested in clinical trials. More work needs to be performed to optimize these drug delivery systems and to better understand the mechanisms underlying the enhanced nanoparticle delivery.

Targeting

A second major benefit in utilizing nanoparticles in drug delivery of natural compounds is their ability to target specific tissues or organs. Targeting is beneficial for a number of reasons. First, targeting can improve drug bioavailability by increasing the fraction of the drug that reaches the tissue of interest. Second, targeted drug delivery can reduce toxic side effects of the drug because it is mainly being released in a localized area of the body. Due to the different types of nanoparticles, a number of targeting approaches are possible, which can fall under two general categories. The first is active targeting, where a targeting ligand is attached to the surface of a nanoparticle. The second type is passive targeting, in which the nanoparticle reaches the targeted area without specific chemical interaction but, instead, relies upon physical transport of the particles due to their intrinsic properties, such as size, shape, and surface charge.¹⁰³ These targeting methods and their advantages are summarized in Table 3.

Active targeting is usually accomplished by functionalizing the nanoparticles with a protein, peptide, antibody, or small molecule. This functionalization allows the particle to be localized and internalized by specific tissues or organs. The use of monoclonal antibodies conjugated onto nanoparticles has shown promise in targeting the blood–brain barrier (BBB). However, this has not been used in conjunction with natural compounds.¹¹² Conjugation of

Table 3 Summary of targeting techniques

Natural product	Disease	Nanodevice	Advantage	References
Curcumin	Macrophage-related diseases	SA- and PEG-DSPE-conjugated lipid nanoparticle	<ul style="list-style-type: none"> • Particles are more likely to go to the bone marrow and be phagocytized by macrophages 	104, 105
Curcumin	Cancer	Iron oxide nanoparticles	<ul style="list-style-type: none"> • Uptaken by cancer cells when induced by magnetic field 	106
Curcumin	Colorectal adenocarcinoma cells	PLGA-lectin-PEG nanoparticles with RNA aptamers	<ul style="list-style-type: none"> • The RNA aptamers target epithelial cell adhesion molecules that are expressed in colorectal adenocarcinoma cells 	107
Eugenol	Fungal skin infections	SA hydrogel containing eugenol-loaded solid lipid nanoparticles	<ul style="list-style-type: none"> • Solid lipid nanoparticles were able to increase the amount of eugenol delivered to the infected cells by at least six-fold compared to other models 	108
Gambogic acid	Colon cancer	Vitamin E-containing telodendrimers	<ul style="list-style-type: none"> • EPR effect allows the nanoparticle to be targeted to the tumor cells 	109
Oncocalyxone A	Guided by magnetic field	Iron oxide nanoparticles coated in oleic acid and block copolymer	<ul style="list-style-type: none"> • Nanoparticles show high uptake rates in cancer cells • The iron oxide nanoparticles can be guided by magnetic field 	110
Quercetin	Tumor cells overexpressing folate receptors	PEG-conjugated PLGA nanoparticles with folic acid	<ul style="list-style-type: none"> • Contains folic acid on the surface • Nanoparticle will more likely bind with cancer cells, which express more folic acid receptors than healthy cells 	111
N/A	Targeting the BBB	Monoclonal antibodies conjugated to nanoparticles	<ul style="list-style-type: none"> • Reduces the interaction of the drug with healthy cells • Monoclonal antibodies have shown promise in targeting the BBB • Potentially useful for natural compound delivery for Alzheimer's, Parkinson's, brain infections, brain cancers, etc 	112
N/A	HIV	Polyacrylate-based nanoparticle carrying BTA moieties	<ul style="list-style-type: none"> • The BTA targets amyloid formation • SEVI targeted in HIV • This technique could be used to target other amyloid proteins, such as beta-amyloid or amylin 	113
N/A	Liver	Gold nanoparticles with sizes of 10 nm, 50 nm, 100 nm, and 250 nm	<ul style="list-style-type: none"> • The liver contained 46% of the 10 nm particles, 21% of the 50 nm particles, 44% of the 100 nm particles, and 31% of the 250 nm particles 	114
N/A	Lysosome	Nanoparticles with a negative surface charge at pH 4	<ul style="list-style-type: none"> • Changing the size of the particles can affect its distribution in the liver and other organs • Intracellular targeting has a significant advantage for treating diseases that affect only certain organelles, such as mitochondrial diseases • Could further be utilized for the delivery of natural compounds 	115

Abbreviations: BBB, blood brain barrier; BTA, benzothiazole aniline; DSPE, distearoylphosphatidylethanolamine; EPR, enhanced permeability and retention; N/A, not applicable; PEG, polyethylene glycol; PLGA, poly(lactic-co-glycolic acid); SA, surface amphiphile; SEVI, semen-derived enhancer of virus infection.

nanoparticles with folic acid (FA) has shown promise in the treatment of cancer cells. Because many types of cancers overexpress FA receptors on the cell surface, attaching FA to a nanoparticle will enable it to target cancer cells. This technique was used in the case of quercetin encapsulated in PLGA nanoparticles that were stabilized by PEG, which increases the nanoparticle biocompatibility and the circulation lifetime. The nanoparticles were then conjugated with FA. HeLa cells were used as a FA-expressing cancer cell line. Non-PEGylated non-FA-targeted nanoparticles, PEGylated non-FA-targeted nanoparticles, and PEGylated FA-targeted nanoparticles were all tested on the HeLa cell line. The cell viability was lowest with the PEGylated FA-targeted nanoparticles (56.63% cell viability) compared to the controls: quercetin alone (84.36%), non-PEGylated non-FA targeted nanoparticles (83.22%), and PEGylated non-FA targeted nanoparticles (81.27%). Cellular uptake of the nanoparticles was also measured, and the PEGylated FA-targeted nanoparticles had a significantly higher cellular uptake than the other nanoparticle models.¹¹¹ Sou et al^{104,105} developed another targeting technique, using curcumin lipid nanoparticles to target bone marrow macrophages. Surface modification of the lipid nanovesicles with an anionic amphiphile, L-glutamic acid, *N*-(3-carboxy-1-oxopropyl)-, 1,5-dihexadecyl ester, resulted in significant targeting of vesicles to the bone marrow. Further incorporation of PEG-lipid passively enhanced the distribution of succinic acid vesicles into the bone marrow.^{104,105} Curcumin has also been incorporated into PLGA-lecithin-PEG nanoparticles with covalently attached RNA aptamers against epithelial cell adhesion molecules. This allows the nanoparticle to target colorectal adenocarcinoma cells. These nanoparticles successfully targeted colorectal cancer cells and enhanced cellular uptake of curcumin.¹⁰⁷

Functionalizing nanoparticles with small molecules for targeted delivery of natural compounds is a relatively new development. Thus, more research work is needed to apply previously developed targeting techniques to the field of natural compounds. For example, benzothiazole aniline (BTA) is a known amyloid-binding compound and has been shown to prevent beta-amyloid protein aggregations.¹¹⁶ It also inhibits semen-derived enhancer of virus infection, which increases the infectiousness of HIV. Polyacrylate-based nanoparticles functionalized with BTA were used to impede semen-derived enhancer of virus infection mediation in HIV-infected cells.¹¹³ Certain natural compounds, such as curcumin, have demonstrated anti-Alzheimer properties.¹¹⁷ The BTA-based nanoparticle described above could potentially be modified to

improve the delivery of these natural compounds for better-quality treatment of Alzheimer's disease.

Targeting can also be achieved using external forces. The use of magnetic fields to direct a delivery system has gained some attention. Iron oxide nanoparticles were prepared and loaded with curcumin and incubated with MDA-MB-231 cells, a breast cancer cell line, in the presence of an external magnetic field generated by a neodymium permanent magnet. The magnetic field significantly increased the uptake and targeting of the cancer cells.¹⁰⁶ A similar study¹¹⁰ was done with oncocalyxone A, an extract from the Brazilian plant *Auxemma oncocalyx* with antitumor activity. Iron oxide nanoparticles coated in oleic acid and oncocalyxone A were incorporated into the hydrophobic cores of block copolymer micelles. Incorporated iron oxide allows the nanoparticles to be directed by a magnetic field to the tumor.¹¹⁰

Passive targeting is often an effective and less-expensive option that is most often used in tumor treatment. Many tumors exhibit the enhanced permeability and retention (EPR) effect caused by leaky vasculature in the tumor.¹¹ This results in a buildup of nanoparticles preferentially in the tumor compared to healthy tissue. An example is the delivery of encapsulated gambogic acid and vitamin E-containing telodendrimers for colon cancer treatment.¹⁰⁹ Gambogic acid has been shown to inhibit the growth of several types of cancer lines, including colon cancer. Dendrimers are hierarchically branched molecules on the nanoscale (Figure 1A). The telodendrimers were made of a PEG-containing, dendritic oligomer of cholic acid and vitamin E. These telodendrimers self-assembled to form spherical nanoparticles similar to micelles. After the telodendrimer was optimized, it was labeled with a fluorescent lipophilic cationic indocarbocyanine dye and injected into mice. The telodendrimers showed a high uptake in the tumor, whereas the dye alone had a higher uptake in the liver, lung, and spleen, but a lower uptake in the tumor.

The reticuloendothelial system can also be passively targeted. For example, the biodistribution of gold nanoparticles with sizes ranging from 10 nm to 250 nm was studied in rat models. Gold uptake in the liver, spleen, lung, kidney, testis, thymus, heart, and brain was quantitated using inductively coupled plasma mass spectrometry. The liver was found to have the highest percentages of the injected dose, containing 46% of the 10 nm particles, 21% of the 50 nm particles, 44% of the 100 nm particles, and 31% of the 250 nm particles.¹¹⁴ This experiment showed the strength of passive targeting. Up to 46% of the nanoparticles can be targeted to the liver

without the addition of any targeting molecules. Although this type of experiment has not been performed using natural compounds, it could be used as a potential targeting mechanism in the future.

Another strategy for targeting is to manipulate the lipophilicity of the nanoparticles. This technique is especially important in targeting the brain. The BBB favors crossing over of lipophilic molecules. By adjusting this property, control is placed on where the nanoparticles go, and therefore, this technique can be used to target the distribution toward specific locations.⁵ Stearic acid hydrogel containing eugenol-loaded SLN was targeted to the epidermis to treat fungal skin infections. These nanoparticles were compared against a eugenol-hydroxypropyl- β -cyclodextrin complex in hydrogel, a less-lipophilic nanoparticle, and an almond oil solution of eugenol. The SLN hydrogel showed an accumulation of 62.65%, compared to the other models, with values of 9.77% and 3.45%, respectively. This is another example of varying the characteristics of the nanoparticles in order to better target the area of interest.¹⁰⁸

Nanoparticles can also be targeted to certain organelles within the cell by manipulating the surface charge. In one study, nanoparticles that were negatively charged at pH 4 (pH of the lysosome) remained in the lysosome, while nanoparticles that were positively charged were released into the cytoplasm.¹¹⁵ Furthermore, nanoparticles with surface modification to carry a positive charge may allow targeting to the mitochondria.¹¹⁸

Controlled release

A third benefit of using nanoparticles to deliver natural compounds is that the release of the drug can be controlled. The amount and rate at which a drug is released from a nanoformulation depends on a multitude of factors, including particle type and size, amount of drug encapsulated, natural compound used, and the microenvironment.²⁴ Recent examples of natural product-based nanoparticles and their properties for controlled release are summarized in Table 4.

The type of nanoparticle used prominently affects the drug release profile. If a polymer nanoparticle is used, the types of polymers can be further adjusted to affect the release profile. Polymers that are biocompatible, such as PEG, increase the time the nanoparticles stay in the body without being excreted or detected by the immune system. Therefore, PEG is often used to increase the release time in a delivery system. In one study, the release of curcumin encapsulated in copolymers of *N*-isopropylacrylamide and *N*-vinyl-2-pyrrolidone with the

addition of poly(ethyleneglycol)monoacrylate (PEG-A) was measured. This copolymer particle allowed for a sustained release of the drug. After 24 hours, only 40% of the drug had been released.¹²¹ The release patterns of quercetin encapsulated in PLA nanoparticles were studied over a period of 96 hours. Within the first half hour, 40%–45% of the quercetin was released. This quick burst was attributed to the quercetin at the surface of the particle diffusing into the surroundings. Over the next 96 hours, the release was slower and reached a maximum of 87.6%. This slower release was attributed to the diffusion of the quercetin from deeper within the nanoparticle.¹²⁸

The release rate of pentacyclic triterpenediol, a natural product with anticancer properties isolated from *Boswellia serrata*, encapsulated in an SLN was studied by Bhushan et al.¹²⁷ The release profile was similar to that of many polymer nanoparticles, with an initial burst followed by a slow, controlled-release pattern.¹²⁷ Another type of nanoparticle has three phases in the release profile. Mesoporous silica nanoparticles, which are silica nanoparticles with holes along the surface to increase surface area and drug loading capacity, present a unique way to increase the release time of a drug. This type of nanoparticle can also be modified for the specific drug and release profile desired. Mesoporous silica nanoparticles, covalently bonded to rhodamine B, were studied.¹³⁰ The particles had a large surface area and pore volume and a positively charged surface (due to the rhodamine B). This was ideal for loading salvianolic acid B, a negatively charged natural product. Mesoporous nanoparticles with and without rhodamine B were compared. Three phases of release were observed over a 144-hour period. The first phase was an initial burst, in which nanoparticles without rhodamine B released more drugs. The second phase included a slower drug release, in which both nanoparticles released around the same amount of drug. In the third phase, the nanoparticles without rhodamine B stopped releasing significant amounts of salvianolic B, while the nanoparticles with rhodamine B continued to release the drug.¹³⁰

The hydrophilic/lipophilic properties of a polymer can also be manipulated to adjust the drug release profile. Ellagic acid, a naturally occurring phenol, was encapsulated in PLGA nanoparticles. The PLGA polymer was combined with didodecyltrimethylammonium bromide (DMAB), PVA, or polyvinyl alcohol with chitosan. The release rates for these three nanoparticles were evaluated over 25 days. The PVA nanoparticles showed the fastest release; 50% of the drug was released over the first 6 days. Polyvinyl alcohol

Table 4 Natural product-based nanoparticles for controlled release

Natural product	Nanodevice	Properties	References
Cinnamic acid	Lecithin–lipid nanoliposomes with PL-DHA, salmon, or soya lecithin phospholipids	<ul style="list-style-type: none"> • Entrapment efficiency may depend on liposome size <ul style="list-style-type: none"> ◦ Salmon: 91.4% ◦ PL-DHA: 76.4% ◦ Soya lecithin: 68.6% 	119
Coumarin-6 ginsenoside, salvianolic acid B	PLGA nanoparticles	<ul style="list-style-type: none"> • Natural product–PLGA interactions may significantly affect the entrapment efficiency (percentage) • Coumarin-6: 51.6% • Ginsenoside: 93.56% • Salvianolic acid B: 92.88% 	120
Curcumin	NIPAM/NVP/PEG polymer nanoparticles	<ul style="list-style-type: none"> • PEG extends release time: only 40% of the drug released after 24 hours 	121
Curcumin	PLGA nanoparticles	<ul style="list-style-type: none"> • Tested in gastric environment (pH 2) and in intestinal environment (pH 7) <ul style="list-style-type: none"> ◦ Intestinal: 77% of the drug released in 7 days ◦ Gastric: 48% released in 7 days 	67
Curcumin	Eudragit S100 polymer nanoparticles	<ul style="list-style-type: none"> • Polymer dissolves at pH 7 • Targets intestines and treats inflammatory bowel disease 	122
Curcumin	PLA and PVA microspheres with Fe ₃ O ₄ paramagnetic nanoparticles	<ul style="list-style-type: none"> • Without Fe₃O₄: 90.35% of the drug released after 72 hours • With Fe₃O₄: 49% released after 72 hours • Curcumin interacts with large-surface-area nanoparticles and release is slowed down 	123
Curcuminoids	SLN in cream	<ul style="list-style-type: none"> • Topical delivery • SLNs with curcuminoids: 70% of the drug released after 8 hours • Free curcuminoids: 90% released after 8 hours 	124
EGCG	Polysaccharide nanoparticles	<ul style="list-style-type: none"> • Fast release time: 46% released in the first 10 minutes, 100% released in 3 hours 	125
Ellagic acid	PLGA nanoparticles with DMAB, PVA, or PVA-CS	<ul style="list-style-type: none"> • Release rate affected by hydrophobicity of polymer (PVA is least hydrophobic) <ul style="list-style-type: none"> ◦ PVA: 50% of the drug released in the first 6 days ◦ PVA-CS: 38% released in the first 6 days ◦ DMAB: 24% released in the first 6 days 	52
Eugenol and <i>trans</i> -cinnamaldehyde	PLGA nanoparticles	<ul style="list-style-type: none"> • <i>Trans</i>-cinnamaldehyde: 87% of the drug released in 72 hours • Eugenol: 64% released in 72 hours 	126
Pentacyclic triterpenediol	SLN	<ul style="list-style-type: none"> • Two phases: initial quick burst followed by slow release 	127
Quercetin	PLA nanoparticles	<ul style="list-style-type: none"> • Two phases: <ul style="list-style-type: none"> ◦ Quick burst: 40%–45% released in 30 minutes ◦ Slow release: 87.6% released in the next 96 hours 	128
Quercetin	Lecithin-based cationic nanocarrier	<ul style="list-style-type: none"> • Interactions between nanoparticle and natural product lead to a higher entrapment efficiency • Entrapment efficiency is as high as 91.3% 	129
Salvianolic acid B	Mesoporous silica nanoparticle with and without RhB	<ul style="list-style-type: none"> • RhB carries a positive charge that attracts negatively charged salvianolic acid B • Three phases: <ul style="list-style-type: none"> ◦ Quick burst (nanoparticles without RhB > nanoparticles with RhB) ◦ Slow release (nanoparticles without RhB = nanoparticles with RhB) ◦ Slower release (nanoparticles without RhB < nanoparticles with RhB) 	130

Abbreviations: CS, chitosan; DMAB, didodecyltrimethylammonium bromide; EGCG, epigallocatechin gallate; NIPAM, *N*-isopropylacrylamide; NVP, *N*-vinyl-2-pyrrolidone; PEG, polyethylene glycol; PLA, poly-L-lactic acid; PL-DHA, phospholipids containing docosahexaenoic acid; PLGA, poly(lactic-co-glycolic acid); PVA, polyvinyl alcohol; RhB, rhodamine B; SLN, solid lipid nanoparticles.

with chitosan was the second fastest, with 38% of the drug released after 6 days and DMAB was the slowest, with 24% being released in 6 days. The release rate was thought to be affected by the hydrophilic nature of each of the polymers. PVA has hydrophilic groups in the polymer, which allow

water molecules to penetrate and increase the release of ellagic acid.⁵² Other polymer types have been tested and have had drug release analyses performed, including PLGA and PCL, to deliver camptothecin.¹³¹ PLGA nanoparticles were used to deliver phytochemical tropical fruit-derived

natural products,¹³² and PLA nanoparticles were used to deliver quercetin.¹²⁸

The microenvironment is another factor that can be manipulated in order to control the release profile of a drug. Xie et al⁶⁷ tested the release profile of curcumin-loaded PLGA nanoparticles in different microenvironments in vitro. An artificial gastric environment was created using phosphate-buffered saline at pH 2 (adjusted with HCl), and an artificial intestinal environment was created using phosphate-buffered saline at pH 7.4. The PLGA nanoparticles were agitated in suspension in the two environments over a period of 7 days, and the amount of curcumin released was measured by high-performance liquid chromatography. More curcumin was released in the artificial intestinal environment than in the gastric environment throughout the entire period. At the end of the 7 days, 77% of curcumin was released in the intestinal environment, whereas only 48% was released in the gastric environment.⁶⁷ The environment that a drug delivery system will encounter in its journey to the target site is an important factor to consider when designing a nanoparticle. As described above, the release profile of a drug is dependent on a number of factors, one of which is pH. For example, nanoparticles that are used to treat inflammatory bowel disease can be designed to have maximum release in the intestinal environment to increase the amount of drug that reaches the target site. Gugulothu et al¹²² utilized this idea when the team designed a pH-sensitive polymer nanoparticle. This nanoparticle dissolves at pH 7, the pH of the intestinal tract. If orally administered, the majority of this polymer (Eudragit S100) will stay intact until it reaches the intestines, where it will deliver the drug. Curcumin and celecoxib, an anti-inflammatory drug, were both encapsulated in the pH-sensitive polymer in order to better treat inflammatory bowel diseases such as ulcerative colitis and Crohn's disease.¹²²

Controlled release can also be achieved by manipulating the environment within the particle. The release of curcumin from a polymer microsphere was controlled with the addition of Fe₃O₄ (magnetite) nanoparticles. The curcumin and the magnetite nanoparticles were encapsulated in PLA and PVA/PEG polymers and the release rate of the curcumin was measured and compared to that from the microsphere without the magnetite nanoparticles. The microspheres that did not contain magnetite released 90% of the curcumin after 72 hours. The magnetite-containing microspheres, however, only released 49% of the curcumin after 72 hours. Those microspheres, like the normal microspheres, exhibited an initial burst of curcumin, but this was followed by a slower

release rate, which was thought to be due to the interaction of the curcumin and the magnetite nanoparticles that have a large surface area with which curcumin could bind.¹²³

Polymer nanoparticles are not the only type of nanoparticle in which release kinetics has been studied. In one study, a cream containing SLNs carrying curcuminoids was compared to free curcuminoids. The SLN slowed the release kinetics. After 12 hours, the SLN had released 70% of the curcuminoids, whereas 90% of the free curcuminoids were released within 8 hours.¹²⁴

The type of natural compound contained in the nanoparticle also affects the release kinetics. In one study,¹²⁶ eugenol and *trans*-cinnamaldehyde were encapsulated in PLGA nanoparticles and the release profiles were measured over 72 hours. The structure and size of the nanoparticles were analogous, but the release profiles were quite different. Both nanoparticles had two phases of release, an initial burst and a slow release. At the end of the 72 hours, 87% of the *trans*-cinnamaldehyde had been released, whereas only 64% of the eugenol was released. The release constants were also measured. For the burst phase, the rate constants were $1.76 \times 10^{-4} \text{ s}^{-1}$ for cinnamaldehyde and $4.10 \times 10^{-4} \text{ s}^{-1}$ for eugenol. During the second, slow release phase, the constants were $2.75 \times 10^{-6} \text{ s}^{-1}$ and $1.65 \times 10^{-6} \text{ s}^{-1}$, respectively. These data show that eugenol is released at a much faster rate than cinnamaldehyde during the initial burst, but at a slower rate during the second phase. This difference in the release of the compounds was believed to be due to the interactions of cinnamaldehyde and eugenol with the PLGA.¹²⁶

Drug release from nanoparticles can be triggered using ultrasound, light, and other physical and chemical environmental changes.^{133,134} Recently, a new light-sensitive polymer based on the quinone-methide system was developed, which degrades when exposed to irradiation at 350 nm and 750 nm.¹³³ Nanoparticles made with this polymer released an encapsulated drug (in this case, the Nile Red dye) when exposed to the specific light wavelength(s). This technology combines targeting and controlled release to make a delivery system that could greatly reduce the drugs' interaction with other tissues in the body.

An important aspect that affects the release of a drug is the entrapment efficiency or encapsulation efficiency (EE) defined as $EE = (\text{total concentration} - \text{supernatant concentration}) / \text{total concentration}$. The entrapment efficiency is important because a nanoparticle with a low efficiency means wasted compound and higher costs. Larger entrapment efficiency also means more drug release in vivo. It is therefore important

to have a nanoparticle with the highest entrapment efficiency. A study by Bouarab et al¹¹⁹ investigated the entrapment efficiency of cinnamic acid encapsulated within lecithin–lipid nanoliposomes. Three different phospholipids were studied, a phospholipid derivative of docosahexaenoic acid, as well as salmon and soya lecithin. Salmon lecithin showed the highest entrapment efficiency, estimated to be $\sim 91.40\% \pm 1.39\%$, then came phospholipids containing docosahexaenoic acid, with $76.4\% \pm 0.98\%$, and soya lecithin with $68.63\% \pm 1.21\%$. The entrapment efficiency could be correlated to the liposome size, whereby the larger liposomes have higher entrapment efficiency. Another property that affects the EE of a natural compound in a nanoparticle is the interaction between the particle and the compound. Quercetin was encapsulated in a lecithin-based cationic nanocarrier (LeciPlex) and had a high encapsulation rate of 91.3%. The authors correlated this high EE to the strong interactions between quercetin and the LeciPlex.¹²⁹ The natural compound used also affects the encapsulation efficiency. Cai et al¹²⁰ encapsulated different natural products within PLGA nanoparticles and found that each compound had a different encapsulation efficiency. Coumarin-6, a phenylpropanoid, had the lowest EE, 51.6%, whereas ginsenoside and salvianolic acid B had the highest EE, 93.56% and 92.88%, respectively. The highest encapsulation efficiencies may be due to stronger interactions between the compound and the PLGA.¹²⁰

Controlling the release of a drug from the nanoparticle is a powerful tool. Drug release kinetics can affect how much of the drug arrives at its targeted location. Optimizing the nanoparticle based on the target location, properties of the natural compound, and preferred nanoparticle type may take a significant amount of time, but the benefits will be significant.

Issues

One of the major problems with nanoparticle delivery systems is their potential toxicity. This is a major concern with nanoparticles, partly because they can cross biological membranes, such as cellular membranes and, in some special cases, the BBB.^{112,135} Cells with phagocytosis ability can take up nanoparticles between 100 nm and 1,000 nm. Nanoparticles < 100 nm, however, can be taken up by cells via endocytosis and could potentially cause systemic toxicity and harm if their biodistribution is not controlled and if they contain toxic polymers or drugs or if they form toxic metabolites. Nanoparticles that can be taken up by cells need to undergo extensive screening, to ensure no harm will come

to healthy cells.¹³⁶ Although numerous studies have claimed that nanoparticles composed of biocompatible polymers (PLGA, PEG, etc), phospholipids (liposomes and micelles), and other materials are safe and show no toxicity to healthy cells, additional studies are clearly needed.¹³⁷ Many of the studies discussed in this review have not completed a toxicity study on healthy cell lines.

Another issue that arises when using nanoparticles as drug delivery systems is that the nanoparticle will undergo changes in the body. It is known that the surface of nanoparticles change as the particles move through different membranes, tissues, and organs in the body. As the nanoparticle moves, proteins become attached to the surface and change the shapes and surface charges of the particles. Interactions with surfaces may also strip the nanoparticles of ligands that were originally present on its surface. The nanoparticle that arrives at the targeted location may thus not be the same as the nanoparticle originally administered.¹³⁸ This must be taken into account, because the changes could affect the bioavailability, targeting, and release kinetics of the drug.

Targeted nanoparticle delivery of natural compounds is a significant benefit. Although most natural compounds show low toxicity, high-dose-induced side effects are a major reason for low patient compliance. Targeted delivery can reduce side effects and limit potential toxicity. However, targeting has challenging issues that must be overcome. The ultimate goal for a targeted drug delivery system is to create a specific drug–carrier combination that enhances the delivery or uptake in a specific area of the body. This will result in a higher therapeutic effect and lower side effects. In order to achieve this goal, the delivery system must have targeting capability and a sufficiently long circulatory half-life. The half-life of nanoparticles is often shortened by the immune macrophage system in the liver and spleen. Although some techniques have been used to lessen the detection of the nanoparticles by the macrophages, such as PEGylation and adjustment of size and charge, it takes multiple tests just to find what properties could work for the specific nanocarrier formulation.¹³⁹ Another problem with targeting a certain tissue is the fact that only a small percentage of the nanoparticles that are administered will reach the target tissues.

In addition to general issues with nanoparticles as drug delivery systems, there are specific issues that are often harder to overcome. When treating tumor cells, targeting using the EPR effect has gained significant attention in nanomedicine. The idea that no additional ligands need to

be added to the nanoparticles makes their use simple and less expensive. The EPR effect, however, does not work for the entire tumor tissue. The leaky vasculature does not extend into the necrotic core of a tumor. Therefore, the nanoparticles tend to aggregate at the periphery of a tumor and do not treat its entirety. Although this treatment can shrink the tumor, it will often not fully eliminate the tumor. The tumor will continue to spread after treatment.¹⁴⁰ Targeting to certain organs in the body, such as the brain, also has specific issues. The BBB is one of the tightest barriers in the body. The goal of nanodelivery is not only to get through this barrier, but also to deliver the drug to specific cells in the brain. For example, in order to get to targeted neuronal cells, the nanoparticles will have to encounter endothelial cells in the BBB, glial cells, and healthy neuron cells. The nanoparticles need to be able to navigate through and around these cells without damaging them.¹³⁸ Special design will be necessary to overcome the issues associated with the targeted area, in order to enhance the effectiveness of the administered drugs.

The issues do not end once the particle has been specifically delivered to the cells. Nanoparticles with sizes >20 nm are normally brought into the cell by endocytosis. Many of these particles are then degraded in lysosomes and no therapeutic effects are achieved. As discussed above, the charge of the nanoparticle can influence the degradation of the nanoparticle once in the endosome/liposome.¹³⁹ These charges, however, are often unfavorable when crossing the biological membranes to make it into the cells. Another area of concern is with the makeup of the nanoparticles. It is important that after the drug has been delivered, the nanoparticle either is eliminated afterward or is biodegradable, especially in the treatment of chronic human diseases. Accumulation of non-degradable nanoparticles in the body over time could lead to unwanted toxicity and cell death.

Engineering nanoparticles that have controlled release properties has another set of challenges. This review mainly discussed polymeric nanoparticles in the section titled "Controlled release". Other types of nanoparticles are less easily manipulated into releasing drugs in a controlled fashion. Liposomes, eg, have a tendency to randomly burst and release the drug in vivo.¹⁴⁰

One of the leading issues currently facing the continued development of nanoparticle drug delivery systems is the transition from bench to bedside. Targeted nanoparticles have been developed over the past 30 years, yet only a small number of them have reached clinical trials. The FDA has approved no targeted nanoparticles and only seven nanomedicine-based

cancer treatments have been approved as of June 2014.^{140,141} This is in part due to the difficulty in reproducing nanoparticle synthesis on a scale needed for commercialization, the lack of understanding regarding how nanosurfaces interact with biosurfaces, the lack of technological platforms necessary to screen large quantities of nanoparticles, and insufficient knowledge about the fate of the nanoparticles once they enter organs, tissues, and cells.¹⁴⁰

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