Flavonoids-Based Delivery Systems towards Cancer Therapies

Subjects: Biochemistry & Molecular Biology

Contributor: Miguel Ferreira , Diana Costa , Ângela Sousa

Cancer is the second leading cause of death worldwide. Cervical cancer, for instance, is considered a major scourge in low-income countries. Its development is mostly associated with the human papillomavirus persistent infection and despite the availability of preventive vaccines, they are only widely administered in more developed countries, thus leaving a large percentage of unvaccinated women highly susceptible to this type of cancer. The treatments are based on invasive techniques, being far from effective. Therefore, the search for novel, advanced and personalized therapeutic approaches is imperative. Flavonoids belong to a group of natural polyphenolic compounds, well recognized for their great anticancer capacity, thus promising to be incorporated in cancer therapy protocols. However, their use is limited due to their low solubility, stability and bioavailability. Several types of flavonoid-based delivery systems are being developed for anticancer therapy, namely for cervical cancer. The consideration of these new forms of delivery systems allows for the use of fewer flavonoids as well as permits the use of ligands that efficiently target these systems to cancer cells, therefore reducing the risk of toxicity in healthy cells and improving their therapeutic effect. A variety of delivery systems for the encapsulation of these drugs can be explored, depending on the material considered and the properties exhibited by the drug.

anticancer activity cervical cancer delivery systems flavonoids

1. Lipid-Based Delivery System

Delivery systems based on lipids include three large groups: liposomes, solid lipid-based nanoparticles and emulsions, and in each of these groups, there may still be divisions into subgroups [1].

1.1. Liposomes

Liposomes are spherical vesicles typically made up of emulsifiers and a bioactive compound dissolved in an organic solvent. Liposomes are usually made of at least one lipid layer which allows them to be used very often for the encapsulation of both hydrophilic and hydrophobic drugs [2][3]. The constitution of liposomes essentially includes phospholipids and may also contain cholesterol or even a hydrophilic polymer such as polyethylene glycol (PEG). In this case, they can be called stealth liposomes, as the junction with this type of polymer prolongs the circulation time of liposomes increasing their efficiency. Regarding the formation of liposomes with phospholipids and cholesterol, their use guarantees structural and biological stability, thus giving rise to biocompatible transport systems displaying high efficiency of encapsulation for all types of flavonoids, which is always superior to 80% and in many cases greater than 95% [4]. In addition, liposome stability also reduces the ability of systems to form

aggregates, be toxic for therapeutic application and increase the capability of controlled release of the encapsulated drugs. However, they still show some physical and chemical instability leading to aggregation issues over time, as well as some drug degradation over the storage period [4][5][6]. Nonetheless, the use of this type of system is also favoured by its size and polydispersity index (PdI) which vary between 100 and 200 nm and 0.1 and 0.25, respectively. These favourable characteristics confer these systems' ability to pass through the pores present in blood capillaries more easily and accumulate in tumors, which usually feature a much higher number of pores in the surrounding blood capillaries [4][5].

In vitro and in vivo studies demonstrated a large number of benefits of liposome application, namely due to their high capacity to encapsulate flavonoids, leading to the increase in therapeutic effect and low toxicity. Viability studies in cervical cancer cell lines (HeLa) demonstrate that, by encapsulating flavonoids into liposomes, a lower concentration of flavonoids is needed to obtain 50% inhibition (IC_{50}) in cellular viability, which can decrease from 200 µM in the case of free quercetin to concentrations around 100 µM after a 24 h incubation. For increased incubation times, an IC_{50} can be obtained for concentrations of 14 µM using liposomes made of triglycerides, lecithin, PEG and folic acid [5][7]. Furthermore, in vivo studies show that quercetin-loaded liposomes made of PEG, cholesterol and soybean phosphatidyl-choline induce a reduction in tumor size of about three times compared to the administration of free quercetin [2][7]. Other liposomes constituted of soybean phosphatidylcholine and cholesterol show a tumor volume decrease of about 50% [2]. In the context of other cancers, the use of liposomes has also been studied and shows equally a high rate of encapsulation, low toxicity and high percentage of cell inhibition in in vitro studies [8][9][10]. Overall, a wide variety of emulsifiers have already been tested in anticancer therapies, such as lecithin or PEG derivatives. In some cases, chitosan coatings are explored in order to increase their bioavailability and stability in vivo, thus opening novel possibilities to improve the application of these systems in studies related to cervical cancer.

1.2. Lipid-Based Nanoparticles

Another type of delivery system widely investigated is solid lipid-based nanoparticles, which are characterized by forming solid lipid systems at room temperature. They can be divided into solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). The main differentiating feature between SLNs and NLCs is the fact that SLNs form totally solid systems with a perfect crystal structure, while NLCs have a non-ideal crystal structure leading to the development of systems with a solid and a liquid zone, increasing drug loading and decreasing water content [11][12][13]. Compared to liposomes, SLNs makes it possible to avoid the need to use organic solvents, therefore reducing the cytotoxicity they present while maintaining a high capacity to encapsulate both hydrophobic and hydrophilic drugs [6]. Therefore, SLNs have high bioavailability, low cost/easy production on a large scale, and high capacity to maintain a controlled release [6]. In terms of constitution, both SLNs and NLCs are mainly composed of non-ionic surfactants such as Polysorbate 80®, Poloxamer 188, Tyloxa-pol and sometimes, made up of lecithin or phosphatidylcholine as their amphoteric nature improves the stability exhibited by the system [14]. In the case of NLCs, liquid lipids are used to make the liquid part of the system, such as oleic acid, olive oil, almond oil and cetiol® [6]. Despite all their advantages and their high tolerance in in vitro and in vivo tests for being composed of

natural compounds, the toxicity of surfactants and other excipients necessary for their production must be considered. In addition, there is still a risk of aggregation and recrystallization [1][15].

The application of SLNs and NCLs in in vitro and in vivo assays related to cervical cancer is not yet fully tested. However, taking into account studies in other types of cancer, their use proved that it was possible to obtain a high rate of encapsulation (greater than 90%) and a drug loading greater than 10% and, in some cases, greater than 20% [11][16][17][18][19]. A significant reduction in cell viability in vitro and a significant reduction in tumor volume in in vivo experiments were also proved [11][16][17][18][19]. These data support the fact that the application of SLNs and NCLs in anticancer therapies against cervical cancer may be of special interest and deserves to be researched.

1.3. Emulsions and Nanoemulsions

Additionally, to these two types of delivery systems, there is also the group of emulsions and nanoemulsions. This class benefits from the interaction between water and oils through the addition of an emulsifier such as polyglyceryl-10 laurate or PEG 660-stearate to form systems that contribute to flavonoid solubilization and increase their bioavailability [20][21]. Although both emulsions and nanoemulsions have the disadvantage of not being thermodynamically stable systems, dissociating over time, their application results in a high rate of flavonoids encapsulation; normally, greater than 80% decreased aggregation while avoiding gravitational separation [20]. Being distinguished by their size, emulsions have sizes greater than 200 nm while nanoemulsions have sizes smaller than 200 nm. Depending on the intended delivery site for the flavonoids, the use of different ratios of emulsifiers can be tested so that systems may present a suitable size range to more easily reach the target site and accumulate therein [3][22].

Nanoemulsions constituted of soybean phosphatidylcholine and cholesterol have been tested in cervical cancer cells and only showed a 10% viability reduction by using a concentration of 200 µg/mL, although they showed low toxicity, potentiating further studies to increase its therapeutic capacity [20]. In other types of cancer, emulsions and nanoemulsions have shown the same physical characteristics in terms of size, encapsulation rate and stability. Despite this, they possess a high anticancer potential, which is demonstrated by the significant decrease in viable cells. For instance, in melanoma cells, a significant reduction for concentrations above 50 µM of drug encapsulated in the emulsion of lecithin, castor oil and PEG 660-stearate was obtained and in human colorectal carcinoma cells, it was possible to record a reduction of viability around 60% for concentrations of 25 µM drug encapsulated in an emulsion of Labrasol®/Tween®, lecithin and Miglyol® 812 [21][23][24]. Thereby, formulations with new emulsifiers and different ratios can be tested to improve flavonoid encapsulation, potentiate their anticancer effect, and increase therapeutic index. In this way, significant advances can be achieved toward the development of anticancer therapies based on these molecules.

2. Polymer-Based Nanoparticles

Polymers have been widely used for flavonoid encapsulation. Polymer-delivery systems were the first and most applied/explored vectors. In general, these delivery systems are constituted of nanoparticles and spherical walls

with an outer polymer and a core composed of a hydrophobic surfactant that provides high stability, solubility and bioavailability to the vast majority of flavonoids [25][26][27]. In order to obtain a high final concentration, the formation of this type of system makes use of the flavonoids dissolution in an organic compound, normally ethanol, which is later removed by evaporation under vacuum, or by spray or freeze-drying to reduce the systems cytotoxicity [3][28]. The characteristics of these polymer-based nanoparticles can vary greatly according to the type of polymer considered and can be divided according to their nature, thus being distributed in natural, synthetic or inorganic-based polymers [1][29].

2.1. Natural Polymers

Systems based on natural polymers, also called biopolymers, form very diversified systems according to the materials used in their composition in the case of biopolymers are proteins and polysaccharides [1][3]. These polymers guarantee high biocompatibility and biodegradability in addition to low cytotoxicity, making their use in in vitro and in vivo assays widely researched [28][30][31]. However, the use of systems made purely on the basis of proteins or polysaccharides is unusual. In many cases, a combination with another type of biopolymer or with a synthetic or inorganic-based polymer is considered [31]. The use of a polysaccharide such as chitosan is very common, appearing in conjugation with a protein, a polysaccharide or another type of polymer. It was found that this approach provides greater biocompatibility, biodegradability and stability to the systems. In addition, chitosan has mucoadhesive properties that contribute to enhanced delivery of systems to specific/mucosal target sites [31][32][33][34]. The conjugation of a biopolymer with another type of polymer is therefore very common and leads to the formation of systems with better performance for both in vitro and in vivo assays. Generally, they present sizes below 200 nm and high stability and controlled drug release, which facilitates delivery to target cells [31][32][35][36]. Natural polymeric systems based on polysaccharides appear as an important way to guarantee greater bioavailability of systems, often applied in conjugation with other polymers or inclusion complexes [37][38][39].

The use of proteins such as BSA (bovine serum albumin), silk fibroin, keratin and gliadin have already been tested on cervical cancer cell lines. Only silk fibroin has been used without any other associated compound, essentially due to the fact that it is a copolymer bearing hydrophobic and hydrophilic blocks, which facilitates the encapsulation of flavonoids and increases their stability [28][30][31][32][35][40]. However, this polymeric system made of silk fibroin showed a relatively large size compared to other systems as well as a relatively high IC_{50} of 250 µg/mL [30]. The use of polysaccharides in cervical cancer was based only on the conjugation of chitosan with another polymer, such as quinoline or gliadin, and the simple use of focoidan, a polysaccharide with chitosan-like properties, which showed a relatively low IC_{50} of 20 µg/mL, despite the considerably high size of 221 nm [31][32]. In addition to the size reduction that occurs due to the conjugation of various types of polymers, this conjugation also leads to a higher encapsulation rate, normally around 80%, which is higher than the encapsulation rate of 21.81% registered in systems consisting solely of one protein [28][30][31][32][35][40].

2.2. Synthetic Polymers

Another type of polymer-based nanoparticles results from synthetic polymers where PEG is the most prominent, often used in conjunction with other types of systems to increase flavonoid solubility and allow a better encapsulation rate [26][41]. In PEG-based systems, the rate of encapsulation is generally high, normally above 90%, although they present a low rate of degradation and low compatibility [26][41][42]. However, the reduction of these negative effects can be significantly mitigated through the formation of systems consisting of a mixture of polymers in order to make their use viable in in vitro and in vivo assays [1][26][29].

In vitro and in vivo studies on anticancer therapies against cervical cancer have already been carried out, with formulated PEG systems conjugated with poly lactide-co-glycolide, poly e-caprolactone conjugated with PEG 1000 succinate, and also gelatine modified pluronic systems [25][26][40][41][43]. In addition, other carriers were also tested in which other types of compounds were used in conjugation with PEG or any of its derivatives. Systems where PEG is not the main compound are mentioned in the section pertaining to this type of compound. Cell viability assays show that it is possible to obtain reduced IC_{50} values close to 10 µM using systems consisting only of synthetic polymers, namely made of PEG and poly lactide-co-glycolide, due to the high blood circulation time that these systems achieve. They also revealed a high capacity to be conjugated with specific ligands, as is the case of folic acid, which promotes an active targeting of systems towards cancer cells, considering that these cells have a higher number of folic acid receptors compared to healthy ones [26]. In in vivo studies, the use of poly e-caprolactone and PEG 1000 succinate systems show a tumor weight reduction four times greater than the administration of the drug in its free form [43].

2.3. Inorganic Polymers

Delivery systems based on inorganic polymers are a class exhibiting larger diversity of applications, namely in targeted drug delivery, tissue repair, hyperthermia and magnetic resonance imaging [44]. This type of carrier is essentially made up of gold, copper and even iron oxide nanoparticles that commonly form delivery systems as nanoparticles or nanotubes [14][44][45][46][47]. Although these systems have a high aggregation capacity, undergo oxidation and display low stability and biocompatibility, their coverage with polymers such as PEG significantly overcome these disadvantages, providing a place for flavonoids and ligands to bind and thus turning these systems viable [44].

Being characterized by having very small sizes compared to other systems, inorganic polymeric vehicles generally have sizes below 50 nm as well as encapsulation rates between 70 and 80% [14][44][45][46][47]. Iron oxide magnetic nanoparticles coated with various polymers, such as BSA, a-cyclodextrin, citric acid, poly citric acid, PEG or 3-aminopropyl triethoxysilane have been explored to transfect HeLa cells [44][45][48]. Cell viability assays varied greatly according to the system used, and an IC_{50} of 10 µg/mL was obtained for the process mediated by nanoparticles based on iron oxide and BSA [40].

Polymeric carriers have been also widely researched for other cancers, and several systems have already been tested to encapsulate flavonoids and target them to cancer cells. Following this, various polymers are highlighted, namely PEG and chitosan, both mentioned above [27][34][36][42][49]. The incorporation of chitosan in the formation of

systems and its conjugation with most types of polymers was tested, for instance, the common conjugation with tripolyphosphate and functionalization with PEG or some type of inclusion complex in order to increase the stability and solubility of encapsulated flavonoids [33][50][51][52]. In addition to the compounds already mentioned, poly(lactic acid), poly(lactic-co-glycolic acid) and polycaprolactone have been also studied, and these systems are also normally conjugated with PEG or one of its derivatives [27][29][42][53][54][55][56]. In vitro assays mediated by these systems showed a decrease in cell viability similar to the one recorded in in vitro assays on HeLa cells, indicating a high coverage of this type of system for any cancer cell.

3. Micelles

Micelles are constituted by amphiphilic molecules and, depending on the polymeric or lipid nature of these molecules, the formulated systems can be classified as polymeric micelles or lipid micelles [1][57][58]. Due to the molecules that make up the micelles, they offer a hydrophobic core with a high capacity to encapsulate therapeutic agents with low solubility and high hydrophobicity [57][58][59]. For that reason, micelles are ideal for the encapsulation of flavonoids, assuring increased stability of flavonoids as well as a suitable and more consistent release profile [57][58][59]. On the other hand, the outer part of micelles, constituted by the hydrophilic zone of molecules, offers high protection and stability, leading to increased bioavailability of the system [57]. In terms of advantages, this type of delivery system is highly favoured by its small size, often below 100 nm, as well as its high thermodynamic stability, a high drug loading capacity, an increased cellular uptake, and an easy large-scale production. However, formulations vary immensely according to the considered ratios of components, and it may be necessary to perform an intensive study until the ideal ratio is revealed [1][57][58].

In vitro experiments on cervical cancer cells demonstrate that the delivery mediated by micelles constituted of chondroitin sulfate and cholesterol led to a 20% viability reduction at a concentration of 200 µg/mL [60]. In addition, these micelles show a relatively low percentage of encapsulation although they present a high percentage of drug loading, 30.6% and 23.4%, respectively [60]. In vitro assays in other types of cancer, mediated by micelles show better results: an IC_{50} of 110 µM and 21.24 µM in breast cancer and lung cancer cells were achieved, respectively [57][58][59][61][62]. Furthermore, these studies also indicated more favourable physical conditions of formed micelles, with sizes smaller than 100 nm and an encapsulation efficiency greater than 80% [57][58][59][61][62]. The performance of micelles in in vivo studies has also been monitored, and a size reduction higher than three times compared to the use of flavonoid in its free form was observed [63]. The application of micelles for flavonoid encapsulation and delivery needs to be further studied before its broad use. The set of properties displayed by these systems must be optimized to increase their performance as a targeted delivery vector and this certainly will have repercussions on their therapeutic potential against cervical cancer.

4. Inclusion Complexes

Inclusion complexes are defined as delivery systems characterized by having a host molecule with the ability to trap another molecule using non-covalent forces. Flavonoids can be “trapped” into these complexes due to their

capacity to establish hydrophobic interactions with them [1][3][29][38]. The main advantage of inclusion complexes is the fact that they have a cone shape open on both sides with a hydrophilic external surface and a hydrophobic internal surface. Flavonoids can thus be “trapped” in the internal cavity which considerably supports the improvement of their solubility, stability and bioavailability [38]. However, inclusion complexes have some disadvantages, namely a limited encapsulation rate for larger flavonoids, such as glycosylated compounds. Furthermore, they generally have a relatively large size (greater than 200 nm) that limits their use for controlled delivery in in vitro and in vivo studies [37][39][64][65][66]. Following this, due to its low versatility, and the fact that there are cheaper methods of flavonoid encapsulation, its use is somewhat conditioned.

This group of delivery systems is essentially made up of cyclodextrins, of which β-cyclodextrins are the most common. They can be modified in order to change their physical and chemical properties to form more suitable systems according to the site of delivery [38][64][65]. β-cyclodextrins can change their characteristics through chemical modifications so they have more negative or positive charges or a greater or lesser degree of replacement [38][64][65]. There are several types of β-cyclodextrins, with β-cyclodextrin, carboxymethyl-β-cyclodextrin, sulfobutyl ether-β-cyclodextrin and hydroxypropyl-β-cyclodextrin being the most scientifically employed [38][39][64][66][67]. Cyclodextrins can be conjugated with polymers, such as chitosan, to increase their stability, reduce their size and increase their bioavailability [38][39][67]. In addition, conjugation with biotin is also very common due to its receptors being highly expressed in cancer cells, which may contribute to efficiently targeting cancer cells [66]. Other types of delivery systems that are sometimes tested for flavonoid encapsulation are α-cyclodextrins, γ-cyclodextrins and β-lactoglobulins [45][68][69].

In vitro studies on HeLa cells show a viability reduction to 11.5% after an incubation period of 48 h, using inclusion complexes constituted of β-cyclodextrin with a chrysin concentration of 100 µM [70]. In other studies related to other types of cancer, a great reduction of cell viability compared to the use of flavonoids in the free form has also been observed [64][66][70][71]. Therefore, although it is necessary to evaluate the disadvantages already mentioned, the use of inclusion complexes for flavonoids loading/encapsulation and delivery can be considered and investigated since they easily promote flavonoids solubilization in aqueous solutions, where most of them cannot be dissolved.

5. Other Types of Delivery Systems

Other delivery systems may also emerge as an alternative for the encapsulation and targeting of flavonoids to cancer cells. For instance, dendrimers appear as one of the most promising vehicles.

Dendrimers are a group made up of polymeric materials that have a highly branched architecture with numerous functional groups and an interior cavity that allows the encapsulation of drugs, such as flavonoids [44][72]. Poly(amidoamine) dendrimers are the main and most common type of dendrimers studied, having already been used in in vitro assays in numerous types of cancer and showing significant results, namely a reduction of viability in HeLa cells to 40% using baicalin flavonoid concentration of 25 µg/mL [44][73].

However, their application still has some limitations due to toxicity concerns. Approaches such as PEG conjugation have been considered to minimize this effect and promote a better association with specific ligands [72][74]. In the future, new strategies still need to be investigated to ensure increased stability and bioavailability of these systems to improve their effect on flavonoids encapsulation and subsequent delivery to target cells.

References

1. Wang, W.; Sun, C.; Mao, L.; Ma, P.; Liu, F.; Yang, J.; Gao, Y. The biological activities, chemical stability, metabolism and delivery systems of quercetin: A review. Trends Food Sci. Technol. 2016, 56, 21–38.
2. Li, J.; Shi, M.; Ma, B.; Niu, R.; Zhang, H.; Kun, L. Antitumor activity and safety evaluation of nanaparticle-based delivery of quercetin through intravenous administration in mice. Mater. Sci. Eng. C 2017, 77, 803–810.
3. Caballero, S.; Li, Y.O.; McClements, D.J.; Davidov-Pardo, G. Encapsulation and delivery of bioactive citrus pomace polyphenols: A review. Crit. Rev. Food Sci. Nutr. 2021, 1–17.
4. Li, J.; Li, Z.; Gao, Y.; Liu, S.; Li, K.; Wang, S.; Gao, L.; Shi, M.; Liu, Z.; Han, Z.; et al. Effect of a drug delivery system made of quercetin formulated into PEGylation liposomes on cervical carcinoma in vitro and in vivo. J. Nanomater. 2021, 2021, 9389934.
5. Saraswat, A.L.; Maher, T.J. Development and optimization of stealth liposomal system for enhanced in vitro cytotoxic effect of quercetin. J. Drug Deliv. Sci. Technol. 2020, 55, 101477.
6. Katopodi, A.; Detsi, A. Solid lipid nanoparticles and nanostructured lipid carriers of natural products as promising systems for their bioactivity enhancement: The case of essential oils and flavonoids. Colloids Surf. A Physicochem. Eng. Asp. 2021, 630, 127529.
7. Ding, B.; Chen, P.; Kong, Y.; Zhai, Y.; Pang, X.; Dou, J.; Zhai, G. Preparation and evaluation of folate-modified lipid nanocapsules for quercetin delivery. J. Drug Target. 2014, 22, 67–75.
8. Patel, G.; Thakur, N.S.; Kushwah, V.; Patil, M.D.; Nile, S.H.; Jain, S.; Banerjee, U.C.; Kai, G. Liposomal delivery of mycophenolic acid with quercetin for improved breast cancer therapy in SD rats. Front. Bioeng. Biotechnol. 2020, 8, 631.
9. Hu, J.; Wang, J.; Wang, G.; Yao, Z.; Dang, X. Pharmacokinetics and antitumor efficacy of DSPE-PEG2000 polymeric liposomes loaded with quercetin and temozolomide: Analysis of their effectiveness in enhancing the chemosensitization of drug-resistant glioma cells. Int. J. Mol. Med. 2016, 37, 690–702.
10. Rezaei-Sadabady, R.; Eidi, A.; Zarghami, N.; Barzegar, A. Intracellular ROS protection efficiency and free radical-scavenging activity of quercetin and quercetin-encapsulated liposomes. Artif.

Cells Nanomed. Biotechnol. 2016, 44, 128–134.

11. Bazylińska, U.; Pucek, A.; Sowa, M.; Matczak-Jon, E.; Wilk, K.A. Engineering of phosphatidylcholine-based solid lipid nanocarriers for flavonoids delivery. Colloids Surf. A Physicochem. Eng. Asp. 2014, 460, 483–493.
12. Fathi, M.; Varshosaz, J.; Mohebbi, M.; Shahidi, F. Hesperetin-loaded solid lipid nanoparticles and nanostructure lipid carriers for food fortification: Preparation, characterization, and modeling. Food Bioprocess Technol. 2013, 6, 1464–1475.
13. Bose, S.; Du, Y.; Takhistov, P.; Michniak-Kohn, B. Formulation optimization and topical delivery of quercetin from solid lipid based nanosystems. Int. J. Pharm. 2013, 441, 56–66.
14. Tan, M.; Zhu, J.; Pan, Y.; Chen, Z.; Liang, H.; Liu, H.; Wang, H. Synthesis, cytotoxic activity, and DNA binding properties of copper (II) complexes with hesperetin, naringenin, and apigenin. Bioinorg. Chem. Appl. 2009, 2009, 347872.
15. Scalia, S.; Haghi, M.; Losi, V.; Trotta, V.; Young, P.M.; Traini, D. Quercetin solid lipid microparticles: A flavonoid for inhalation lung delivery. Eur. J. Pharm. Sci. 2013, 49, 278–285.
16. Sun, M.; Nie, S.; Pan, X.; Zhang, R.; Fan, Z.; Wang, S. Quercetin-nanostructured lipid carriers: Characteristics and anti-breast cancer activities in vitro. Colloids Surf. B Biointerfaces 2014, 113, 15–24.
17. Liu, Y.; Zhang, H.; Cui, H.; Zhang, F.; Zhao, L.; Liu, Y.; Meng, Q. Combined and targeted drugs delivery system for colorectal cancer treatment: Conatumumab decorated, reactive oxygen species sensitive irinotecan prodrug and quercetin co-loaded nanostructured lipid carriers. Drug Deliv. 2022, 29, 342–350.
18. Firoozeh, N.; Mahmoud, O.; Layasadat, K.; Mohammadreza, A.; Esrafil, M.; Ali, K. Effects of quercetin-loaded nanoparticles on MCF-7 human breast cancer cells. Medicina 2019, 55, 114.
19. Yostawonkul, J.; Surassmo, S.; Iempridee, T.; Pimtong, W.; Suktham, K.; Sajomsang, W.; Gonil, P.; Ruktanonchai, U.R. Surface modification of nanostructure lipid carrier (NLC) by oleoyl-quaternized-chitosan as a mucoadhesive nanocarrier. Colloids Surf. B Biointerfaces 2017, 149, 301–311.
20. Ni, S.; Hu, C.; Sun, R.; Zhao, G.; Xia, Q. Nanoemulsions-based delivery systems for encapsulation of Quercetin: Preparation, characterization, and cytotoxicity studies. J. Food Process Eng. 2017, 40, e12374.
21. Dora, C.L.; Silva, L.F.C.; Mazzarino, L.; Siqueira, J.M.; Fernandes, D.; Pacheco, L.K.; Maioral, M.F.; Santos-Silva, M.C.; Baischl, A.L.M.; Assreuy, J.; et al. Oral delivery of a high quercetin payload nanosized emulsion: In vitro and in vivo activity against B16-F10 melanoma. J. Nanosci. Nanotechnol. 2016, 16, 1275–1281.

22. Qian, C.; McClements, D.J. Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. Food Hydrocoll. 2011, 25, 1000–1008.
23. Ting, Y.; Chiou, Y.-S.; Pan, M.-H.; Ho, C.-T.; Huang, Q. In vitro and in vivo anti-cancer activity of tangeretin against colorectal cancer was enhanced by emulsion-based delivery system. J. Funct. Foods 2015, 15, 264–273.
24. Ragelle, H.; Crauste-Manciet, S.; Seguin, J.; Brossard, D.; Scherman, D.; Arnaud, P.; Chabot, G.G. Nanoemulsion formulation of fisetin improves bioavailability and antitumour activity in mice. Int. J. Pharm. 2012, 427, 452–459.
25. Van Thoai, D.; Nguyen, D.T.; Dang, L.H.; Nguyen, N.H.; Nguyen, V.T.; Doan, P.; Nguyen, B.T.; Van Thu, L.; Tung, N.N.; Quyen, T.N. Lipophilic effect of various pluronic-grafted gelatin copolymers on the quercetin delivery efficiency in these self-assembly nanogels. J. Polym. Res. 2020, 27, 369.
26. Elgogary, R.I.; Rubio, N.; Wang, J.T.-W.; Al-Jamal, W.T.; Bourgognon, M.; Kafa, H.; Naeem, M.; Klippstein, R.; Abbate, V.; Leroux, F.; et al. Polyethylene glycol conjugated polymeric nanocapsules for targeted delivery of quercetin to folate-expressing cancer cells in vitro and in vivo. ACS Nano 2014, 8, 1384–1401.
27. Wang, Q.; Bao, Y.; Ahire, J.; Chao, Y. Co-encapsulation of biodegradable nanoparticles with silicon quantum dots and quercetin for monitored delivery. Adv. Healthcare Mater. 2013, 2, 459–466.
28. Deepika, M.S.; Thangam, R.; Sheena, T.S.; Sasirekha, R.; Sivasubramanian, S.; Babu, M.D.; Jeganathan, K.; Thirumurugan, R. A novel rutin-fucoidan complex based phytotherapy for cervical cancer through achieving enhanced bioavailability and cancer cell apoptosis. Biomed. Pharmacother. 2019, 109, 1181–1195.
29. Yousefi, M.; Shadnoush, M.; Sohrabvandi, S.; Khorshidian, N.; Mortazavian, A.M. Encapsulation systems for delivery of flavonoids: A review. Biointerface Res. Appl. Chem. 2021, 11, 13934–13951.
30. Fuster, M.G.; Carissimi, G.; Montalbán, M.G.; Víllora, G. Improving anticancer therapy with naringenin-loaded silk fibroin nanoparticles. Nanomaterials 2020, 10, 718.
31. Filho, I.K.; Machado, C.S.; Diedrich, C.; Karam, T.K.; Nakamura, C.V.; Khalil, N.M.; Mainardes, R.M. Optimized chitosan-coated gliadin nanoparticles improved the hesperidin cytotoxicity over tumor cells. Braz. Arch. Biol. Technol. 2021, 64, 200795.
32. Rahimi, S.; Khoee, S.; Ghandi, M. Preparation and characterization of rod-like chitosan–quinoline nanoparticles as pH-responsive nanocarriers for quercetin delivery. Int. J. Biol. Macromol. 2019, 128, 279–289.

33. Kim, E.S.; Kim, D.Y.; Lee, J.-S.; Lee, H.G. Quercetin delivery characteristics of chitosan nanoparticles prepared with different molecular weight polyanion cross-linkers. Carbohydr. Polym. 2021, 267, 118157.
34. Singh, A.; Dutta, P.K.; Kumar, H.; Kureel, A.K.; Rai, A.K. Synthesis of chitin-glucan-aldehyde-quercetin conjugate and evaluation of anticancer and antioxidant activities. Carbohydr. Polym. 2018, 193, 99–107.
35. Kunjiappan, S.; Panneerselvam, T.; Somasundaram, B.; Sankaranarayanan, M.; Chowdhury, R.; Chowdhury, A. Design, in silico modeling, biodistribution study of rutin and quercetin loaded stable human hair keratin nanoparticles intended for anticancer drug delivery. Biomed. Phys. Eng. Express 2018, 4, 025019.
36. Pedro, R.D.O.; Hoffmann, S.; Pereira, S.; Goycoolea, F.M.; Schmitt, C.C.; Neumann, M.G. Self-assembled amphiphilic chitosan nanoparticles for quercetin delivery to breast cancer cells. Eur. J. Pharm. Biopharm. 2018, 131, 203–210.
37. Nguyen, H.T.; Goycoolea, F.M. Chitosan/cyclodextrin/TPP nanoparticles loaded with quercetin as novel bacterial quorum sensing inhibitors. Molecules 2017, 22, 1975.
38. Krauland, A.H.; Alonso, M.J. Chitosan/cyclodextrin nanoparticles as macromolecular drug delivery system. Int. J. Pharm. 2007, 340, 134–142.
39. Fülöp, Z.; Saokham, P.; Loftsson, T. Sulfobutylether-β-cyclodextrin/chitosan nano- and microparticles and their physicochemical characteristics. Int. J. Pharm. 2014, 472, 282–287.
40. Zhang, X.; Xu, M.; Zhang, Z.; Hu, X.; Hao, L.; Lin, Q.; Wang, S.; Jiang, W. Preparation and characterization of magnetic fluorescent microspheres for delivery of kaempferol. Mater. Technol. 2017, 32, 125–130.
41. Yamina, A.M.; Fizir, M.; Itatahine, A.; He, H.; Dramou, P. Preparation of multifunctional PEG-graft-halloysite nanotubes for controlled drug release, tumor cell targeting, and bio-imaging. Colloids Surf. B Biointerfaces 2018, 170, 322–329.
42. Qureshi, W.A.; Zhao, R.; Wang, H.; Ji, T.; Ding, Y.; Ihsan, A.; Mujeeb, A.; Nie, G.; Zhao, Y. Co-delivery of doxorubicin and quercetin via mPEG–PLGA copolymer assembly for synergistic anti-tumor efficacy and reducing cardio-toxicity. Sci. Bull. 2016, 61, 1689–1698.
43. Mei, L.; Zhang, H.; Zeng, X.; Huang, L.; Wang, Z.; Liu, G.; Wu, Y.; Yang, C. Fabrication of genistein-loaded biodegradable TPGS-b-PCL nanoparticles for improved therapeutic effects in cervical cancer cells. Int. J. Nanomed. 2015, 10, 2461–2473.
44. Mashhadi Malekzadeh, A.; Ramazani, A.; Tabatabaei Rezaei, S.J.; Niknejad, H. Design and construction of multifunctional hyperbranched polymers coated magnetite nanoparticles for both targeting magnetic resonance imaging and cancer therapy. J. Colloid Interface Sci. 2017, 490, 64–73.

45. Ghafelehbashi, R.; Yaraki, M.T.; Saremi, L.H.; Lajevardi, A.; Haratian, M.; Astinchap, B.; Rashidi, A.M.; Moradian, R. A pH-responsive citric-acid/α-cyclodextrin-functionalized Fe3O4 nanoparticles as a nanocarrier for quercetin: An experimental and DFT study. Mater. Sci. Eng. C 2020, 109, 110597.
46. Payne, J.N.; Badwaik, V.D.; Waghwani, H.K.; Moolani, H.V.; Tockstein, S.; Thompson, D.H.; Dakshinamurthy, R. Development of dihydrochalcone-functionalized gold nanoparticles for augmented antineoplastic activity. Int. J. Nanomed. 2018, 13, 1917–1926.
47. Simon, A.T.; Dutta, D.; Chattopadhyay, A.; Ghosh, S.S. Quercetin-loaded luminescent hydroxyapatite nanoparticles for theranostic application in monolayer and spheroid cultures of cervical cancer cell line in vitro. ACS Appl. Bio Mater. 2021, 4, 4495–4506.
48. Alpsoy, L.; Baykal, A.; Kurtan, U.; Ülker, Z. Synthesis and characterization of carboxylated luteolin (CL)-functionalized SPION. J. Supercond. Nov. Magn. 2017, 30, 2797–2804.
49. Liu, Y.; Liu, C.; Tang, C.; Yin, C. Dual stimulus-responsive chitosan-based nanoparticles co-delivering doxorubicin and quercetin for cancer therapy. Mater. Lett. 2021, 305, 130826.
50. Baksi, R.; Singh, D.P.; Borse, S.P.; Rana, R.; Sharma, V.; Nivsarkar, M. In vitro and in vivo anticancer efficacy potential of Quercetin loaded polymeric nanoparticles. Biomed. Pharmacother. 2018, 106, 1513–1526.
51. Konecsni, K.; Low, N.H.; Nickerson, M.T. Chitosan–tripolyphosphate submicron particles as the carrier of entrapped rutin. Food Chem. 2012, 134, 1775–1779.
52. Bi, F.; Yong, H.; Liu, J.; Zhang, X.; Shu, Y.; Liu, J. Development and characterization of chitosan and D-α-tocopheryl polyethylene glycol 1000 succinate composite films containing different flavones. Food Packag. Shelf Life 2020, 25, 100531.
53. Saha, C.; Kaushik, A.; Das, A.; Pal, S.; Majumder, D. Anthracycline drugs on modified surface of quercetin-loaded polymer nanoparticles: A dual drug delivery model for cancer treatment. PLoS ONE 2016, 11, e0155710.
54. Karthick, V.; Panda, S.; Kumar, V.G.; Kumar, D.; Shrestha, L.K.; Ariga, K.; Vasanth, K.; Chinnathambi, S.; Dhas, T.S.; Suganya, K.S.U. Quercetin loaded PLGA microspheres induce apoptosis in breast cancer cells. Appl. Surf. Sci. 2019, 487, 211–217.
55. Sunoqrot, S.; Abujamous, L. pH-sensitive polymeric nanoparticles of quercetin as a potential colon cancer-targeted nanomedicine. J. Drug Deliv. Sci. Technol. 2019, 52, 670–676.
56. Pandey, S.K.; Patel, D.K.; Thakur, R.; Mishra, D.P.; Maiti, P.; Haldar, C. Anti-cancer evaluation of quercetin embedded PLA nanoparticles synthesized by emulsified nanoprecipitation. Int. J. Biol. Macromol. 2015, 75, 521–529.

57. Sheu, M.-T.; Chen, L.-C.; Chen, Y.-C.; Su, C.-Y.; Hong, C.-S.; Ho, H.-O. Development and characterization of self-assembling lecithin-based mixed polymeric micelles containing quercetin in cancer treatment and an in vivo pharmacokinetic study. Int. J. Nanomed. 2016, 11, 1557–1566.
58. Patra, A.; Satpathy, S.; Shenoy, A.K.; Bush, J.A.; Kazi, M.; Hussain, M.D. Formulation and evaluation of mixed polymeric micelles of quercetin for treatment of breast, ovarian, and multidrug resistant cancers. Int. J. Nanomed. 2018, 13, 2869–2881.
59. Cote, B.; Carlson, L.J.; Rao, D.A.; Alani, A.W.G. Combinatorial resveratrol and quercetin polymeric micelles mitigate doxorubicin induced cardiotoxicity in vitro and in vivo. J. Control. Release 2015, 213, 128–133.
60. Yu, C.; Gao, C.; Lü, S.; Chen, C.; Huang, Y.; Liu, M. Redox-responsive shell-sheddable micelles self-assembled from amphiphilic chondroitin sulfate-cholesterol conjugates for triggered intracellular drug release. Chem. Eng. J. 2013, 228, 290–299.
61. Zhao, M.-H.; Yuan, L.; Meng, L.-Y.; Qiu, J.-L.; Wang, C.-B. Quercetin-loaded mixed micelles exhibit enhanced cytotoxic efficacy in non-small cell lung cancer in vitro. Exp. Ther. Med. 2017, 14, 5503–5508.
62. Khonkarn, R.; Mankhetkorn, S.; Hennink, W.E.; Okonogi, S. PEG-OCL micelles for quercetin solubilization and inhibition of cancer cell growth. Eur. J. Pharm. Biopharm. 2011, 79, 268–275.
63. Gao, X.; Huang, N.; Shi, H.; Ren, L.; Xu, G.; Gou, H.; Gong, D. Enhancing the anti-colon cancer activity of quercetin by self-assembled micelles. Int. J. Nanomed. 2015, 10, 2051–2063.
64. Trapani, A.; Lopedota, A.; Franco, M.; Cioffi, N.; Ieva, E.; Garcia-Fuentes, M.; Alonso, M.J. A comparative study of chitosan and chitosan/cyclodextrin nanoparticles as potential carriers for the oral delivery of small peptides. Eur. J. Pharm. Biopharm. 2010, 75, 26–32.
65. Trapani, A.; Garcia-Fuentes, M.; Alonso, M.J. Novel drug nanocarriers combining hydrophilic cyclodextrins and chitosan. Nanotechnology 2008, 19, 185101.
66. Zhu, P.; Chen, L.; Zhao, Y.; Gao, C.; Yang, J.; Liao, X.; Liu, D.; Yang, B. A novel host-guest complex based on biotin functionalized polyamine-β-cyclodextrin for tumor targeted delivery of luteolin. J. Mol. Struct. 2021, 1237, 130339.
67. Liu, B.; Li, W.; Zhao, J.; Liu, Y.; Zhu, X.; Liang, G. Physicochemical characterisation of the supramolecular structure of luteolin/cyclodextrin inclusion complex. Food Chem. 2013, 141, 900–906.
68. D'Onofre Couto, B.; Novaes da Costa, R.; Castro Laurindo, W.; Moraes da Silva, H.; Rocha da Silva, C.; Sélia dos Reis Coimbra, J.; Barbosa Mageste, A.; de Cássia Dias, S.; José Boggione Santos, I. Characterization, techno-functional properties, and encapsulation efficiency of self-assembled β-lactoglobulin nanostructures. Food Chem. 2021, 356, 129719.

69. Zu, Y.; Wu, W.; Zhao, X.; Li, Y.; Zhong, C.; Zhang, Y. The high-water solubility of inclusion complex of taxifolin-γ-CD prepared and characterized by the emulsion solvent evaporation and the freeze drying combination method. Int. J. Pharm. 2014, 477, 148–158.
70. Sundararajan, M.; Thomas, P.A.; Venkadeswaran, K.; Jeganathan, K.; Geraldine, P. Synthesis and characterization of chrysin-loaded β-cyclodextrin-based nanosponges to enhance in-vitro solubility, photostability, drug release, antioxidant effects and antitumorous efficacy. J. Nanosci. Nanotechnol. 2017, 17, 8742–8751.
71. Liu, M.; Liao, R.; Zhao, Y.; Yang, B. Host—Guest Inclusion System of Luteolin with Polyamine-β-cyclodextrin: Preparation, Characterisation, Anti-oxidant and Anti-cancer Activity. Aust. J. Chem. 2015, 69, 174–182.
72. Luong, D.; Kesharwani, P.; Deshmukh, R.; Mohd Amin, M.C.I.; Gupta, U.; Greish, K.; Iyer, A.K. PEGylated PAMAM dendrimers: Enhancing efficacy and mitigating toxicity for effective anticancer drug and gene delivery. Acta Biomater. 2016, 43, 14–29.
73. Lv, T.; Yu, T.; Fang, Y.; Zhang, S.; Jiang, M.; Zhang, H.; Zhang, Y.; Li, Z.; Chen, H.; Gao, Y. Role of generation on folic acid-modified poly(amidoamine) dendrimers for targeted delivery of baicalin to cancer cells. Mater. Sci. Eng. C 2017, 75, 182–190.
74. Chauhan, A.S. Dendrimers for drug delivery. Molecules 2018, 23, 938.