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Review

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EGCG as a therapeutic agent: a systematic review of recent advances and challenges in nanocarrier strategies

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Abstract: Epigallocatechin-3-gallate (EGCG), a bioactive polyphenol abundant in green tea, has garnered significant attention for its diverse therapeutic applications, ranging from antioxidant and anti-inflammatory effects to potential anticancer properties. Despite its immense promise, the practical utilization of EGCG in therapeutic settings as a medication has been hampered by inherent limitations of this drug, including poor bioavailability, instability, and rapid degradation. This review comprehensively explores the current challenges associated with the application of EGCG and evaluates the potential of nanoparticle-based formulations in addressing these limitations. Nanoparticles, with their unique physicochemical properties, offer a platform for the enhanced stability, bioavailability, and targeted delivery of EGCG. Various nanoparticle strategies, including polymeric nanoparticle, micelle, lipid-based nanocarrier, metal nanoparticle, and silica nanoparticle are currently employed to enhance EGCG stability and pharmacological activity. This review concludes that the particle size of most of these formulated nanocarriers falls within 300 nm and their encapsulation efficiency ranges from 51% to 97%. Notably, the pharmacological activities of EGCG-loaded nanoparticles – such as antioxidative, anti-inflammatory, anticancer, and antimicrobial effects – are significantly enhanced compared to those of free EGCG. By critically analyzing the existing literature and highlighting recent advancements, this article provides valuable insights into the promising prospects of nanoparticle-mediated EGCG formulations, paving the way for the development of more effective and clinically viable therapeutic strategies.

Key words: EGCG, Nanoparticle, Nanocarrier, Nanosystem, Nanoformulation, Stability, Biological activity

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1 Introduction

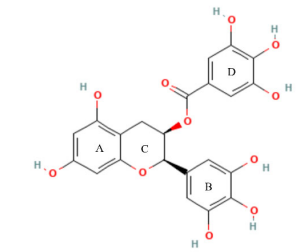

Fig. 1 2D structure image of CID 65064 (Epigallocatechin Gallate)

Within the catechin family, epigallocatechin-3-gallate (EGCG) stands out as a highly potent antioxidant.

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While sharing structural similarities with other catechins, EGCG contains an additional gallic acid moiety, amplifying its antioxidant and anti-inflammatory abilities. Within the flavonoid family, EGCG is derived from the flavan-3-ol subgroup. Notably, during its synthesis, it undergoes structural modification where the original hydroxyl (-OH) group at position 3 on the tetrahydropyran moiety is replaced by a galloyl group. This results in the incorporation of a benzenediol ring (A), a tetrahydropyran moiety (C), a pyrogallol ring (B), and a galloyl group (D), collectively forming the distinctive chemical structure of EGCG (Botten et al., 2015), as illustrated in Fig. 1. The antioxidative property of EGCG is attributed to its potent ability to scavenge various free radicals (Chandra and Arora, 2018; He et al., 2018), to inhibit metal-mediated oxidation (Zwolak, 2021), as well as to stimulate the synthesis of endogenous antioxidant enzymes, amplifying the cellular defense against oxidative stress (Wada et al., 2019).

The above antioxidative properties of EGCG contribute to a diverse range of biological activities, such as anti-inflammatory, anti-cancer, cardioprotective, and neuroprotective effects, making it a promising candidate for therapeutic applications for inflammatory or oxidative stress-associated diseases (Alam et al., 2022; Furniturewalla and Barve, 2022; Mohd Sabri et al., 2022; Parn et al., 2022; Youn et al., 2022; Khor et al., 2023). Over the past decades, the protective effects of EGCG against metabolic disorders have garnered significant interest, particularly concerning its roles in modulating carbohydrate and lipid metabolism, as evidenced by its inhibitory effects on α-glucosidase, α-amylase, and lipase (Jiang et al., 2021; Wen et al., 2022; Ma et al., 2024).

Nevertheless, the practical application of EGCG as a therapeutic agent has been hindered by its poor stability in the intestinal environment, low permeability and active efflux, as well as rapid degradation (Cai et al., 2018; Cano et al., 2019). EGCG is prone to degradation in harsh conditions (alkaline medium, enzymatic degradation) due to its susceptibility to oxidation, which underscores the importance of preventing its uncontrolled degradation within biological systems. Such degradation can lead to inconsistent drug release kinetics, jeopardizing the therapeutic efficacy and patient outcomes, potentially resulting in inadequate treatment and unintended side effects. The encapsulation of EGCG within nanocarriers has been shown to effectively mitigate its degradation by providing a protective environment, shielding the molecule from external factors such as pH changes and enzymatic activity. In addition, surface modifications and coatings can be applied to nanocarriers to further enhance their stability and protect the EGCG encapsulated within them, which helps regulate drug release kinetics and prevent premature degradation. Overall, nanocarriers serve as an effective tool for controlling drug degradation and ensuring the stability and efficacy of the encapsulated EGCG under various physiological conditions. This review aims to critically assess and consolidate the current state of research on nanoparticle-mediated strategies for formulating EGCG, with a focus on overcoming the inherent limitations of this drug to unlock its full therapeutic potential.

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1.1 EGCG: limitations as a therapeutic agent

The attainment of optimal drug bioavailability and absorption holds as a pivotal aim in drug development and formulation. Drug bioavailability and therapeutic effects are profoundly influenced by various factors, including solubility, permeability, and the intricacies of first-pass metabolism.

1.1.1 Poor stability under intestinal conditions

Orally consumed EGCG undergoes a drastic pH change in the body, from a strong acidic condition in the stomach (pH 2) to an alkaline environment in the intestine (pH 8). EGCG is unstable under alkaline condition and quickly undergoes degradation via oxidation (Zhu et al., 1997; Cano, et al., 2019). The structural vulnerability of EGCG lies in its pyrogallol ring that comprises three hydroxyl groups, rendering its high susceptibility to autooxidation, poor stability, and rapid degradation (Severino et al., 2009). Autooxidation occurs in the alkaline medium of the intestine together in the presence of molecular oxygen. Two hydrogen atoms of the hydroxyl groups are snatched away by the oxygen molecule, resulting in the simultaneous formation of hydrogen peroxide and several intermediate products of pyrogallol oxidation (Omoruyi et al., 2020). This leads to the impaired integrity of the structure of EGCG and its poor bioavailability in the systemic circulation.

1.1.2 Low permeability and active efflux

The absence of a specific carrier or receptor for EGCG on the surface of small intestinal epithelial cells

necessitates reliance on passive diffusion—comprising both paracellular and transcellular transport—as the primary route of EGCG absorption (Cai, et al., 2018). However, eight hydroxyl groups on the benzenediol, pyrogallol, and galloyl groups in EGCG could form hydrogen bonding with water molecules, leading to high hydrophilicity and resulting in poor membrane permeability across biological membranes (Dahan and Miller, 2012). Besides, EGCG absorptions into the small intestine encounter active hindrance from efflux transporters such as P-glycoprotein (P-gp). These transporters actively pump EGCG out of the cells, leading to its poor bioavailability (Dai et al., 2020b). This limitation in permeation across the small intestine prevents a substantial amount of EGCG from reaching target tissues and executing its therapeutic functions effectively.

1.1.3 Rapid metabolism

EGCG undergoes a series of metabolic activities upon ingestion. Esterase enzymes, present in the saliva, hydrolyze the ester bond between the tetrahydropyran moiety and the galloyl group in EGCG, causing the formation of a degalloylated EGCG derivative (Higdon and Frei, 2003). Subsequent metabolic processes in the small intestine and liver, including methylation, sulphation, and glucuronidation, lead to the biotransformation of EGCG into O-methylated, sulphated, and glucuronidated conjugates (Rietveld and Wiseman, 2003; Mokra et al., 2022). These metabolites exhibit different structures and biological activities as compared to their parent compound, therefore their efficacy in achieving therapeutic outcomes may not reach that of the original compound. This underscores the potential impact of metabolism on the therapeutic effectiveness of EGCG (Xu et al., 2004; Dai, et al., 2020b).

1.2 Nanocarriers as stability and biological activity enhancers

While EGCG shows great promise as a therapeutic agent, addressing the above limitations is essential for its successful development and application in clinical settings. Researchers continue to explore innovative formulations and strategies to overcome these challenges and harness the potential benefits of EGCG in various health conditions. This review aims to discuss the existing nanoparticle strategies, including polymeric nanoparticle, micelle, lipid-based nanocarrier, metal nanoparticle, and silica nanoparticle, emphasizing their role in optimizing the therapeutic efficacy of EGCG via various experimental models.

Nanocarriers are nano-sized structures, typically ranging from tens to hundreds of nanometers in size, to encapsulate therapeutic agents such as drugs, proteins or nucleic acids for targeted drug delivery. They can be designed using various materials such as lipids, polymers or inorganic substances, each offering unique advantages in terms of stability, biocompatibility, and drug release kinetics (Table 1). Once loaded with the desired drug, nanocarriers can be administered through various routes such as oral, intravenous or topical, depending on the target tissue and the desired therapeutic outcome. Upon reaching the target site, nanocarriers can passively accumulate via enhanced permeability and the retention (EPR) effect or actively target specific cells or tissues through surface modifications or ligand conjugation. Once internalized by the target cells, nanocarriers release the encapsulated drug either through diffusion, degradation or triggered release mechanisms, resulting in localized therapeutic effects while minimizing potential systemic toxicity.

Various nanocarriers, each with distinct structures and compositions, have been constructed and investigated to encapsulate EGCG (Table 2). These serve as protective vehicles, addressing the limitations of EGCG and facilitating its efficient delivery to target sites. In this paper, we carried out an extensive search for studies delving into the exploration and comparative analysis of EGCG-loaded nanocarriers.

Table 1 Advantages and disadvantages of different nanocarriers

Type of nanocarrier	Advantage(s)	Disadvantage (s)
Polymeric nanoparticle	Higher stability than lipid-based nanocarrier, controlled release behavior, incorporation of biodegradable materials	Toxicity issue by prolonged retention of synthetic polymer, difficult scale-up
Micelle	Enhanced solubility of lipophilic drug, small particle size, biocompatible, biodegradable	Unstable below critical micelle concentration (CMC)

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Liposome	Encapsulation of both hydrophilic and lipophilic drugs, protection against drug degradation	Drug leakage, low drug loading, large particle size
Solid lipid nanoparticle	Low toxicity, biodegradable, targeted drug delivery	Lipid polymorphism-induced drug leakage, initial burst release behavior
Nanostructured lipid carrier	Enhanced physical stability, high drug loading capacity, biocompatible	Selective drug choice
Gold nanoparticle	Large surface area, less invasive, exhibit localized plasmon surface resonance (LPSR) phenomenon	Weak optical signal
Silver nanoparticle	Low toxicity, biocompatible	Off-target drug delivery
Selenium nanoparticle	High stability	Biocompatibility depends on synthesis method
Zinc oxide nanoparticle	Large surface area, high photostability, enhanced redox capacity	Off-target drug delivery
Silica nanoparticle	Tunable particle size, excellent compatibility within living systems and large surface area	Scattered particle size distribution

The above are extracted from (Talluri et al., 2015; Kahraman et al., 2017; Khan and Roy, 2019; Sharma et al., 2019; Lu et al., 2021; Huang, et al., 2022; Milligan and Saha, 2022; Sampath, et al., 2024)

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2 Search strategy

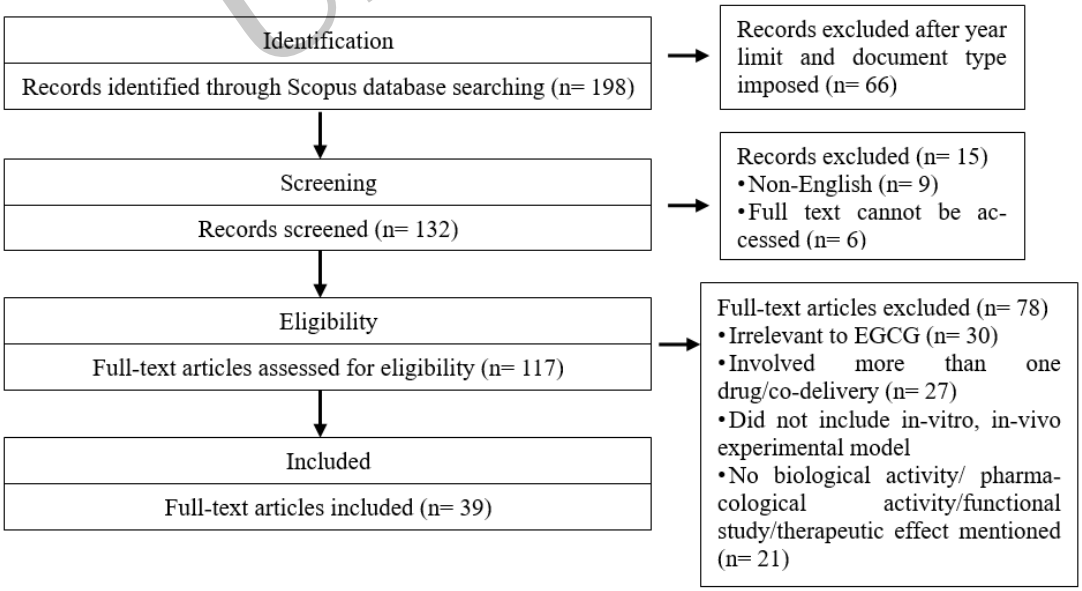

Fig. 2 PRISMA Flowchart of Study Selection

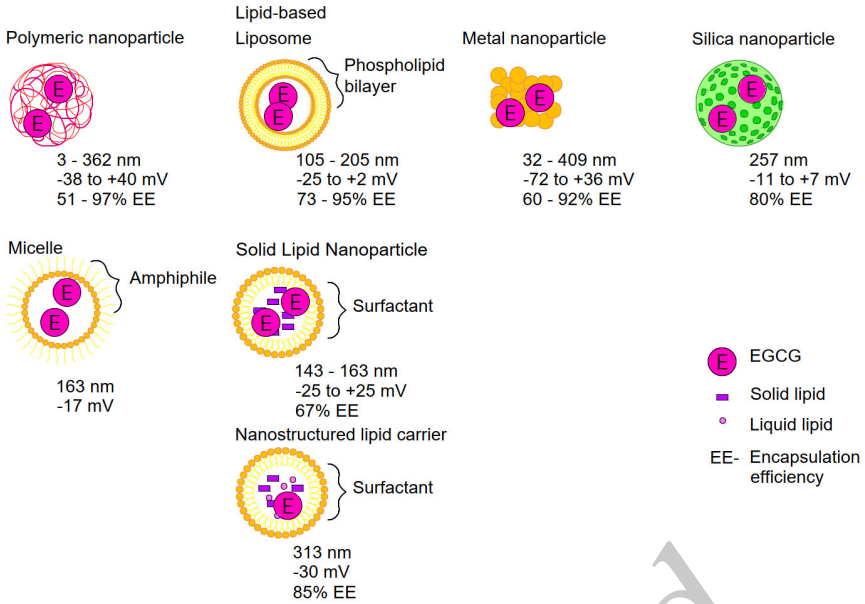

Fig. 3 Illustration of various EGCG nanocarriers

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Data collection was conducted by entering the key terms "EGCG" OR "Epigallocatechin-3-gallate" OR "Epigallocatechin gallate" AND "Micelle" OR "Liposome" OR "Nanoparticle" OR "Nanotube" OR "Silica" OR "Dendrimer" OR "Niosome" OR "Nanosuspension" OR "Nanomicelle" OR "Nanocrystal" OR "Nanosphere" OR "Nanocapsule" OR "Metal Nanoparticle" AND "in-vitro" OR "in-vivo" into the Scopus database. The search was constrained to articles published between 2018 and 2023, focusing on the document type. Full-text articles published in English and relevant to EGCG, featuring a single active ingredient, utilizing at least one in-vitro or in-vivo experimental model, and including functional studies or measured therapeutic effects (determination of biological or pharmacological activities) were included in the final review. The initial search of the database yielded 132 records. Following screening by title and abstract, 117 full-text articles were analyzed. Among these, 78 papers were excluded, leaving 39 papers meeting the inclusion criteria that were included in this review (Fig. 2). The key findings are illustrated in Table 2 and Fig. 3.

3 Main findings

In this paper, the database search revolved around exploring various nanocarrier systems employed for the formulation of EGCG, which found five types of nanocarriers, namely, polymeric nanoparticle, micelle, lipid-based nanocarrier, metal nanoparticle, and silica nanoparticle. Each nanocarrier was examined for its unique structural features and advantages in encapsulating EGCG. The studies selected highlight the potential of these nanocarrier systems to enhance the stability, bioavailability, therapeutic efficacy, and safety profile of EGCG-loaded formulations, providing a comprehensive overview of the current advancements in EGCG nanoparticle technology.

3.1 Polymeric nanoparticles and EGCG

A polymeric nanoparticle is defined as a colloidal particle made up of repeated subunits, with dimensions falling within the nanometer range. Synthetic polymers such as polyethylene glycol (PEG), poly(lactic-co-glycolic acid) (PLGA), poly(n-butyl cyanoacrylate) (PBCA), and polyvinylpyrrolidone (PVP) have been utilized as nanocarriers for EGCG (Singh et al., 2018a; Singh et al., 2018b; Liu et al., 2019; Hu et al., 2021; Minnelli et al., 2023). These synthetic polymers offer precise control over nanoparticle properties such as size, shape, and surface characteristics. Nevertheless, prolonged retention of these synthetic polymers within

biological systems may induce certain toxicity issues, prompting a gradual shift towards the application of natural alternatives like chitosan, gelatin, hordein, soybean polysaccharides in the EGCG-nanocarrier formulation (He et al., 2020; Moreno-Vásquez et al., 2021; Zhou et al., 2022; Zhang et al., 2023b).

The EGCG-loaded polymeric nanoparticles discussed in this study are in the size range of 50 to 362 nm, indicating a notable degree of variance. However, the overall size range falls within acceptable limits (Table 2). The above variation could be attributed to the varying interactions between different polymeric ingredients utilized and loaded with EGCG. Stronger chemical interactions between EGCG and polymeric ingredients tend to pull the outer polymeric shell closer to the core, resulting in smaller particle sizes. The studies discussed in this paper reveal that the encapsulation efficiency of polymeric nanoparticles spans from 46% to 97%, highlighting their efficacy as potent EGCG delivery vehicles. The production method also plays a crucial role in developing an effective nanocarrier. When utilizing identical polymeric components (PLGA and PEG), the nanoprecipitation technique yields a 49% encapsulation efficiency, whereas the double emulsion-solvent evaporation method achieves a significantly higher efficiency of 95% (Cano et al., 2018; Alserihi et al., 2021).

Encapsulating EGCG within a polymeric nanoparticle yields enhanced anti-inflammatory effects, as evidenced by the reduced expression of pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) and the increased expression of anti-inflammatory markers such as interleukin-10 (IL-10) and arginase-1 (Arg-1). Moreover, this formulation exhibits superior antioxidant activity compared to its free form, characterized by an increased capacity for scavenging reactive oxygen species (ROS) (Tian et al., 2022). These augmented biological effects can be attributed to the enhanced stability of EGCG and protection against drug degradation provided by nanoparticle encapsulation. Besides, the improved cellular uptake and permeability of EGCG across Caco-2 cells, coupled with the suppression of multidrug resistance protein 2 (MRP2) and P-gp-mediated efflux transport, contribute to the increased intracellular retention of EGCG, thereby amplifying its therapeutic effectiveness (Zhang et al., 2023a; Zhang, et al., 2023b).

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3.2 Micelles and EGCG

A micelle is a spherical structure with a core-shell configuration, formed by the self-aggregation of amphiphilic molecules at the critical micelle concentration (CMC) and critical micelle temperature (CMT) (Koopaie, 2020). Micelles can be mainly categorized into two types, that are, regular micelles with hydrophilic shell and hydrophobic core, and inverse micelles with hydrophobic shell and hydrophilic core. The choice of solvent determines the type of micelle to be synthesized (Perumal et al., 2022).

Most nano-systems encapsulate drugs within the center of nanoparticles, utilizing pharmaceutical excipients as carriers. However, a novel delivery platform integrating micelles has been developed by transforming the role of EGCG from bioactive natural ingredient to bioactive drug carrier, thereby enhancing its bioavailability. This involves structural modification through the conjugation of EGCG with 9-fluorene methoxy carbonyl (Fmoc) moiety, yielding EGCG-Fmoc. Due to its amphiphilic nature, EGCG-Fmoc can undergo self-assembly in the medium and form a micellar system. The EGCG-Fmoc micelle is characterized as a spherical particle with small particle size (~163 nm) and homogenous distribution (~0.09). Its relatively small size takes advantage of the EPR effect, facilitating penetration into tumor cells. The negative zeta potential found on the surface of micelle prevents particle aggregation, indicating its stability enhancing impact. The anti-proliferation effect of EGCG-Fmoc micelle on a breast cancer cell line was significantly improved as compared to EGCG alone, suggesting the enhanced bioavailability and therapeutic effects of EGCG through the self-assembly system. Lower half-maximal inhibitory concentration (IC50) was found in EGCG-Fmoc (45.21 μg/mL) than that in EGCG (63.88 μg/mL), signifying the enhanced anti-cancer effect achieved by the EGCG-Fmoc micelle. The self-aggregated EGCG-Fmoc micellar system demonstrated an exceptional advantage, allowing the co-delivery of EGCG with other pharmaceutical ingredients without the need of an inert carrier (Liu et al., 2023).

An EGCG-loaded reverse micelle has been synthesized using Tween 80 and Span 80. Unfortunately, the

study did not provide details on key characterizations such as particle size, surface charge, and encapsulation efficiency. However, the reverse micelle approach effectively increased cytotoxicity of EGCG against HeLa cells and demonstrated deeper penetration into rat skin, owing to the enhanced lipophilicity of EGCG (Rosita et al., 2019).

3.3 Lipid-based nanocarriers and EGCG

The utilization of lipids as formulating materials for nanocarriers ensures biocompatibility, making them particularly suited to drug delivery applications. Three types of lipid-based nanocarriers, namely, liposome, solid lipid nanoparticle, and nanostructured lipid carrier, have been examined in this review to highlight their unique features and potential applications in EGCG delivery.

3.3.1 Liposomes and EGCG

A liposome is a spherical vesicle comprising an aqueous core enclosed by a phospholipid bilayer. The presence of this bilayer enables the biocompatibility of liposome and facilitates its transport across the plasma membrane (Dymek and Sikora, 2022). Compared to micelles, liposomes typically have a larger diameter, primarily due to the enlargement of the overall particle size caused by the presence of the outer bilayer. One of the key advantages of liposomes is their versatility in encapsulating various types of drugs. Both hydrophilic and lipophilic drugs can be efficiently loaded into liposomes. Hydrophilic drugs are encapsulated within the aqueous core of the liposome, where they are solubilized and protected from degradation. On the other hand, lipophilic drugs can be incorporated into the phospholipid bilayer, where they are stably embedded (Laddu et al., 2021; Nsairat et al., 2022).

All liposomal formulations discussed in this review utilize phospholipids, glycerol, and cholesterol as their primary ingredients for formulation. Among the various methods for liposome synthesis, thin-film hydration has emerged as a popular choice, with three out of four studies opting for this technique. The results indicate favorable outcomes, as the size achieved through thin-film hydration (ranging from 105 to 161 nm) is smaller compared to that obtained through reverse phase evaporation (205 nm). This size difference may also be attributed to the incorporation of high molecular weight and additional ingredients (1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), cholesteryl hemisuccinate (CHEMS), magnesium salt, and Poloxamer-407) in this anionic liposome formulation (Minnelli et al., 2018). However, it is important to note that the size of 205 nm still falls within an acceptable range. Furthermore, reverse phase evaporation demonstrated superior encapsulation efficiency, with 95% of EGCG encapsulated within the liposomal core, marking the highest among all liposomal formulations discussed.

The attenuation of oxidative stress-induced cellular damage and the reduced levels of reactive oxygen species suggest that the antioxidant efficacy of EGCG is heightened when formulated within liposomes compared to its natural form (Xia et al., 2023). The slower release rate of encapsulated EGCG from the liposome extends its presence and circulation within the body, thereby augmenting its therapeutic impact (Minnelli, et al., 2018). Further evidence of this effect was provided by the amplified inhibition of the transforming growth factor-β (TGF-β) signaling pathway, which was associated with Pulmonary Arterial Hypertension (PAH). This effect was observed with liposomal EGCG but not with free EGCG at 1 μM concentration (Haddad et al., 2023).

3.3.2 Solid lipid nanoparticles and EGCG

Solid Lipid Nanoparticle (SLN) is a spherical particle characterized by a surfactant monolayer enveloping a solid lipid core. Its structure resembles that of a liposome, albeit with a notable difference: the phospholipid bilayer is replaced by a surfactant monolayer, and the drug is entrapped within this solid lipid matrix. Emulsification is required to blend the immiscible solid lipid and the surfactant, resulting in the formation of an emulsion. Various ingredients have been explored for their suitability in formulating SLN as a nanocarrier for

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EGCG, such as glyceryl monostearate, stearic acid, lecithin soy, Sofitsan S100, Lipoid S75, etc.

Researchers have developed a positively charged SLN formulation encapsulating EGCG, employing ingredients such as glycerol, Sofitsan S100, Lipoid S75, and cationic lipids (dimethyldioctadecylammonium bromide, DDAB). The anti-proliferative effect of this formulation was evaluated across various cell lines, with notable outcomes observed specifically in fibroblast SV-80 cells, characterized by a lower IC50 value following 48 h of incubation and reduced cell viability. However, the researchers suggested that the enhanced anti-proliferative effect may not be anticipated in other cell lines, which could be attributed to the superior antioxidant property and protective effects of the formulation on these cell lines (except SV-80). As a result, comparable cell viability was observed between the group treated with EGCG-DDAB-SLN and the group treated solely with EGCG (Silva et al., 2019).

A research group has selected glycerol monostearate (GMS), stearic acid (SA), and soy lecithin as lipid sources for synthesizing EGCG-SLN. After formulation optimization, a preparation with 5% (w/w) EGCG to lipid ratio (particle size: 157 nm; polydispersity index (PDI): 0.268; drug encapsulation efficiency of 67.2%) was chosen as the optimum for subsequent assessments. Notably, a formulation with 3% (w/w) EGCG to lipid ratio was considered as a better option due to its smaller particle size (112 nm), lower PDI (0.14), and higher encapsulation efficiency (89%). However, the authors do not explicitly state the rationale behind selecting the 5% (w/w) EGCG to lipid ratio preparation as the optimum choice. The anti-proliferation ability of EGCG-SLN was assessed against a cancer cell line. Reduced breast cancer cell viability and lowered IC50 value of EGCG-SLN indicated the improved cytotoxicity of EGCG towards cancer cells, which can be related to drug encapsulation within the lipid formulation, leading to increased stability and enhanced therapeutic effects (Radhakrishnan et al., 2016).

To further enhance the specific targeting of EGCG-SLN, a research group developed bombesin-conjugated EGCG-SLN. Bombesin, a tumor-specific peptide molecule, acts as a ligand targeting and interacting specifically with overexpressed gastrin releasing peptide receptor (GRPR) in breast cancer. The conjugation of bombesin onto EGCG-SLN is anticipated to significantly improve the anti-tumor and anti-cancer efficacies of EGCG. Compared to non-conjugated formulation, bombesin-conjugated EGCG-SLN exhibited larger particle size (163 nm) and reduced zeta potential (due to neutralization of negative charge on SLN surface), proving the successful interaction between bombesin and the lipid particle. In-vitro cytotoxicity results revealed 1) lower percentage of cells viable in conjugated EGCG-SLN group, 2) less EGCG-SLN required to exert half of its maximal inhibitory effect, and 3) increased cell apoptosis. Additionally, negligible wound closure in cancer cells was observed, indicating the improved anti-angiogenic effect of EGCG. These in-vitro outcomes aligned with the in-vivo assessment results, where conjugated EGCG-SLN 1) extended lifespan of tumor-bearing mice, 2) retained normal body weight, 3) reduced tumor growth. The incorporation of bombesin onto the EGCG-SLN surface increases its target-specific ability towards cancer cells. This implies enhanced cellular uptake and internalization through receptor (bombesin)-mediated endocytosis in the conjugated formulation, further augmenting the therapeutic benefits of EGCG (Radhakrishnan et al., 2019).

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3.3.3 Nanostructured lipid carriers and EGCG

Issues have been identified with solid lipid nanoparticles (SLN), including low drug loading capacity and drug expulsion during storage. In response to these challenges, a nanostructured lipid carrier (NLC) has been presented. NLC is a colloidal spherical particle encapsulating a lipid blend (solid and liquid lipids). The combination of solid and liquid lipids introduces imperfections into the lipid matrix, enhancing its capacity to accommodate drugs. Moreover, the amorphous nature of the lipid matrix in NLC prevents drug expulsion caused by crystallization, a common occurrence in the crystalline matrix of SLN, which contributes to the preservation of NLC storage stability (Müller et al., 2002).

A combination of Precirol ATO 5 (solid lipid), Mygliol 812 (liquid lipid), and Tween 60 (surfactant) was utilized in the synthesis of NLC, which was further functionalized with folic acid (FA). The resulting NLC exhibited a size of 313 nm, PDI of 0.2, and zeta potential of -30 mV, indicating the successful synthesis of a

highly stable formulation, which achieved a notable encapsulation efficiency of 85%. This EGCG-loaded formulation demonstrated significant cytotoxic effects on three types of breast carcinoma cells, as evidenced by reduced cell viability and the controlled proliferation of cancer cells. Conversely, human normal mammary epithelial cells (MCF10A cells) treated with EGCG-loaded formulation showed no sign of cytotoxicity, displaying cell viability comparable to the control group. This indicated the ability of the formulation to selectively target cancer cells, possibly attributed to the FA functionalization on the surface of nanocarrier, targeting the overexpressed folate receptor found in various cancer cell subtypes (Farabegoli et al., 2022). This targeting mechanism ensures the safety of the formulation towards normal cells while effectively exerting anti-cancer effects.

3.4 Metal nanoparticles and EGCG

A metal nanoparticle is a nano-sized structure composed of metallic elements such as gold (Au), silver (Ag), copper (Cu), zinc (Zn), etc. These nanoparticles have attracted considerable attention in drug formulation due to their customizable physical and chemical properties, surface modification capability, biocompatibility, and stability (Klebowski et al., 2018; Yaqoob et al., 2020).

3.4.1 Gold nanoparticles and EGCG

Among the reviewed articles, gold nanoparticles (AuNPs) stand out as the most prominent and commonly used ingredient in formulating EGCG-metal nanoparticles (Table 2). This preference is largely due to their inertness and low toxicity compared to other metal types (Hammami et al., 2021). AuNPs possess the remarkable ability to be precisely tailored into diverse shapes and structures, including nanospheres, nanorods, and nanocubes. This morphological versatility allows for fine-tuning of their physicochemical properties, such as surface area, plasmonic characteristics, and stability (Hu et al., 2020). The widespread applications of AuNPs in the medical field stem from their 1) excellent biocompatibility (therefore low toxicity), 2) high inertness, and 3) potential for surface modification (Pissuwan et al., 2020).

All the formulated gold-EGCG nanoparticles listed in Table 2 exhibit small particle sizes below 150 nm. This characteristic is likely a contributing factor to their enhanced membrane penetration and therapeutic efficacy. One study synthesized gold nanoparticles using two different methods (chitosan reduction and the Turkevitch method), followed by conjugation with EGCG. In the chitosan reduction method, chitosan serves as the reducing agent and is mixed with chloroauric acid (HAuCl4) solution to form EGCG-ChAuNPs. In the Turkevitch method, HAuCl4 is reduced by sodium citrate and functionalized with cysteamine, yielding EGCG-CystAuNPs. The particle sizes of EGCG-ChAuNPs and EGCG-CystAuNPs are 125 nm and 111 nm, respectively, implying the effect of reducing agent on particle size (Cunha et al., 2022). The Turkevich method has been evaluated as effective for producing spherical particles < 30 nm. However, as the size of the AuNPs increases beyond 30 nm, the particles lose their spherical shape coupled with wider size distribution, indicating the incompatibility of this method with larger nanoparticles (Dong et al., 2020). This is especially evident in the case of CystAuNPs, which have a size of 54 nm and a PDI value of 0.6, indicating their heterogeneity. Conjugation with EGCG could increase the size of nanoparticles but significantly reduce the PDI to 0.2, suggesting that the addition of EGCG facilitates control over the properties of AuNPs. However, most studies included in this review did not assess their encapsulation efficiency; therefore, the challenge remains to accurately determine the extent to which EGCG is loaded onto the nanoparticles, thereby limiting our understanding of the efficiency of EGCG encapsulation in these formulations.

Loading EGCG onto gold nanoparticles has enhanced its anticancer effects, as evidenced by 1) the decreased viability of cancer cells, 2) increased apoptosis in cancer cells, 3) the reduced expression of anti-apoptotic proteins including B-cell lymphoma 2 (BCL2) and B-cell lymphoma-extra large (BCL-xL), and 4) the heightened expression of the pro-apoptotic protein such as BCL2-associated protein x (Bax) (Chavva et al., 2019; Mostafa et al., 2020; Cunha, et al., 2022). This improvement can be attributed to 1) enhanced stabilization, 2) increased cellular uptake, 3) reduced P-gp-mediated efflux, and 4) controlled release of EGCG from the gold

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nanoformulation (Chavva, et al., 2019).

3.4.2 Silver nanoparticles and EGCG

Silver nanoparticles (AgNPs) have emerged as versatile nanomaterials with diverse bio-applications, notably as potent agents against bacterial and viral infections. One study employed a chemical reducing agent, namely sodium citrate, for the production of EGCG-modified silver nanoparticles (EGCG-AgNPs) via a seed growth-mediated method (Krzyzowska et al., 2023). While this kind of chemical synthesis method offer advantages such as simplicity and cost-effectiveness, the use of chemical reducing agents may not be environmentally friendly and pose unforeseen risks to biological systems. In response to these drawbacks, the utilization of biological agents has emerged as a promising alternative. Another study, which utilized aminated guar gum (AGG) as the reducing agent for the synthesis of hydrogel-silver-EGCG (HG-Ag-EGCG), exemplifies an environmental-friendly approach while demonstrating the potency of HG-Ag-EGCG as an antibacterial agent (Kar et al., 2019).

Research shows that reduction with sodium citrate yields superior results in characterization. The particle size and zeta potential of EGCG-AgNPs and HG-Ag-EGCG were 32 nm and 217 nm, and -67 mV and -2.6 mV, respectively. The significantly smaller particle size may offer advantages in cellular penetration and uptake, while the 25-fold larger absolute zeta potential may contribute to the much higher stability of EGCG-AgNPs via electrostatic repulsion, preventing particle aggregation. Nevertheless, these differences do not affect the biological effects of HG-Ag-EGCG. Several outcomes were observed, including 1) reduced bacterial growth curve, 2) increased wound closure percentage, 3) higher vascular endothelial growth factor (VEGF), and 4) reduced levels of pro-inflammatory cytokine (IL-6) compared to HG-EGCG (Kar, et al., 2019). Similarly, enhanced antiviral response was noted in EGCG-AgNPs compared to its free counterpart, as evidenced by 1) inhibition of herpes simplex type 1 and type 2 (HSV-1 and HSV-2) infections, 2) reduced attachment and penetration of HSV-1 and HSV-2 to human keratinocytes, and 3) activated early antiviral response (Krzyzowska, et al., 2023).

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3.4.3 Selenium nanoparticles and EGCG

The development of selenium nanoparticles (SeNPs) has unveiled their promising biological applications, including antimicrobial, antioxidant, and anticancer properties as well as their utility in drug delivery systems, owing to their biocompatibility and bioavailability (Sampath et al., 2024). However, preclinical trials on rodent models have indicated potential adverse effects of SeNPs, such as weight loss and liver toxicity, emphasizing the need to establish effective therapeutic dosages while mitigating the side effects (Ryabova et al., 2024).

The particle size of selenium nanoparticles in two studies fell between 89 and 91 nm, showing consistent results despite variations in the starting materials and production methods (Wang et al., 2022; Alrashdi et al., 2023) (Table 2). In one study, mice with pentylenetetrazole (PTZ)-induced acute epileptic seizures were treated with EGCG or EGCG-SeNPs. The results showed reduced seizure duration and increased seizure latency in the EGCG-SeNPs treated group, indicating enhanced anti-convulsant effects, along with augmented antioxidant and anti-inflammatory functions. These effects could be attributed to the enhanced cellular uptake of EGCG through its incorporation into high surface area and nanosized SeNPs, thereby augmenting its therapeutic effects (Alrashdi, et al., 2023).

Another study demonstrated the antioxidant and anti-inflammatory properties of EGCG-SeNPs using models of oxidative stress induced by hydrogen peroxide (H2O2) and inflammation induced by lipopolysaccharide (LPS). The neuroprotective effects were evidenced by 1) higher Basso, Beattie, and Bresnahan (BBB) scores, 2) improved integrality of spinal cord structure with decreased lesion voiding, and 3) enhanced demyelination and nerve fibers preservation (Wang, et al., 2022).

3.4.4 Zinc oxide nanoparticles and EGCG

Zinc oxide nanoparticles (ZnO NPs) are chemically inert, possess a large surface area, exhibit high

photostability, and demonstrate an enhanced redox capacity, making them highly versatile for applications in drug delivery (Nagar et al., 2022; Zhou et al., 2023). While ZnO NPs have been deemed generally safe by the Food and Drug Administration (FDA), they still possess dose-dependent toxicity risks (Nagar, et al., 2022; Pushpalatha et al., 2022). Therefore, the future development of ZnO NPs should be accompanied by a thorough scrutiny of their potential hazardous effects, toxicity mechanisms and impacts on human health.

Due to the low bioavailability of EGCG, researchers have sought to develop EGCG-zinc oxide co-crystalline nanoparticles (EGCG-ZnO), harnessing their potential as effective anticancer agents. These negatively charged particles, with a relatively large size of 409 nm, have been successfully synthesized. The selective anticancer property of hybrid EGCG-ZnO has been demonstrated by the reduced viability of cancer cells without affecting the viability of normal cells when treated with the same concentration of EGCG-ZnO. Essentially, a safe concentration (12.5 μg/mL in the cited study) of EGCG-ZnO particle effectively killed cancer cells without causing any harm to normal cells. This effectiveness may stem from the synergism exhibited by the hybrid particles upon their simultaneous uptake into cancer cells, whereas the same phenomenon could not be observed in the simple physical mixture of EGCG and ZnO due to differences between EGCG and ZnO in their cellular penetration ability and penetration pathways. Consequently, simultaneous uptake is not achievable, which constrains the potential of EGCG-ZnO to achieve synergistic anticancer effects (Samutprasert et al., 2018).

3.5 Silica nanoparticles and EGCG

The favorable properties of silica nanoparticles (SiNPs), such as tunable particle size, excellent compatibility within living systems, and large surface area, have led to their widespread adoption (Huang et al., 2022). SiNPs have been successfully utilized in various applications including drug delivery systems, biomedical imaging, photodynamic and photothermal therapies, and bone regeneration (Selvarajan et al., 2020; Li et al., 2021). However, it is important to note that SiNPs may induce toxicity concerns in organisms, necessitating comprehensive toxicity evaluations during both development and specific application phases (Huang, et al., 2022).

The Stöber method, commonly employed for the production of SiNPs, involves the combination of tetraethyl orthosilicate (TEOS) with a mixture of water, alcohol and ammonia. Subsequent removal of water or alcohol molecules from the silicic acid molecules results in the formation of Si–O–Si condensate. Initially, primary SiNPs aggregate upon reaching a supersaturation point, followed by crystalline growth, yielding stable silica nanoparticles (Li, et al., 2021). A mesoporous silica nanoparticle (MSN) was synthesized using an ammonia-based catalysis technique, with TEOS as the primary component. This nanoparticle was then coated with chitosan and further functionalized with the AS1411 aptamer. The resultant entity efficiently loaded EGCG onto its structure, hence dubbed as SiO2@CS-EGCG-Ap. Its particle size was measured as 257 nm, with PDI approaching 1, indicating considerable heterogeneity. Incorporating the aptamer altered the surface charge of nanoparticle from +7.14 mV to -11.4 mV, a significant shift affirming successful conjugation with the targeting ligand. Notably, 80% of EGCG was effectively encapsulated, showcasing the high efficiency of SiO2@CS NPs. SKOV-3, an ovarian cancer cell line, served as the focal point of this investigation. SiO2@CS-EGCG-Ap demonstrated higher cellular uptake (50%) compared to its unconjugated counterpart (29.6%), attributed to the aptamer specific recognition of nucleolin on the cell surface, facilitating macropinocytosis. This heightened uptake translates into enhanced anticancer effects, as evidenced by 1) a lower IC50, 2) suppressed cell proliferation, 3) diminished cancer cell viability, 4) increased nuclei fragmentation, and 5) reduced expression of extracellular signal-regulated kinase 2 (ERK2) and human telomerase reverse transcriptase (hTERT), both pivotal in cellular growth and proliferation (Alizadeh et al., 2020).

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Table 2 Characteristics and biological effect of EGCG-loaded nanocarrier system

Type of nanocarrier	Composition	Production method	Particle size (nm)	Zeta potential (mV)	Encapsulation efficiency (%)	Experimental model	Biological effect (as compared to free EGCG)	Reference
Polymeric nanoparticle	Type-A Gelatin, Poly-γ-Glutamic Acid	Solvent injection	155.1 ± 7.3	−23.9 ± 0.9	51.2–72.4	Human colorectal adenocarcinoma cells (Caco-2 cell)	Enhanced antioxidant activity	(Zhang, et al., 2023b)
	Poly-lactic poly-glycolic copolymer (PLGA), Poloxamer 407	Double emulsion/solvent evaporation	134 ± 4	N/A	58 ± 5	Lung adenocarcinoma epithelial cell line (A549) and Human Dermal Fibroblasts (HDF) cells	Enhanced anticancer activity	(Minnelli, et al., 2023)
	Hordein	Liquid–liquid dispersion	160 ± 10	20.7 ± 0.4	91.2 ± 0.2	Human liver cancer cell line (HepG2)	Enhanced antioxidant activity	(He, et al., 2020; Song et al., 2022)
	Cationic soluble soybean polysaccharide (CSSPS)	Mixing followed by centrifugation and ultrafiltration	152 ± 3.35	+19 ± 0.86	87.53 ± 0.27	*In-vitro* - normal cell (human umbilical vein endothelial cells (HUVECs)) and various tumor cell lines (HCT-116, MCF-7, HepG2, K562, A549, and DU145), *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus*, *ex-vivo* - salmon	Enhanced antioxidant, antitumor and antimicrobial activities	(Zhou, et al., 2022)
	Glycine, formadelhyde, Pluronic F-127	One-step polyphenolic condensation reaction (Mannich condensation)	72.4 ± 9.7	−38	N/A	*In-vitro* - RAW264.7 cell line, mouse peritoneal macrophages extracted from Balb/c mice, *in-vivo* - chronic periodontitis rat	Enhanced anti-inflammation, antioxidant and anti-osteoclastogenic effects	(Tian, et al., 2022)
	Fluronic F-68, poly(n-butylcyanoacrylate) (PBCA)	Self-polymerization method	156.03 ± 4.82	−31.05 ± 2.41	90.32 ± 3.45	Silica-induced pulmonary fibrosis Sprague Dawley rat model	Enhanced anti-fibrosis effect	(Yao et al., 2022)
	Poly(n-butyl cyanoacrylate) (PBCA), ethyl acetate (EtOAc)	Interfacial polymerization	EGCG-PBCA NP water (50.9 ± 3.1) & EGCG-PBCA NP oil (55.7 ± 2.8)	EGCG-PBCA NP water (−21.7 ± 1.3) & EGCG-PBCA NP oil (−24.6	EGCG-PBCA NP water (76.30 ± 1.81) & EGCG-PBCA NP oil (74.60	*Bacillus subtilis*, *Staphylococcus sureus*, *Escherichia coli*, and *Micrococcus tetragenus*	Enhanced anti-bacterial activity	(Hu, et al., 2021)

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			± 3.0)	± 2.32)			
Chitosan	Nanoprecipitation	362.93 ± 28.36	40.87 ± 0.64	N/A	Gram-negative (*Pseudomonas fluorescens* ATCC 13525) and gram-positive strains (*Staphylococcus aureus* ATCC 25923)	Enhanced anti-bacterial and antioxidant effects	(Moreno-Vásquez, et al., 2021)
Lysozyme, pectin	Mixing	~200	−28.5 ± 1.42	71.77 ± 8.01	N2 wild-type *Caenorhabditis elegans*	Enhanced antioxidant activity	(Zhang et al., 2021)
Hyaluronan, fucoidan, polyethylene glycol, gelatin	Mixing and centrifugation	217.00 ± 14.00	−33.60 ± 1.30	52.08 ± 5.37	Raw264.7 cells	Enhanced anti-inflammation effect	(Ho et al., 2020)
Poly (lactic-co-glycolic acid), folate peptide	Nanoprecipitation followed by conjugation with Folate peptide (FA-Pep-1)	169 ± 5.6	−25.6 ± 3.81	52.3 ± 2.05	*In-vitro* - breast adenocarcinoma cell lines (MDA-MB-231 and MCF-7), *In-vivo* – Sprague Dawley rat and MDA-MB-231 tumor bearing nude mice	Enhanced antitumor activity	(Kazi et al., 2020)
Chitosan, β-lactoglobulin (β-Lg)	Ionic cross linking	157.2 ± 5.15	30.51 ± 5.31	76.29 ± 1.45	Human colorectal adenocarcinoma cells (Caco-2 cell)	Enhanced cellular antioxidant activity	(Dai et al., 2020a)
Polyvinylpyrrolidone (PVP), iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$)	Ultrasonication and dialysis	3.2±1.2	-10	N/A	Rat pheochromocytoma cells (PC12 cells)	Enhanced anti-fibrillation of Aβ40	(Liu, et al., 2019)
Poly (lactic-co-glycolic acid)-polyethylene glycol (PLGA-PEG) , Tween®80 (surfactant), ethyl acetate (oil phase)	W/O/W double emulsion-solvent evaporation	168.5 ± 9.9	−23.3 ± 5.3	>95	*In-vitro* - astrocytes, mouse brain microvascular endothelial cells (bend.3) and rat pheochromocytoma cells (PC12) cells, *in-vivo* - wild-type (WT) C57BL/6J mice, *ex-vivo* - rat neural progenitor cells (NPCs)	Enhanced anti-seizure and neuroprotection effects	(Cano, et al., 2018; Kühne et al., 2019)
Poly(lactic-co-glycolic acid) and pluronic® F127 (PLGA/PF127)	Emulsion/solvent evaporation	129.4 ± 15.5	N/A	86.27 ± 2.42	Nerve-like cells (NLCs)	Enhanced antioxidant activity	(Hoyos-Ceballos, et al., 2018)

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	Polylactic acid (PLA), polyethylene glycol (PEG)	Double emulsion solvent evaporation	317.8	−24.5	96.25±1.01	Male Swiss Albino Wistar rat induced with aluminum chloride and neuroblastoma cell line (SH-SY-5Y cells)	Enhanced anti-fibrillation, neuroprotection and anti-oxidant effects	(Singh, et al., 2018a; Singh, et al., 2018b)
	Type A gelatin, hyaluronic acid	Magnetic stirring	253.4±7.3	9.2±1.8	97.8±0.5	*In-vitro* - human corneal epithelium cells (HCECs), *in-vivo* - Wistar rat and New Zealand White rabbits with healthy eyes	Enhanced anti-inflammation effect	(Huang et al., 2018)
	Eudragit S100 (ES100), poly(lactic-co-glycolic acid) (PLGA)	Emulsion evaporation	91.3±0.8	-21.3	80.8±1.6	Sprague Dawley rats	Enhanced renal protection	(Zhang and Zhang, 2018)
Micelle	EGCG, 9-Fluorenylmethoxycarbonyl chloride (Fmoc-Cl), Pluronic F-68 (P-F68)	Esterification followed by solvent evaporation	163.1 ± 2.54	− 17.07 ± 0.25	N/A	Breast cancer cell (4T1 cells)	Enhanced anticancer activity	(Liu, et al., 2023)
	Tween 80 and Span 80	Direct dissolution	N/A	N/A	N/A	*In-vitro* - Henrietta Lacks (HeLa) cancer cells, *in-vivo* - Wistar rat	Enhanced anticancer activity	(Rosita, et al., 2019)
Lipid-based Liposome	Soybean phospholipids (SPC), cholesterol (chol), glucose ligand	Thin-film hydration and sonication	158.70 ± 1.30	2.37 ± 0.53	73.05 ± 1.00	Rat adrenal pheochromocytoma cells (PC12) and mouse brain microvascular endothelial cells (bend.3)	Enhanced antioxidant activity	(Xia, et al., 2023)
	1,2-Dipalmitoyl-sn-glycerol-3-phosphate-rac-(1-glycerol) (DPPG, sodium salt), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), choles-	Thin-film hydration	105	-25.5	90.5	Transfection cell line (HEK293T cells)	Enhanced anti-hypertension effect	(Haddad, et al., 2023)

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	terol							
	L-α-phosphatidylcholine (PC), phosphatidylserine (PS), cholesterol (CH) and α-tocopherol (vitamin E, VE)	Thin-film hydration	142.9 - 161.5	N/A	60.2 - 76.8	*In-vitro* - BV2 cells, *in-vivo* - Lipopolysaccharide (LPS)-induced Parkinson's disease rat	Enhanced anti-inflammation effect	(Cheng et al., 2021)
	1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and cholesteryl hemisuccinate (CHEMS), magnesium salt, poloxamer-407	Reverse Phase Evaporation (REV)	205.2 ± 8.9	−17.6 ± 4.1	95.0 ± 4.8	Adult human retinal pigment epithelial (ARPE-19) cells	Enhanced antioxidant activity	(Minnelli, et al., 2018)
Solid lipid nanoparticle	Glycerol, Sofitsan S100, Lipoid S75, cationic lipids (DDAB), ascorbic acid, Poloxamer 188 (P188)	Multiple emulsion (w/o/w)	143.7 ± 0.450	+25.70 ± 1.420	N/A	Human epithelial colorectal adenocarcinoma (Caco-2), human lung fibroblast (SV-80), human retina retinoblastoma (Y-79), human liver hepatocellular carcinoma (HepG2) and human breast adenocarcinoma (MCF-7)	Enhanced anti-proliferation effect	(Silva, et al., 2019)
	Glycerol mono-stearate (GMS), stearic acid, lecithin soy, Pluronic F68, bombesin	Double emulsification-evaporation followed by conjugation with bombesin	163.4 ± 3.2	−25.2 ± 2.8	67.2 ± 3.5	*In-vitro* - MDA-MB-231 human breast cancer cell lines and B16F10 mouse melanoma cells, *in-vivo* - tumor-bearing female C57/BL6 mice	Enhanced anticancer, anti-angiogenic, antitumor effects	(Radhakrishnan, et al., 2019)
Nanostructured lipid carrier	Solid lipid (Precirol ATO 5), liquid lipid (Mygliol 812), surfactant (Tween 60), folic	High-shear homogenization and ultra-sonication	313	−30	85	Breast carcinoma cell lines (MCF-7, MDA-MB-231, MCF-7TAM), and human normal mammary epithelial cells (MCF10A Cells)	Enhanced anticancer activity	(Farabegoli, et al., 2022)

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	acid (FA)							
Metal nanoparticle								
Gold nanoparticle	Chitosan, cysteamine, tetrachloroauric (III) acid ($HAuCl_4$)	Gold reduction (EGCG-ChAuNPs), Turkevitch method (EGCG-CystAuNPs)	EGCG-ChAuNPs (125 ± 13), EGCG-CystAuNPs (111 ± 1)	EGCG-ChAuNPs (36 ± 6), EGCG-CystAuNPs (−24 ± 1)	EGCG-ChAuNPs (60 ± 2), EGCG-CystAuNPs (78 ± 1.3)	Human pancreatic cancer cell lines (BxPC3)	Enhanced anticancer activity	(Cunha, et al., 2022)
	Hydrogen tetrachloroaurate(III) tetrahydrate ($HAuCl_4·4H_2O$)	Gold reduction	38.6- 135.2	–30.4 to –6.4	N/A	Cancer cells (MDA-MB-231, HeLa), and embryonic mouse fibroblast cell line (NIH3T3 cells)	Enhanced antioxidant and anticancer effects	(Gan, et al., 2022)
	Chloroauric acid ($HAuCl_4$)	Gold reduction	33.8 ± 2.2	−24.9 ± 0.3	92.6 ± 0.6	*Ex-vivo* - Venous blood samples were collected from male volunteers, *in-vivo* - Ehrlich's tumor bearing mice	Enhanced anticancer and antitumor effects	(Safwat et al., 2020)
	Sodium-tetrachloroaurate ($NaAuCl_4$)	Gold reduction	35	N/A	N/A	Human HCC (Hepatocellular carcinoma) HepG2 cells	Enhanced anticancer and antitumor effects	(Mostafa, et al., 2020)
	Gold (III) chloride trihydrate ($HAuCl_4·3H_2O$)	Gold reduction	90.3	–72.57	N/A	Human normal and cancer cell lines (MCF-10A, hTERT-HPNE [HPNE], RWPE1, MDA-MB-231, MIA PaCa, and PC3), normal immortalized keratinocytes (HaCaT), A375SM cell line	Enhanced anticancer effect	(Chavva, et al., 2019)
	Chloroauric acid ($HAuCl_4$)	Direct reduction	35.6	− 19.5	N/A	*In-vitro* - bone marrow macrophages (BMMs), *in-vivo* - LPS-induced mouse calvarial bone erosion	Enhanced anti-osteoclastogenic and antioxidant effects	(Zhu et al., 2019)
Silver nanoparticle	Silver nitrate ($AgNO_3$), sodium citrate ($C_6H_5Na_3O_7·2H_2O$), sodium borohydride ($NaBH_4$)	Seed growth-mediated method followed by incubation and stirring	32 ± 11	−67 ± 2	N/A	*In-vitro* - Human VK2-E6/E7 vaginal epithelial cells (ATCC® CRL-2616), Vero cells (ATCC® CCL-81) and HaCaT keratinocytes, *in-vivo* - Herpes simplex virus 1 and 2 (HSV-1 & HSV-2) infected C57BL/6 mice	Enhanced antiviral effect	(Krzyzowska, et al., 2023)
	Aminated guar	Solution mixing	217 ± 10	−2.6 to −1.59	N/A	*In-vitro* - *Escherichia coli* K12	Enhanced	(Kar, et al.,

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	gum (AGG), Sodium alginate (SA), glycerol, calcium chloride ($CaCl_2$), silver nitrate ($AgNO_3$)	and casting				(MTCC-1302), *Pseudomonas aeruginosa* (MTCC-424), *Bacillus subtilis* (MTCC-441), and *Staphylococcus aureus* (lab strain), *in-vivo* - wound-bearing Wistar rat	anti-bacterial and wound-healing effects	2019)
Selenium nanoparticle	Sodium selenite (Na_2SeO_3)	Mixing and magnetic stirring	89.3	−30.6	N/A	Pentylenetetrazole (PTZ)-treated male albino Swiss mice	Enhanced anti-convulsant, antioxidant and anti-inflammation effects	(Alrashdi, et al., 2023)
	Selenious acid	Dialysis	91.3 ± 35.7	N/A	N/A	*In-vitro* - Rat pheochromocytoma cells (PC12 cells), BV2 microglia, *in-vivo* - Spinal cord injury (SCI) Sprague-Dawley rat	Enhanced anti-inflammation and neuroprotection effects	(Wang, et al., 2022)
Zinc oxide nanoparticle	Zinc oxide	Co-crystallization	409.5 ± 9.2	−20.03 ± 0.23	N/A	Normal fibroblast cells (WI-38) and prostate cancer cells (PC-3)	Enhanced anticancer activity	(Samutprasert, et al., 2018)
Silica nanoparticle	AS1411 aptamer, chitosan, silica	Ammonia-based catalysis (mesoporous silica nanoparticle preparation) followed by ultrasonication and centrifugation	257	+ 7.14 ±5.9 mV (before conjugation), and after conjugation of the aptamer, its surface charge becomes − 11.4 ± 3.28	80	Ovarian cancer (SKOV-3) cell lines	Enhanced anticancer activity	(Alizadeh, et al., 2020)

N/A: Data is not available.

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4 Summary, challenges, and future prospects

From the reviewed studies on advanced formulations of EGCG, it seems that polymeric nanoparticles emerge as the most well studied and optimal choice of EGCG-loaded nanocarriers. This preference can be attributed to their relatively small size, high encapsulation efficiency, and favorable zeta potential compared to other nanocarriers. Moreover, the good safety profiles of EGCG-polymeric nanoparticles have been vigorously demonstrated across numerous studies (Table 3). In the comparison between various polymeric nanoparticles discussed in the involved studies (Table 2), one configuration stands out: EGCG enclosed within poly(n-butylcyanoacrylate) (PBCA)/Fluronic F-68 nanoparticle. This leads the pack primarily due to its favorable particle size (156 nm), stable surface charge potential (-31 mV), and impressive encapsulation efficiency (90%). While certain studies may boast higher encapsulation efficiencies, they often compromise other critical factors, such as zeta potential. Nonetheless, the choice of nanocarriers is highly dependent on the specific disease model and its desired therapeutic outcomes, and the main goal is to achieve optimized efficacy while mitigating any risks of potential toxicity.

Table 3 Toxicity Assessment of the EGCG-loaded nanocarriers

Composition	Experimental model	Toxicity assay/Dosage	Finding (s)	Reference
Polymeric nanoparticle				
Poly-lactic poly-glycolic copolymer (PLGA), poloxamer 407	Human Dermal Fibroblasts (HDF) cells	Cytotoxicity MTT assay (20 - 80 μM)	Low cytotoxicity in 20 -80 μM concentration range	(Minnelli, et al., 2023)
Cationic soluble soybean polysaccharide (CSSPS)	Human umbilical vein endothelial cells (HUVECs)	Cytotoxicity MTT assay (10 – 100 μg/mL)	High cell viability in 10 – 100 μg/mL concentration range	(Zhou, et al., 2022)
Chitosan, β-lactoglobulin (β-Lg)	Caco-2 cell	Cytotoxicity MTT assay (25 – 125 μg/mL)	High cell survival rate in 25 – 125 μg/mL concentration range	(Dai, et al., 2020a)
Poly (lactic-co-glycolic acid)-polyethylene glycol (PLGA-PEG) , Tween®80 (surfactant), ethyl acetate (oil phase)	Astrocytes and bEnd.3 cells	Cytotoxicity Alamar Blue assay (0.08 – 2.50 mg/mL)	>80% cell viability at concentration <1.25 mg/mL	(Cano, et al., 2018)
Poly(lactic-co-glycolic acid) and Pluronic® F127 (PLGA/PF127)	Nerve-like cells (NLCs)	Cytotoxicity Fluorescent microscopy and/or by flow cytometry (assessment of the ROS generation and mitochondrial membrane potential) (40 - 100 μg/mL of PLGA/PF127 NPs	No significant ROS production and alteration in mitochondrial membrane potential at concentration range of (40 - 100 μg/mL of PLGA/PF127 NPs & 10 - 50 μM of EGCG)	(Hoyos-Ceballos, et al., 2018)

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		& 10 - 50 μM of EGCG)		
Type A gelatin, hyaluronic acid	Human corneal epithelium cells (HCECs)	Cytotoxicity CCK-8 assay and live/dead staining (2 – 200 μg/mL)	High cell viability in 2 – 20 μg/mL concentration range	(Huang, et al., 2018)
Liposome				
Soybean phospholipids (SPC), cholesterol (chol), glucose ligand	Mouse brain microvascular endothelial cells (bEnd.3)	Cytotoxicity MTT assay (2.5 – 160 μM)	High cell viability up to 160 μM	(Xia, et al., 2023)
1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and cholesteryl hemisuccinate (CHEMS), magnesium salt, poloxamer-407	Adult human retinal pigment epithelial (ARPE-19) cells	Cytotoxicity MTT assay (22 – 66 μM of EGCG & 35 – 110 μg/mL of lipid)	High cell viability in concentration range of (22 – 66 μM of EGCG & 35 – 110 μg/mL of lipid)	(Minnelli, et al., 2018)
Gold nanoparticle				
Hydrogen tetrachloroaurate(III) tetrahydrate ($HAuCl_4 \cdot 4H_2O$)	NIH3T3 cells (embryonic mouse fibroblast cell line)	Cytotoxicity CCK-8 assay (2.5 – 10 μM)	High cell viability in 2.5 – 10 μM concentration range	(Gan, et al., 2022)
Chloroauric acid ($HAuCl_4$)	Bone marrow macrophages (BMMs)	Cytotoxicity CCK-8 assay (5 – 25 μM)	High cell viability in 5 – 25 μM concentration range	(Zhu, et al., 2019)
Silver nanoparticle				
Aminated guar gum (AGG), sodium alginate (SA), glycerol, calcium chloride ($CaCl_2$), silver nitrate ($AgNO_3$)	*In-vitro* - Murine Skin Keratinocyte cell lines (MSC-P5) *In-vivo* - Wound-bearing Wistar rat	Cytotoxicity MTT assay *(Dosage not stated)*	*In-vitro* - 75 - 80% cell viability *In-vivo* - No histo-architecture alteration on liver and kidney, normalized liver and kidney functions (comparable serum level of urea, creatinine, AST, ALT to control group)	(Kar, et al., 2019)
Zinc oxide nanoparticle				
Zinc oxide	Normal fibroblast cells (WI-38)	Cytotoxicity MTT assay (0.0275 – 0.44 μg/mL of EGCG & 3.125 – 50 μg/mL of ZnO)	High cell viability in concentration range of (0.0275 - 0.11 μg/mL of EGCG & 3.125 – 12.5 μg/mL of	(Samutprasert, et al., 2018)

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ZnO)

Nanocarriers are often constructed 1) from biocompatible materials to minimize the risk of immune response, 2) from biodegradable materials to minimize long term accumulation effects, 3) with good membrane penetration via its appropriate surface properties (size, charge, surface chemistry), 4) to allow surface modifications facilitating receptor-mediated update, and 5) to enable controlled release to adjust to the diffusion coefficient, physiological stimuli (pH, presence of enzymes or biomolecules), dissolution and degradation rates. Nevertheless, a significant gap exists in the literature regarding the comprehensive evaluation of EGCG-loaded nanocarriers. Specifically, exploration has been limited regarding 1) how the design and construction of delivery vehicles facilitate membrane penetration, 2) the potential acute and long-term effects stemming from high load capacity of the nanocarriers, 3) thorough toxicity assessments of the nano-complex in biological system, and 4) the microstructure and interface characteristics of EGCG nanocarriers.

Among the analyzed studies, four investigated the membrane internalization mechanisms of EGCG nanocarriers. These carriers exhibit the ability to permeate intestinal epithelial cells and enter systemic circulation. Two of these studies illustrated the intestinal absorption of EGCG-polymeric nanoparticles by disrupting membrane tight junctions, thereby facilitating their passage through the paracellular pathway (Huang et al., 2020; Zhang, et al., 2023b). Furthermore, two studies demonstrated the cellular uptake of EGCG gold nanoparticles via laminin receptors (Chavva, et al., 2019; Gan et al., 2022). More research is evidently needed to provide further insights into how EGCG-loaded nanocarriers enhance membrane permeability, including the clarification of the roles played by their microstructure and interface characteristics.

Among the discussed studies, 12 out of 39 studies assessed the toxicity profile of EGCG-loaded nanocarriers using various cell lines, while only one study performed this in an animal model (Wistar rats). These studies reported low cytotoxicity and high cell viability, and no adverse effects on the liver and kidney functions of Wistar rats (Table 3). The findings indicate that the selection of chemical constituents and excipients in these studies is appropriate and the EGCG-loaded nanocarriers exhibit a favorable safety profile. The utilization of biocompatible and biodegradable materials with well-established safety profiles is prioritized in pharmaceutical formulation, including the construction of EGCG nanoparticle formulations reviewed in this study.

Potential toxicity due to the high load capacity of nanocarriers could be mitigated by implementing a controlled release strategy that minimizes exposure to high concentrations of EGCG. Nonetheless, such toxicity is unlikely to occur, as the dose-dependent analysis of EGCG is well established and the determination of maximum tolerable dose for either rodent models or human trials is possible based on previous concrete findings (Parn, et al., 2022; Siblini et al., 2023). Moreover, several studies reviewed in this paper have shown that EGCG-loaded polymeric and lipid nanoparticles exhibit controlled release of EGCG in simulated gastric and intestinal fluid conditions, maintaining low toxicity and high cell viability (Cano, et al., 2018; Hoyos-Ceballos et al., 2018; Minnelli, et al., 2018; Minnelli, et al., 2023). Future assessment should include comprehensive toxicity studies involving multiple in-vivo models over longer periods, detailed dose-response analyses, and mechanistic studies to understand the interactions between nanocarriers and biological systems. Certainly, long-term monitoring in both animal and human trials is essential to evaluate the safety, efficacy and potential delayed or cumulate adverse effects.

On the other hand, it is crucial to apply standardized evaluation methods for EGCG nanocarriers in the future to ensure consistency and reliability across different research endeavors. Beyond standardizing particle size and distribution, surface charge (zeta potential), drug encapsulation efficiency, in-vitro cell viability, and uptake across studies discussed in this article, additional assessments such as drug release kinetics, in-vivo biodistribution and pharmacokinetics, immunogenicity, and biocompatibility, targeting efficiency in disease models, as well as stability and shelf-life studies are needed for a comprehensive understanding of the properties and performance of nano-delivery vectors. As nanoparticles move towards clinical translation, scalability and

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manufacturing processes should also be standardized to ensure reproducibility and quality control. Moreover, variations in individuals' genetic background and polymorphisms may exert significant influence on the response to EGCG nanocarriers, particularly its pharmacokinetics and pharmacodynamic profiles, presenting a substantial challenge in the assessment of efficacy and safety in human trials and clinical settings. In addition, it has been demonstrated that EGCG may influence various drug pharmacokinetic and pharmacodynamic profiles (Tan et al., 2021; Siew-Keah et al., 2023). Therefore, it is mandatory to perform a systematic re-assessment of drug-drug interactions of both the free form of EGCG and EGCG loaded in nanoparticles. On the other hand, it would be beneficial to conduct a comparative study assessing the impact of each nanocarrier on the stability and biological activity of EGCG. All parameters, including the preparation method and drug-to-excipient ratio, should be standardized, with the only variable being the excipient used in each carrier—given that these nanocarriers are differentiated by their distinct excipients. Such approach would allow for a relatively equitable comparison of the structural, physicochemical properties as well as pharmacokinetic indexes. In order to ensure the advancement of future clinical trials, addressing these challenges is imperative. Strategies must be devised to navigate the intricacies of genetic diversity, perhaps via tailored dosing regimens, patient stratification based on genetic markers, or the integration of personalized medicine approaches.

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Author contributions

Chee Ning WONG was involved in investigation, writing – original draft, editing, and visualization. Siew-Keah LEE was involved in conceptualization, writing – review, editing and supervision. Yang Mooi LIM, Kai Bin LIEW, Yik-Ling CHEW, Ang-Lim CHUA were involved in investigation, final review, and proof-reading. All authors have read and approved the final manuscript and, therefore, had full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Chee Ning WONG, Yang Mooi LIM, Kai Bin LIEW, Yik-Ling CHEW, Ang-Lim CHUA and Siew-Keah LEE declare that they have no conflict of interest. This review does not contain any studies with human or animal subjects performed by any of the authors.

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