Special Report

Therapeutic Delivery

Delivering natural products and biotherapeutics to improve drug efficacy

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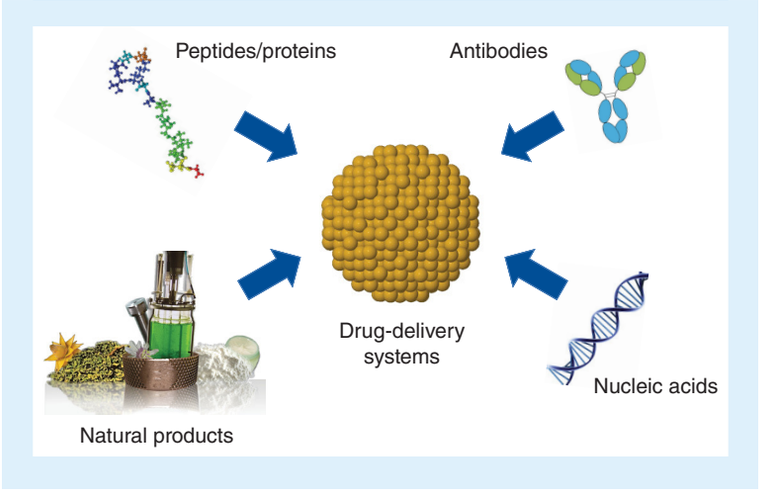
Due to the increasing problem of drug resistance, new and improved medicines are required. Natural products and biotherapeutics offer a vast resource for new drugs; however, challenges, including the cost and time taken for traditional drug discovery processes and the subsequent lack of investment from the pharmaceutical industry, are associated with these areas. New techniques are producing compounds with appropriate activity at a faster rate. While the formulation of these combined with drug-delivery systems offers a promising approach for expanding the drug developments available to modern medicine. Here, various classes of drug-delivery systems are described and the advantages they bring to small molecule and biotherapeutic targeting are highlighted. This is an attractive approach to the pharmaceutical industry and the rising trend in research in this area is examined in brief.



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New medicines are constantly being developed or repurposed, aimed at curing or preventing diseases or conditions where therapeutic product availability is lacking, or to reduce side effects, improve quality of life, reduce the burden on the cost of healthcare systems, while significantly extending patients' lives. However, drug discovery, research and development (R&D) can be an extensive process lasting over 7–10 years, with an average cost of $2.6 billion for each successful drug that reaches the market [1]. These substantial cost and time factors originate from the scientific, technical and regulatory challenges that are needed to fully understand the drug mechanisms of action and physiological interactions for complex diseases at molecular level. Achieving viable commercial success subsequently

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requires investment in highly sophisticated technologies, advanced manufacturing processes and innovative research approaches to tackle the ever-growing cost and time of the entire process.

Traditional medicine has been around for millennia, and in recent decades its use has increased in developing countries, while pharmaceutical companies have embraced synthetic and combinatorial technologies in favor of drug discovery programs based on natural products. However, there is a need for new medicines since growing drug resistance has rendered antibiotics virtually useless [2] and modern medicine is facing a crisis. One hurdle that slows down progress to the market of naturally derived candidate molecules arises when high-throughput screening reveals potentially excellent *in vitro* therapeutic properties that are then shown to be inactive, toxic or nonselective when evaluated *in vivo* or in the clinic [3]. One approach to overcome this is to improve the bioavailability and effects of these compounds, or reduce their toxicities by loading them into different types of delivery systems [4]. These have the ability to deliver the therapeutic agent to a particular site of the body at a specific rate and have the potential to enable multiple molecules with different roles to be included in a single delivery system [5]. Our research group has extensive experience in natural products as well as developing drug-delivery systems [6,7]. More recently, we have expanded our research to include biotherapeutic developments [8,9], and in this paper we discuss how these research fields have benefitted from being combined to meet the need to produce new and better drugs. In addition, there is evidence that delivery systems can help to reduce drug resistance and so have a critical role to expand the arsenal of therapies available to modern medicine.

Drug-delivery systems

Drug delivery refers to the use of a delivery tool or vehicle to carry a therapeutic agent and release it at a specific rate at a particular location [5]. Recently, there has been a significant rise in the use of delivery systems to deliver therapeutic agents for treatment of various diseases with many successful outcomes [10]. Drug-delivery systems can be used to facilitate the delivery of small compounds as well as large molecules such as peptides, nucleic acids, polymers and poorly water-soluble therapeutic agents from natural or synthetic sources [11]. Different types of delivery systems have been effectively used (Figure 1). These include: lipid-based nanoparticles such as liposomes, solid–lipid particles, micelles and niosomes; polymeric nanoparticles such as chitosan and atelocollagen; dendrimers; inorganic nanoparticles such as carbon nanotubes, metal-based nanoparticles, quantum dots and silica nanoparticles [12]. Polymeric nanoparticles (such as poly(lactic-*co*-glycolic acid), polyethylene glycol, polyvinyl alcohol, poly-l-lactic acid, polycaprolactone and chitosan) and liposomes have been the most tested in combination with natural products. The former are the most commonly used due to their biocompatibility, biodegradability and the ease by which they can be functionalized [13]. All these delivery systems can be characterized in terms of particle size, size distribution, surface charge, shape, stability and encapsulation efficiency [14].

Despite the advantages of the use of delivery systems in developing new medicines, some challenges still need to be addressed before their wide application becomes commonplace; these are related to the fast elimination of the delivery system by the reticuloendothelial system, especially for charged molecules [15]. In addition, toxicity and inflammation as a result of the use of specific types of delivery systems can cause tissue damage [16]. It is also critical for the delivery system to be well established and to meet regulatory considerations given to the manufacturing processes involved, such as physicochemical characterization, controlled drug release, stability, storage, large-scale production and manufacturing costs [17].

Impact of drug-delivery systems in natural product research

The plant kingdom provides an abundant source of natural products, which has fueled the drug-discovery process and resulted in a plethora of small drug-like molecules to complex polymers [18,19]. Other natural sources include marine organisms, fungi, microbes and invertebrates (such as insects and reptiles) [20]. Between the years 2000–2006, the natural products field was estimated to produce or be involved in R&D of approximately 50% of all small drug molecules, and 10 out of the 44 approved small molecules by the regulatory authorities in 2014 were derived from natural sources [21,22]. Conventionally, natural products are extracted from source materials, concentrated, fractionated and purified. At the early stages of research, a range of techniques are used to isolate pure natural products, and the pros and cons of these have been comprehensively reviewed elsewhere [18,23,24]. Soxhlet solvent extraction, maceration or infusions are utilized to extract constituents. This is usually followed by TLC and NMR analysis for chemical structure elucidation, and MS as a confirmatory technique; these are examples of the core techniques that are usually utilized [7,25], along with open-column chromatography (gel filtration and vacuum liquid separation). For further separation and isolation of pure compounds, modern chromatography

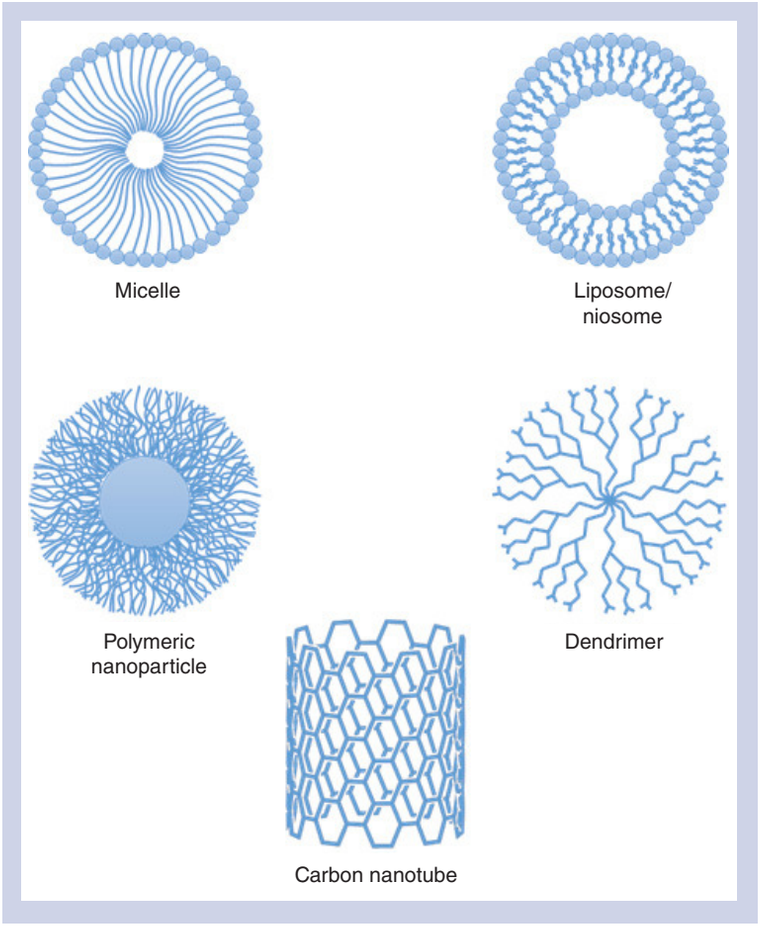

Figure 1. Different types of delivery system, suitable for use with natural products and biotherapeutics.

(medium-pressure and high-pressure liquid chromatography systems) is used. In order to identify a particular compound's location within tissues, techniques such as MALDI-TOF MS are usually employed. Compounds isolated in this way from different natural sources have consistently shown promising therapeutic potential against a range of diseases including cancer, hypertension, diabetes and infections [26]. In evaluating extracts and isolated compounds, a range of bioassays are employed to determine the potential therapeutic activity present [27]. For extensive evaluation of mechanisms of action, new technologies such as molecular biology (polymerase chain reaction and RNA-sequencing) have been introduced into this field. These latter methods can show which genes are affected and therefore aid in tailoring the subsequent research toward specific disease pathways. Metabolomics is also used to advance the understanding and development of any potential lead molecules [28].

Use of advanced techniques in natural product research has increased the rate of identification of bioactive compounds, however, this has not translated into an increase in marketable products. The reasons for this are diverse. Some issues include solubility, bioavailability *in vivo,* hydrophilicity, physical and chemical instability. In addition, poor pharmacokinetics, first-pass metabolism, accumulation in tissues or low targeting efficacy can hinder therapeutic benefit. Drug-delivery systems can be employed to enhance bioavailability and pharmacological activity of molecules by enabling them to cross cellular membranes of target cells. Diseases that have benefitted from combination of drug-delivery systems with natural products include diabetes [29], cancer [30–32], neurodegenerative diseases [33] and infections [13] to name a few. Table 1 provides some examples where delivery systems have been used with selected natural compounds. In addition, Bilia has recently reviewed the role of drug-delivery systems to improve effectiveness of natural products [30].

One extensively studied natural compound encapsulated into different delivery systems to improve bioavailability via different tissues has been curcumin, a yellow pigment present in the spice turmeric (*Curcuma longa)* [34]. A recent review provides details of the application of delivery systems with curcumin in various diseases [35].

Table 1. Some examples of selected natural compounds being investigated with delivery systems.

Plant/constituents	Biological activity	Delivery system used	Efficacy of the delivery system	Ref.
Curcumin	Anticancer	Liposomes	Long systemic residence time and high entrapment efficiency	[40]
Curcumin	Anticancer and antioxidant	Phytosomes	For enhanced antioxidant action and bioavailability	[41]
Curcumin	Anticancer	Emulsion system	To improve absorption	[42]
Curcumin	Anticancer and antioxidant	Transferosomes	To enhance permeation	[43]
Curcumin	Anti-inflammatory	Micropellatization	To enable sustained release and for specific locus targeting	[44]
Quercetin	Anticongestion and antianxiety	Liposomes	To increase efficacy, bioavailability and reduce side effects	[45]
Quercetin	Anti-inflammatory and antioxidant	Microspheres	To enhance permeation	[46]
Quercetin	Antioxidant	Emulsion system	To enhance permeation	[47]
Ginkgo biloba	Brain activator	Nanoparticles	To improve cerebral blood flow and metabolism	[48]
Ginkgo biloba	Antiasthmatic, antidiabetic and cardioprotective	Phytosomes	To improve efficacy	[49]
Wogonin	Anticancer	Liposomes	To increase duration of action	[50]
Embelin	Antifertility and antibacterial	Phytosomes	To enhance solubility	[51]

Drug-delivery systems have also been used to stabilize components and increase therapeutic activity. For example, essential oils are often unstable and susceptible to degradation, hence, encapsulation into colloidal systems is desirable [36]. Sinico *et al.* used multilamellar liposomes to increase therapeutic activity of *Artemisia arborescens* essential oil against Herpes simplex virus type 1. They found that the oil enhanced *in vitro* activity by increasing the cytoplasmic viral barrier penetration of the active components of the plant [37]. Rajendran *et al.* used chitosan nanoparticles to encapsulate a methanolic extract of *Ocimum sanctum.* This formulation was demonstrated to have significant antibacterial activities against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* compared with the free unencapsulated extract [38]. One issue that can be highlighted from this area of research is the extensive use of extracts as opposed to pure compounds that makes it a problem from a regulatory perspective, as relative quantities of the active(s) may not be known. However, often several compounds present in extracts act synergistically and, so it is critical to understand synergy and adverse effects as well as the mechanisms of action when combining extracts of unknown composition and batch-to-batch variation with delivery systems [39].

Often, compounds are found to be biologically active, however, they are also highly water soluble but have low absorption (like flavonoids, tannins and terpenoids) and in some cases such as polysaccharides can have high molecular weight. This renders them unable to freely cross lipid membranes resulting in poor absorption and loss of biological activity when tested *in vivo* [52]. In addition, some compounds are highly toxic when exposed to normal cells [53]. In this respect, delivery systems can be employed to improve absorption, reduce toxicity and increase selectivity of some of these compounds.

Delivery systems can also provide the potential to develop therapeutics with multiple purposes. Radwan *et al.* described several uses of ganoderic acids (triterpenoids) extracted using methanol from the fungus *Ganoderma lucidum,* combined with nanoparticles for cancer therapy [54]. This particular mushroom has been in used in traditional medicine for centuries. By encapsulating a lipophilic near infrared dye within hydrophobic pockets in the polymeric matrix of polyacrylic acid-coated iron oxide nanoparticles (IONPs) together with the anticancer drug, the nanoparticles can be used for therapy as well as imaging. The development of ganoderic acid-infused nanoparticles has also enabled enhanced targeting and reduced toxic side effects by combining the anticancer drug and cancer cell-targeting moieties. The concept being that drug doses used to kill tumor cells can also affect normal cells, but use of a drug-delivery system allows accumulation of the drug in cancer cells only. In this case, folic acid was coencapsulated with gum arabic (GA) to target the folate receptor that is overexpressed in cancer cells, thus enabling higher drug doses of GA to accumulate in the tumors and reduce off-target side effects [27]. A micelle-based delivery system has also been used for enhancing the bioavailability and biodistribution of other mushroom compounds such as *Flammulina velutipes* sterols [54]. In these examples, delivery systems can be seen to

have multiple purposes including their use to enhance stability, provide sustained delivery, reduce toxicity, improve targeting and offer protection from chemical or physical degradation of complex natural compounds [52,53]. The main challenge of working with mushrooms is the need to produce consistent quality of therapeutic molecules. These include difficulty in cultivating mushrooms, requirement of good manufacturing practice of cultivation methods and establishing sound methods of isolation, purification, identification and testing of bioactive compounds to elucidate the mechanisms of action [55]. The greatest problem with this area of research is the lack of standardization and this has limited the clinical trials carried out [56]. Further examples of the applications of nanoparticles for delivery of natural products have been reviewed elsewhere [52,53]. One area that might be of relevance include potent natural antibiotics, which have limited use due to insolubility issues and have required new synthesis procedures to improve their bioavailability or use of delivery systems to improve targeting and reduce the induction of antibiotic resistance [57,58].

Impact of drug-delivery systems in biotherapeutics developments

The introduction of targeting moieties (such as monoclonal antibodies, peptides and proteins) into the surfaces of delivery systems can be employed to target the therapeutic agents to certain tissues, which will increase the selectivity of these plant extract formulations. This can increase the accumulation of the encapsulated product at the desired site, which will enhance its efficacy [59]. This introduces another face of the drug discovery coin that relies on the development of novel biotherapeutics. The shift in prominence toward the development of protein therapeutics (including antibodies) or nucleic acid-based drugs is in part reflected by the growing prevalence of biologic agents in the portfolio of major biopharmaceutical companies. The annual number of first approvals was in the range of 5–8 in 2014 onward, with 53 novel antibody therapeutics in Phase III studies in 2016, and approximately 210 novel antibody therapeutics in each of Phase I and II of clinical development [60]. Financially, the global sales revenue for all monoclonal antibody products was nearly US$75 billion in 2013, and expected to reach $125 billion by 2020. This unprecedented attraction to antibodies originates from the remarkable structural flexibility of these proteins to selectively recognize different antigen classes such as proteins, carbohydrates and lipids and challenging haptens like pharmaceutical small molecules, pesticides and even biomarkers that can contribute to the potential detection of life on other planets like Mars [61,62]. Antibodies not only represent potential therapeutics but can be implemented in diverse bespoke applications such as immunodiagnostics, biosensors, photothermal therapies and nanoparticle conjugation for drug delivery.

Several advancements have been achieved in the application of drug-delivery systems for biotherapeutics delivery. This can be desirable to enhance the onset of therapeutic action and enables administration of biomolecules via noninvasive routes such as oral or inhalation to avoid intravenous administration [63]. For example, biotherapeutics such as growth hormone, glucagon or α_1-antitrypsin can be effectively delivered by inhalation therapy through the use of an appropriate delivery system [64]. Drug-delivery systems can be used to improve the pharmacokinetics of highly degradable compounds such as peptides and proteins through protecting these agents and increasing their half-lives [65]. One important development has been the delivery of insulin by noninvasive oral or inhalation routes. Insulin oral delivery is considered to be the optimum route of delivery as it will go directly to the liver, which is the main site for insulin action [66]. In addition, it will overcome the needle-anxiety barrier that is associated with delivering insulin subcutaneously [67]. However, it is highly challenging to deliver insulin using this approach for a number of reasons, including pH inactivation, harsh chemicals in the digestive tract, enzymatic and cellular barriers [68]. All these can lead to very low insulin bioavailability (<1%) [69]. Recent studies have been carried out using liposomes, microspheres and microemulsions to increase the bioavailability of insulin when it is administered orally. Encapsulation of proteins like insulin in such vehicles will protect them from the harsh stomach environment [69]. In terms of inhaled insulin, the lungs have been targeted for insulin delivery due to the large surface area (100 m^2), and the onset of action following inhalation [70]. Inhaled insulin falls into two formulations, dry powder or liquid. Dry powder formulations are considered to be more stable and require less complex devices for delivery [71]. Pfizer developed a product called Exubera (normal human insulin in dry powder formulation) that was the only approved inhaled insulin therapy to have reached the market [70,71]. It obtained approval by the US FDA in 2006. However, due to very low sales and poor patient acceptance, the product was discontinued in October 2007. A number of reasons were responsible for Exubera's failure, including the bulk and size of the inhaler device, it required weekly cleaning, patients had to perform more than one inhalation per dose to achieve the therapeutic effect [71]. Nevertheless, the potential is there when a suitable medical device is designed to overcome these problems.

Another example of the use of drug-delivery systems with biotherapeutics is antibody–drug conjugates (ADCs). ADCs are considered a prototype of Paul Ehrlich's 'magic bullet' theory for tailored and targeted drug delivery to combat invading microbes or malignant cells. ADCs have proven their value in fatal diseases like cancer, where the standard chemotherapies are notoriously associated with limited selectivity against cancer cells leading to a small therapeutic window that can subsequently limit their efficacy [72]. The ADCs concept relies on combining the high specificity and stability profile of antibodies with the antitumor potency of very cytotoxic small-molecule drugs 'warheads' to create a selective treatment with an increased therapeutic window and reduced off-target toxicity [73]. Albeit the intensive focus of ADCs development has been on cancer, their exploitation has been extended to other applications through the optimization of immunosuppressants [74], and antibody–antibiotic conjugates [75]. The first-generation ADCs, represented by Wyeth's Mylotarg® (gemtuzumab–ozogamicin), revealed disappointing, insufficient potency and toxicity effects that led to withdrawal of this conjugate from the market in 2010. The development of more potent (100–1000 fold) cytotoxic agents, like auristatins and maytansinoid, have led to the approval of two second-generation ADCs, Acetris® (brentuximab–vedotin) and Kadcyla® (trastuzumab–emtansine), by both the FDA and EMA [76,77]. Even with these approvals, the second-generation ADCs are still associated with drawbacks such as heterogeneity resulting from stochastic coupling strategies, limited penetration of solid tumors and the development of resistance [78]. Consequently, third-generation ADCs have emerged with significant focus on site-specific conjugation in order to confirm homogenous ADCs with well-defined antibody–drug ratio [79]. The high potential of these conjugates has revolutionized the biopharma R&D, with currently over 50 ADCs undergoing clinical evaluation. According to a recent market analysis, the ADC global market is projected to display a robust growth represented by a compound annual growth rate of 22% during 2017–2022, primarily driven by a large number of ADC candidates in the pipeline, rising numbers of cancer patients and a wider therapeutic window offered by these ADCs [80]. Despite the wide variances among the recent conjugation approaches, which were comprehensively reviewed elsewhere [81,82], their differences have mainly focused on target selection, warhead optimisation, design of suitable linkers, selection of specific antibodies and site-specific and alternative conjugation strategies to enhance potency [72,83].

ADCs are continuously revealing great potential in both oncological and nononcological indications. A great number of drug candidates have failed in preclinical or clinical stages due to lack of selectivity or toxicity issues. For this specific reason, the ADC strategy provides a valuable opportunity for these molecules to be re-evaluated, especially with the significant advancement of antibody engineering and conjugation technologies.

Another example for the application of drug-delivery systems with biotherapeutics includes delivery of several types of nucleic acids such as plasmids, nucleotides or RNA. Drug-delivery systems enhance the application of these nucleic acid therapeutics by facilitating their accumulation and uptake by the target site. One interesting example is the use of these systems for the delivery of short-interfering RNA (siRNA). siRNA consists of short double-stranded nucleic acids that have the ability to silence specific protein expression through a mechanism called RNA interference [84]. Through this mechanism, siRNA in the cytoplasm of the target cell can cause the degradation of a specific mRNA that is complementary to the antisense strand of the siRNA, which will result in the degradation of this mRNA with the subsequent inhibition of the protein being expressed by this mRNA [85]. This results in significant research for the application of siRNA in treating different diseases such cancer. However, siRNA is a very hydrophilic molecule that cannot pass through the target cell membrane. Moreover, siRNA is highly susceptible to degradation by nuclease enzymes which limit their half-lives [86]. Drug-delivery systems such as liposomes or niosomes have been successfully applied to the delivery of siRNA in which the encapsulation of siRNA into these systems can protect them from the early degradation as well as enhance their uptake by target cells [87]. This is still an area of ongoing research, with very promising outcomes.

Conclusion

Although biotherapeutics have been developed for decades, while natural products have been around for millennia, drug-delivery systems have not been extensively utilized with them. This is particularly true for natural products, where prior to 2015 publications in this area were sparse. Since then, the advantages of combining these systems are being actively explored and are expanding the repertoire of new and safer medicines.

Future perspective

Drug discovery and drug delivery are now proceeding in parallel to improve efficacy of newly discovered drugs, increase their selectivity and bioavailability or reduce their toxicity. Delivery systems not only increase the ef-

fectiveness of active compounds but also enable many compounds that have been discarded because of the lack of efficacy to be reinvestigated. This is an upcoming approach that has a promising future. It also provides an opportunity for pharmaceutical companies to revisit candidate molecules they have previously abandoned. This is particularly relevant to the antibiotic sector, where previously potent antibiotics have had limited use due to issues with solubility. Improved targeting with drug-delivery systems can help revitalize this area, particularly where organisms are multidrug resistant. A recent comprehensive review examines the use of nanoparticles in infection control, showing that there is renewed interest in the field [58].

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Executive summary

The need for new medicines

- Increased drug resistance, for example, antibiotics, cancer chemotherapies.
- Pharmaceutical companies have moved away from drug discovery based on natural product research, however, traditional medicine is thriving in developing countries.
- New developments rising in terms of biotherapeutics.

Delivery of natural products

- Use of drug-delivery systems can improve bioavailability, biodistribution, therapeutic activity and stability of natural products.
- Introduction of multiple components into drug-delivery systems to enable a range of roles (e.g., imaging) as well as therapy.

Delivery of biotherapeutics

- Offers alternative routes of administration.
- Improved targeting of molecules.

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