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Advances in Plant-derived Edible Nanoparticle-based lipid Nano-drug Delivery Systems as Therapeutic Nanomedicines

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Abstract

Plant-derived edible nanoparticles (PDNPs) are nano-sized membrane vesicles released by edible plants, such as grapefruit, ginger, broccoli, and lemon. They are non-toxic, have tissue-specific targeting properties, and can be mass-produced. Thus, they have great potential for clinical application. PDNPs offer multiple advantages over the currently available drug delivery systems, such as their relatively high internalization rate, low immunogenicity, proven stability in the gastrointestinal (GI) tract, and ability to overcome the blood-brain barrier but not cross the placental barrier. In this review, we will discuss these merits of PDNPs and analyze the current issues in PDNP research.

1 Introduction

Nanotechnology is a promising means for delivering drugs to specific targets, as small molecular drugs or therapeutic biomolecules may be packaged into nano-sized particles¹. Naturally occurring nanoparticles (e.g., exosomes released from different mammalian cells) have been used to encapsulate drugs and short interfering RNAs (siRNAs) for the treatment of disease in mouse models²⁻⁶. Small unilamellar liposomes vesicles (SUVs), which are spherical vesicles that are composed of a lipid bilayer and have a diameter up to 100 nm, can carry hydrophobic drugs or biologics to unhealthy tissues⁷⁻¹⁰. Although enormously promising applications have emerged in this field, however, nanomedicine remains limited by major issues with: a) human toxicity or immunogenicity; b) a limited uptake efficiency at target tissues; c) potential adverse environmental impacts; and d) the high cost associated with the large-scale production of nanoscale materials^{8, 11-13}.

Plant-derived edible nanoparticles (PDNPs) are recently discovered nano-sized vesicles that may be isolated from edible plants, such as grapefruit¹⁴, ginger¹⁵, grape¹⁶, and lemon¹⁷. They represent a new wave of non-toxic and environment-friendly vehicles for potential use

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in nanomedicine, as they have obvious benefits compared to artificial nanoparticles and mammalian cell-secreted exosomes. For example, PDNPs have no detectable toxicity or immunogenicity; can show highly efficient uptake at the target site; have a strong capacity to deliver a variety of therapeutic agents¹⁴; and may be produced economically¹⁵. However, we currently lack scalable methods to efficiently isolate and assemble PDNPs of a uniform size. Furthermore, their lipid compositions and bilayer membrane substructures, which critically determine the internalization rate and targeting of different tissues, have not been well studied. Thus, the therapeutic application of PDNPs as a drug delivery system is still in its infancy. In this review, we attempt to summarize what is known about edible plant nanoparticles, especially in the context of nanotechnology, and discuss their potential for use as a safe, sustainable, and diversified platform for therapeutic applications.

2 Preparation of PDNPs

2.1 Isolation and purification of PDNPs

Differential ultracentrifuge (UC) techniques are commonly used to isolate exosomes from mammalian cells¹⁸⁻²⁰, and have been used to isolate PDNPs from plants^{12, 14, 16, 21, 22} [Fig. 1]. In general, freshly squeezed plant juice is submitted to low-speed centrifugation (from below 8,000 to 10,000×g) to remove large particles, such as fiber and other plant debris. The supernatant is then centrifuged at high speed (about 150,000×g) for more than 1 hour to pellet the nano-sized particles. The pellet is then suspended in a small volume of phosphate buffer solution (PBS), and the homogenized suspension is applied to a sucrose step gradient (8%/15%/30%/45%/60%) and centrifuged at above 150,000×g for approximately 2 hours. Distinct types of nanoparticles are isolated based on the high g-force sedimentation and the different velocities of the sucrose gradients. The production yields of PDNPs are very similar across the fruits and root-derived edible plants studied to date, with 100 g of edible plant material producing about 350 mg to 450 mg of nanoparticles¹⁶. In most cases, the 30%/45% layer is harvested as the best source of PDNPs^{15, 23}.

2.2 Lipids extraction and reassembly

A prerequisite for the further development of PDNPs as a drug delivery system is that we devise a means to reproducibly generate uniform-sized nanoparticles². However, this has proven challenging because naturally produced PDNPs range in size from ~50 nm to ~500 nm between and even within species^{15, 16, 24, 25}. Although naturally occurring nanoparticles isolated from ginger and lemon can be directly employed to prevent and treat inflammatory bowel disease (IBD)^{15, 17}, most reports indicate that PDNP lipids should be isolated and reassembled to more uniform-sized nanoparticles, either before or after the cargo is loaded²⁶⁻²⁸.

Lipid profiling has demonstrated that the two major groups of lipids in PDNPs are phospholipids and glycerol lipids. These hydrophobic molecules are prone to accumulate in the organic phase during classical two-phase liquid-liquid extraction (LLE). M Zhang et al.²² used the Bligh and Dyer method (a well-reported LLE method²⁹) to extract total lipids from ginger nanoparticles, and loaded siRNA-CD98 to the lipid/HEPES buffer suspension. The authors passed the extracted lipids through a 200-nm liposome extruder, and

successfully obtained relatively uniform-sized siRNA-CD98/ginger-derived lipid vehicles with an average diameter around 189.5 nm. These reassembled nanoparticles were successfully delivered to colon tissues in a mouse model of ulcerative colitis²². Q Wang et al. used the same protocol to extract total lipids from grapefruit¹⁴. Interestingly, after the grapefruit lipids were sonicated and passed through a high-pressure homogenizer, they reassembled into multilayer flower-like nanoparticles of 200 nm in size. In another intriguing study, Q Wang et al. performed lipid extraction and mixed the reassembled grapefruit lipids with plasma membrane-derived vesicles to create plasma membrane-coated PDNPs. The coated PDNPs were demonstrated to directly home to an inflamed tumor site via an activated leukocyte trafficking pathway²⁷.

2.3 cargo loading

Therapeutic agents, such as small molecular drugs, siRNAs, DNA expression vectors, and proteins, can be loaded to PDNPs that can be directed to target different disease tissues^{14, 22, 26}. The negatively charged nature of PDNPs means that positively charged compounds, such as doxorubicin, can easily be loaded into the PDNP cavity by sonication²⁶ [Fig. 2]. Neutral small molecular agents (e.g., curcumin) and even negatively charged compounds (e.g., folic acid, or FA) can also be trapped in PDNPs without altering their biological activities¹⁴. It seems that the hydrophobic properties of these compounds can overcome the static electronic forces at the PDNP surface, allowing them to become encapsulated. Although some reports have demonstrated that siRNAs and DNAs (which have strong negative charges) could be loaded to PDNPs and showed their desired effects in target tissues, the loading efficiencies of such cargoes were not as high as those obtained for positively charged therapeutics²².

3 Properties

3.1 Chemical properties

The chemical profiles of PDNPs are distinct from those of mammalian cell-derived exosomes. The lipid bilayer of mammalian cell-derived exosomes is rich in glycosphingolipids, cholesterol, and phosphatidylserine, which accounts for their unique rigidity^{6, 30}. Most of the synthetic liposomes studied to date have a similar lipid composition, which helps maintain their stability^{7, 10}. In contrast, PDNPs lack cholesterol. Another difference is that mammalian cell-derived exosomes contain more proteins than PDNPs^{15, 31}. However, mammalian cell-derived exosomes and PDNPs are comparable in their contents of mRNAs, micro RNAs (miRNAs), and non-coding RNAs^{15, 32}.

3.1.1 Lipids—Lipids are essential components of the lipid-bilayer structures of PDNPs, mammalian exosomes, and artificial nanovesicles (e.g., liposomes)³³. The lipid compositions of PDNPs are distinct from those of mammalian cell-derived exosomes and artificially synthesized liposomes^{4, 10}. For example, mammalian exosomes and synthetic liposomes normally contain a high abundance of phosphatidylcholine (PC) and a certain percentage (10-20%) of cholesterol, stabilize the lipid bilayer structure^{6, 34, 35}. In contrast, deep lipidomic analysis of active ginger-derived nanoparticles revealed that they represent a cholesterol-free scenario: the lipids isolated from 30-45% sucrose density gradient UC

comprised 42% phosphatidic acid (PA), 27% digalactosyldiacylglycerol (DGDG), and 19% monogalactosyldiacylglycerol (MGDG)¹⁵. Similarly, no cholesterol was found in lipids isolated from bioactive grapefruit-derived nanoparticles obtained using 30-45% sucrose density gradient UC. However, the lipid profile of grapefruit-derived nanoparticles was entirely different from that of the ginger-derived nanoparticles [Fig. 3], as the former comprised 24% phosphatidylethanolamine (PE), 23% phosphatidylcholine (PC), 13% phosphatidylinositol (PI), and only 10% diacylglycerol (DG)¹⁴. Thin-layer chromatographic (TLC) analysis also demonstrated that the ginger nano-lipids and grapefruit nano-lipids had distinct migration properties¹⁶. The lipid profiles of nanoparticles derived from other plants (e.g., grape, carrot, broccoli, and lemon) have not yet been examined using modern lipidomic techniques such as LC-MS or GC-MS. However, it seems safe to predict that plant-derived lipids are cholesterol-free, as most edible plants and vegetables lack cholesterol.

3.1.2 Proteins—The abundant surface-associated proteins of mammalian cell-derived exosomes play key roles in intercellular communications. On the surfaces of dendritic cell-derived exosomes, for example, integrin α MB2 and tetraspanin are responsible for targeting or adhering, while major histocompatibility complex (MHC) and cluster of differentiation (CD) function as T-cell stimulators^{20, 34}. Mammalian exosomes also reportedly harbor membrane transport and fusion proteins (GTPases, annexins, and flotillin), tetraspanin proteins (CD63, CD81, CD82, CD53, and CD37), heat shock proteins, lipid raft-associated proteins (including glycosylphosphatidylinositol-anchored proteins), and multivesicular bodies (MVB) biogenesis-related proteins (Alix and TSG101)³⁶. In addition to their potential functional roles, these highly concentrated proteins can be used as biomarkers to differentiate diverse subtypes of exosomes³⁷.

Relatively few reports have addressed the proteins of PDNPs, and the available results are inconsistent. One group¹⁵ found that PDNPs have a relatively low protein content compared to mammalian cell-derived exosomes, and that the protein compositions differ between these two types of nanovesicles. The authors found that the proteins of ginger-derived nanoparticles tended to be predominantly cytosolic (mainly actins and proteolysis enzymes). They also identified a few low-abundance membrane proteins, such as membrane channels/transporters (e.g., aquaporin and chloride channels), that appear to contribute to the substructure of PDNPs¹⁵. In contrast, a proteomics approach taken by a different group revealed that nanovesicles derived from lemon (*Citrus limon* L.) juice had a relatively high protein content, and that approximately 57% of the proteins overlapped with those found in mammalian cell-derived exosomes, regardless of the cell origin¹⁷.

It is commonly believed that plants are less advanced than animals³⁸, and do not require complicated ensembles of surface-associated proteins to communicate with different organs (as seen in animals). Thus, it seems logical that the proteins present in PDNPs would be simpler in their profiles and less abundant than those of mammalian exosomes. However, the data are very limited at this time, so it may be too early to draw meaningful conclusions in this regard.

3.1.3 RNAs—RNAs, including mRNAs, miRNAs, and lncRNAs, can be functionally transferred from producing cells to recipient cells via extracellular vesicles, such as mammalian exosomes⁶. Similarly, PDNPs carry a considerable number of miRNAs to fulfill their intercellular communication tasks. Deep sequencing revealed that ginger-derived nanoparticles harbor at least 125 different miRNAs, each containing between 15 and 27 nucleotides¹⁵. Interestingly, in silico prediction showed that 124 of these miRNAs could potentially target and regulate the expression of human genes by binding to their 3'-untranslated regions (3'-UTRs), indicating that there is potential for PDNP-mediated interspecies communication at the molecular level.

3.1.4 Other—Although the secondary metabolites of plants are believed to primarily contribute to the defenses against insects and herbivores and/or to act as growth factors, humans have extensively explored the potentials of such metabolites as a natural resource for curing disease³⁹. More than 50% of the drugs we use today comprise plant secondary metabolites or their derivatives⁴⁰⁻⁴². Accumulating evidence shows that naturally occurring PDNPs also carry secondary metabolites with potential therapeutic applications. For example, ginger-derived nanoparticles carry bioactive secondary metabolites, such as 6-gingerol and 6-shogaol, which can be used to prevent IBD and colitis-associated cancer¹⁵, and broccoli-derived nanoparticles contain sulforaphane compounds that were shown to inhibit colitis in a mouse model by activating the adenosine monophosphate-activated protein kinase (AMPK) pathway in dendritic cells²⁴.

3.2 Physical properties

3.2.1 Size and charge—The normal size distribution of naturally occurring and reassembled nanoparticles ranges from 30 to 500 nm. Structures smaller than 30 nm are unstable because it is difficult to pack lipids inside a strongly curved geometry, and their drug-loading capacity is correspondingly low⁴³. Ginger-derived nanoparticles are spherical lipid bilayers with an average size of ~220 to 290 nm¹⁵, while nanoparticles isolated from grapefruits normally resemble into multilayer flower-like structures of 180 nm to 200 nm in diameter¹⁴. Notably, the size of reassembled PDNPs can be manipulated by changing the dispersity at which the extracted nanoparticles pass through the high-pressure extruder. For example, ginger-derived nanoparticles passed through a 200-nm cut-off extruder had an average size of ~189 nm, which is smaller than the usual range [Fig. 4B.]. They were successfully used to deliver therapeutic compounds, such as siRNA-CD98 and doxorubicin, to targeted colon cancer tissues²⁶. Interestingly, similarly small reassembled grapefruit-derived nanoparticles were detected on day 7 after intravenous injection, indicating that they had an extended retention in the circulatory system, and thus had a good opportunity to penetrate into target tissues¹⁴.

In some cases, the sizes of plant-derived nanoparticles can also be altered in a pH-dependent manner¹⁶. In a neutral pH environment, the average diameters of grape-, ginger-, grapefruit-, and carrot-derived nanoparticles are all below 300 nm. In a solution that mimics the pH of the acidic stomach environment, however, these PDNPs have distinct behaviors: the grape-derived nanoparticles are reduced in size; the ginger-derived nanoparticles are enlarged; the uniform-sized grapefruit-derived nanoparticles separate into subpopulations of two different

sizes; and the two size-distinguished subsets of carrot-derived nanoparticles merge into a single population of uniform size. Similarly, in a solution that mimics the pH of the intestine, the grape-derived nanoparticles become smaller and the ginger-derived nanoparticles become larger. However, the grapefruit-derived nanoparticles maintain a single population and become smaller, while the carrot-derived nanoparticles remain as two subsets, with the larger subset becoming enlarged. In addition to pH, other factors may affect the size distribution, such as the use of different solvents (buffer or water), the concentration of the buffer, and severe temperature changes. Collectively, these data reflect that the chemical (especially lipid) compositions of these nanostructures vary widely.

It is well known that all eukaryotic cells (including those from animals, plants, and fungi) maintain a negative transmembrane potential of -40 to -80 mV¹⁰. Unsurprisingly, PDNPs also maintain a negative potential; their surface charges, as measured by zeta-potential, are around -12 mV (average value, ginger) to -17.1 mV (average values, -2.62 to -39.2 mV, broccoli)^{24,25}. When grape-, grapefruit-, and carrot-derived NPs were dissolved in a solution with an intestine-like pH, there was no change in the surface charge. However, in a solution with a stomach-like pH, these PDNPs exhibited a remarkable reductions in their negative charges¹⁶, at least in part because the negative charges were neutralized by the acidic solution.

3.2.2 Structure—Most PDNPs have a simple lipid bilayer similar to that of the eukaryotic cellular membrane, and are spherical in structure²³ [Fig. 4A.]. These characteristics are shared by mammalian cell-secreted exosomes and other extracellular vesicles (EVs)¹⁸. Synthetic nanoparticles (e.g., liposomes) may represent diverse and complex structures; examples of this are multilamellar vesicles (MLVs), small unilamellar liposome vesicles (SUVs), large unilamellar vesicles (LUVs), and cochleate vesicles⁹ [Fig. 5].

After lipid extraction and reassembly, PDNPs can be fabricated into various multilayer substructures. Q. Wang et al. reported that reassembled grapefruit-derived nanoparticles (GNPs) had a unique multi-layer flower-like structure, and that they could be used to deliver chemotherapeutic agents, siRNAs, and proteins to diverse cell types¹⁴. In another study, GNPs were coated with blood cell-derived plasma membrane to form a desirable multilayer substructure in which the edible fruit lipid-loaded drug was encapsulated within a plasma membrane-derived vesicle; the generated particles successfully homed to the inflamed tumor site²⁷.

3.3 Biological properties

3.3.1 Toxicity—A key issue in the use of synthetic nanoparticles and liposomes is their toxicity^{8, 11}. Researchers have used chemical modification (such as PEGylation or the addition of chitosan) to increase the target tissue bioavailability of nanoparticles or liposomes and reduce their biodistribution to nontarget tissues [Fig. 5]. However, these modifications significantly increase the immunogenicity of such particles, meaning that an immune response is induced and the clearance of these particles is increased upon subsequent injection⁸. In this regard, natural nanoparticles, such as PDNPs and mammalian exosomes, represent an excellent alternative for drug delivery.

As PDNPs come from edible plants, they are non-toxic and non-immunogenic. Supporting this, a study testing the effects of ginger-derived nanoparticles (GDNPs) on Colon-26 and RAW 264.7 cells (epithelial and macrophage cell lines, respectively) showed that treatment with GDNPs (up to 100 mg/mL) for 24 hours did not alter the viability of these cells¹⁵. In the same work, electric cell-substrate impedance sensing (ECIS) assays showed that GDNPs did not affect the integrity of the barrier function of Caco2-BBE monolayers, and propidium iodide (PI)/Annexin V staining revealed that GDNPs (up to 100 mg/mL) did not increase the percentage of apoptotic Colon-26 or RAW 264.7 cells¹⁵. These findings demonstrated that GDNPs appear to be non-toxic in vitro. Moreover, a toxicity evaluation of GDNPs in healthy mice in vivo showed that there was no significant change in colonic myeloperoxidase (MPO) activity or induction of pro-inflammatory cytokines (TNF- α , IL-6, or IL-1b; assessed at the mRNA and protein levels) in mice dosed with GDNPs by gavage for 7 days.¹⁵ The colonic tissues of these mice showed no change in hematoxylin and eosin (H&E) staining, intestinal epithelial cell (IEC) proliferation, or IEC apoptosis. Histological examination of H&E-stained heart, liver, spleen, kidney, and lung found no evidence of morphological or pathological changes in the GDNP gavage group compared with controls¹⁵.

Other in vivo studies demonstrated that reconstruction and surface decoration of GDNPs are safe. In one report, Nanoparticles formed by Nano-lipids extracted from GDNPs were loaded with siRNA-CD98 and orally administrated to mice²². Blood tests revealed no difference in the numbers of white blood cells, red blood cells, and hemoglobin in Nanoparticle- versus PBS-treated mice. The parameters that reflect hepatic and renal function, including blood urea nitrogen, total bilirubin, alanine aminotransferase and total protein, were also quite similar between the siRNA-CD98 group and the PBS group, indicating that reconstructed GDNP Nano-lipids are non-toxic. In another report, the surface of ginger Nano-lipids was further decorated with folic acid and loaded with the anti-cancer drug, doxorubicin²⁶. The generated FA-GDNP/Dox were able to target tumors more efficiently than GDNP/Dox in a mouse colon cancer model (CT-26). Furthermore, H&E staining of sections revealed that the FA-GDNP/Dox-treated group did not show any overt sign of tissue or cellular damage in the heart, liver, spleen, lung, or kidney. Together, these reports indicate that ginger-derived nanoparticles can be used as a non-toxic drug-delivery system.

In an in vitro study of the cytotoxicity of grapefruit-derived nanoparticles, cells treated for more than five passages did not exhibit any reduction of doubling time compared to that of the control group¹⁴. ATPlite assays and PI/Annexin V staining revealed that GDNPs treatment (up to 200 nmol/mL) had no significant effect on the cell proliferation or death rates of A549 and CT26 cells, compared to cationic 1,2-dioleoyl-3-trimethylammoniumpropane/dioleoylphosphatidylethanolamine (DOTAP/DOPE) liposome-treated cells. In the same report, in vivo mouse experiments showed that cationic DOTAP/DOPE liposomes altered the levels of alanine aminotransferase and aspartate aminotransferase at a dose of 50 nmol/mouse and above, whereas the same doses of grapefruit-derived nanoparticles did not induce these signs of liver injury. Pro-inflammatory cytokines were not induced by GDNPs treatment. Histological analysis of tissues from GDNPs-treated animals revealed no pathological change in the lung, kidney, liver, or spleen when compared with untreated mice. The liver hepatocytes appeared normal, and there was no sign of an inflammatory response. In lung

samples, no pulmonary fibrosis was detected. No necrosis was found in any histological sample¹⁴.

Collectively, these studies suggest that PDNPs are very safe, especially compared to synthetic nanomaterials, such as liposomes.

3.3.2 Cell uptake—Efficient uptake of nanoparticles by target cells is crucial for their use as a vehicle for intracellular drug delivery⁴⁴. Q Wang et al. evaluated the uptake efficiency of grapefruit nanoparticles (GNPs) by different cell types¹⁴. The cells were treated with PKH26-labeled GNPs (PKH26–GNPs) and examined by confocal microscopy or fluorescence-activated cell sorting (FACS). Quantitative analyses indicated that the majority of GL26, A549, SW620, CT26, and 4T1 cells internalized the PKH26–GNPs. Remarkably, more than 20% of the B cells and 14% of the T cells took up the GNPs within 12 hours of treatment; for these cells, such an uptake rate cannot be achieved by any current commercially available transfection reagent¹⁴. The same report also demonstrated that the efficiency of GNPs uptake was temperature-dependent: the uptake was very slow at 4°C, but it increased at a higher temperature. Moreover, metabolic energy was found to be required for this process. Comparison of uptake efficiencies revealed that more than 80% of GNPs were taken up, whereas only 40% of the cationic DOTAP/DOPE liposomes were internalized, suggesting that GDNPs have the advantage over synthetic liposomes in this regard.

3.3.3 Natural targeting properties—The ultimate goal of targeted therapy is to tailor nanoparticles such that they accumulate at the target site instead of at disease-unrelated peripheral tissues. Thus, tissue distribution studies are an important step in designing nanoparticles and determining their potential targets⁴. Studies showed that DiR-labeled grapefruit nanoparticles (GNPs) administered by intramuscular injection were predominantly localized in the muscle, while those applied via the intranasal route were located in the lung and brain¹⁴. Different clearance rates were observed in the brain and lung after intranasal administration, with GNPs showing a longer residency in the brain. FACS analysis indicated that intravenously injected GNPs were taken up by splenic liver cells; these particles remained in the liver, spleen, and lung for 20 days, but were cleared from the kidney and brain by days 1 and 5, respectively. Surprisingly, circulating DiR-labeled GNPs were still detectable at 7 days after intravenous injection. Moreover, these particles did not cross the placental barrier in pregnant mice, probably because certain efflux transporters, such as some P-glycoproteins (Pgps) and multidrug resistance-associated proteins (MRPs), are expressed differently on the placental barrier versus the blood-brain barrier (BBB) and other membranes that form physiological barriers⁴⁵. These efflux transporters of placental barrier may recognize the GNPs and immediately pumping them from placental cells back to the systemic circulation. However, they may not be able to identify and translocate synthetic lipids, potentially offering another safety advantage of PDNP over synthetic liposomes¹⁴.

3.4 Stability

Compared to naturally occurring plant nanoparticles or mammalian exosomes, the major advantages of synthetic nanoparticles seem to be their stability and the predictability of their

characteristics⁴⁶. However, excessive stability may have a negative effect by triggering undesirable toxicity⁴. PDNP-extracted lipids, on the other hand, are natural membrane lipids. They are absorbed rather than being metabolized/modified, and their degradation products are natural nutrients (fatty acids and phospholipids).

Moreover, recent studies have challenged the common belief that PDNPs are not as stable as synthetic nanoparticles. For example, Q Wang et al. discovered that grapefruit nanoparticles (GNPs) are more stable than cationic liposomes (DOTAP/DOPE) at 37°C in the presence of 10% bovine serum. The same study showed that GNPs were very stable at 4°C for more than one month, and that a loaded cargo (curcumin) maintained its biological activity during this period¹⁴. In another study, M Zhang et al. found that ginger-derived nanoparticles were very stable in stomach- and intestine-like solutions, and tolerant of freeze/thaw cycles¹⁵.

4. Applications

4.1 Treatment of IBD

Scientists have used our growing understanding of the pathophysiology of inflammatory responses in IBD to develop various therapeutic agents in the past decade. For example, TNF- α inhibitors have been developed to target this pro-inflammatory cytokine and suppress the trafficking of T-lymphocytes into the gut epithelium¹². Indeed, anti-TNF- α agents are among the most potent drugs for the treatment of IBD. However, these agents need to be administered systemically, and their use is limited by deleterious side effects. Thus, we need a targeted carrier system that can deliver such agents specifically to inflamed regions and for prolonged periods. Nanoscale particles isolated from edible plants, such as ginger, grapefruit, and broccoli, appear to be very promising candidates. Such delivery systems could significantly reduce the off-target effects of otherwise useful treatments.

M Zhang et al. showed that orally administered ginger derived nanoparticles (GDNPs) can target the colon, reduce acute colitis, prevent chronic colitis, and accelerate intestinal wound healing¹⁵. The contents of GDNPs (bioactive components gingerols, shogaols, and ginger miRNAs) have been shown to reduce pro-inflammatory cytokines (TNF- α , IL-6, and IL-1b), increase anti-inflammatory cytokines (IL-10 and IL-22), increase the survival and proliferation of intestinal epithelial cells in colitis models, attenuate damaging factors, and promote wound healing. In subsequent research, these researchers specifically delivered siRNA-CD-98 to colon tissues by package the siRNAs into nanolipids extracted from GDNPs, and found that administration of these particles efficiently reduced CD98 expression and prevented inflammation²². In addition, Z Deng et al. recently demonstrated that broccoli-derived nanoparticles can specifically target dendritic cells and regulate intestinal immune homeostasis to prevent DSS-induced colitis²⁴.

4.2 Anti-cancer therapeutics

Cancer is a leading cause of death worldwide⁴⁷, and it has a strong impact on human society. The current chemotherapeutics used against cancer share two intertwined drawbacks: toxicity and a lack of specificity. To improve the targeting efficiency and reduce toxicity, scientists have designed various drug delivery system. The first nanosized drug delivery

system was based on lipid vesicles (introduced in the 1960s), which later became known as liposomes¹. Since then, many polymeric nanoparticles, micelles, and other organic and inorganic biomaterials have been developed as nano-scale drug carriers in the fight against cancer⁴⁴. Four generations of liposomes, including stealth liposomes and PEG-coated liposomes, have been developed to date, in an effort to enhance the targeting efficiency and reduce the required dose of therapeutics. However, there are still many concerns about the adverse health effects of synthetic liposomes⁸.

PDNPs are released from edible plant cells and are not cytotoxic. Among them, GDNPs have shown the capacity to: target the colon; reduce colorectal tumorigenesis by reducing pro-inflammatory cytokine levels, inhibit IEC proliferation; and induce IEC apoptosis. Most importantly, they are free from immunotoxicity^{15, 26}.

The surface modification of PDNPs or the combination of plant-derived nanolipids with other nanosized biomaterials could potentially be used to tune the PDNPs to target different cancer sites without generating adverse immunotoxicity. For example, Q Wang et al. coated grapefruit-derived nanovectors with inflammatory chemokine receptor-enriched plasma membrane, and showed that the resulting particles could deliver the anti-cancer drug, doxorubicin, to an inflamed tumor site²⁷. In two crucial steps of delivery, namely the capturing of free-flowing leukocytes and transmigration, studies showed that leukocyte function-associated antigen-1 (LFA-1) and CXC chemokine receptor 2 (CXCR2), respectively, play key roles.

In another example, intravenously injected grapefruit-derived nanovectors with folic acid (FA)-enriched surfaces were used to deliver paclitaxel (PTX) to the tumor site. FA-enriched Nanoparticles take advantage of the strikingly elevation of FA receptors in a variety of tumor cells; they showed significantly increased targeting efficiency and were able to reduce the tumor volumes in mice xenografted with SW620 or CT26 cells¹⁴.

5. Future and perspectives

Among the nanotechnologies developed to date, PDNPs offer some unique features. Because they originate from edible plants and the human immune system had adapted to stimuli from edible plants, PDNPs can have negligible toxicity. The composition of the PDNP lipid bilayer is distinct from those of mammalian exosomes and synthetic liposomes. With their higher contents of glycolipids and phospholipids and their lack of cholesterol, PDNPs appear to be an ideal drug delivery system for the targeting of diseased tissue. PDNPs also contain abundant plant miRNAs and bioactive secondary metabolites, and thus may offer a novel library for drug discovery.

Currently, PDNPs are used to treat digestive system diseases, such as IBD and colon cancer [Table 1]. The metabolic fate of PDNPs seems to be consistent with that of synthetic liposomes, as both appear to be cleared through the liver and kidney. However, through innovative surface modification and reassembly, PDNPs can be directed to selectively aggregate at hard-to-reach tumors. One example is found in the intranasal administration of folic acid-embedded grapefruit nanoparticles (FA-GNPs) carrying the drug, JSI-124

(Cucurbitacin I). FA-GNPs effectively distribute to the lung and brain of mice; after 72 hours, however, they remain stable in the brain but not the lung, and thus demonstrated brain tumor-targeting ability¹⁴. Another example is the intravenous injection of plasma membrane-coated grapefruit nanoparticles to xenograft breast cancer and colon cancer model mice. Researchers found that an activated leukocyte trafficking pathway allowed these membrane-coated nanoparticles to quickly exit the peripheral circulation and accumulate at the inflamed sites of breast and colon cancer for more than 24 hours²⁷.

In the near future, it can be hoped that more and more scientists will participate in this new field, and that PDNPs will be developed as a mature drug delivery system that benefits patients.

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Mingzhen Zhang received his PhD degree at Huazhong University of Science and Technology. He is now an assistant professor in the Institute for Biomedical Sciences of Georgia State University. His research focuses on developing plant-derived oral drug delivery systems for intestinal inflammation and colitis-associated cancer therapy. He has published over 30 peer-reviewed papers.

Prof. Didier Merlin obtained both his MS and PhD from the University Paris VII, France. He is professor at Georgia State University and Research Career Scientist at Veterans Affairs Medical Center, Decatur, GA. He has established research program that focuses on understanding the pathogenesis of intestinal inflammation and formulating treatment strategies for intestinal inflammatory diseases. He has published over 170 peer-reviewed papers.

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Figure 1. Isolation of plant-derived edible nanoparticles^{12, 14, 16}.

Figure 2.
Lipids extraction, reassembly, and cargo loading of PDNPs²⁶.

Figure 3.

Lipids from ginger and grapefruit derived nanoparticles^{14,26}.

A) Lipid compositions from ginger nanoparticles; B) Lipid compositions from grapefruit nanoparticles C) Representative structures of phospholipids and glycolipids.

Figure 4.
Transmission electron microscopy (TEM) of PDNPs.
A) Typical TEM graph of ginger derived nanoparticles, and B) TEM graph of reassembled ginger Nano lipids loaded with siRNA CD-98.

Figure 5. Structure comparison of PDNPs, exosome, and liposome.
A) Major forms of PDNPs, B) Mammalian exosome, and C) Major forms of the artificial liposome.

Table 1

Drug delivered by plant derived nanoparticles

origin	modification	structure	Size (nm)	cargo	target	ref
Grapefruit	Coated; (with plasma membrane)	Multi-layer	<200	Doxorubicin, Curcumin	colon cancer	27
	Reassembled; embedded with folic acid	Multi-layer flower-like	<200	JSI-124 (Cucurbitacin D)	Brain tumor (GL26)	14
				Paclitaxel	Colon cancer (CT26, SW620)	14
				Luciferase gene siRNA	Colon cancer (CT26, SW620)	14
Ginger	unmodified	bilayer	~220-290	miRNA, gingerol, and shogaol	Colon	15
				Doxorubicin	Colon	26
				siRNA-CD98	Colon	22
Lemon	unmodified	bilayer	50-70	Proteins	Chronic myeloid leukemia	17
Broccoli	unmodified	bilayer	32.4; 18.3-118.2	Sulforaphane	Colon	24