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Cancer therapeutics with epigallocatechin-3-gallate encapsulated in biopolymeric nanoparticles

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Graphical abstract
Abstract

With the recent quantum leap in chemoprevention by dietary products, their use as cancer therapeutics is garnering worldwide attention. The concept of effortlessly fighting this deadly disease by gulping cups of green tea or swallowing green tea extract capsules is appreciated universally. Epigallocatechin-3-gallate (EGCG), a major polyphenol in green tea, has generated significant interest in controlling carcinogenesis due to its growth-inhibitory efficacy against a variety of cancers by targeting multiple signaling pathways. However, the success of EGCG in preclinical studies is difficult to translate into clinical trials due to issues of low solubility, bioavailability and an uncertain therapeutic window. The laborious and expensive journey of drugs from the laboratory to commercialization can be improved by utilizing nanoparticles as anti-cancer drug carriers. Exploitation of biopolymeric nanoparticles in recent years has improved EGCG’s biodistribution, stability and tumor selectivity, revealing its superior chemopreventive effects. This review briefly summarizes recent developments regarding the targets and side effects of EGCG, complications associated with its low bioavailability and critically analyses the application of biopolymeric nanoparticles encapsulating EGCG as a next generation delivery systems.

Keywords: Cancer, Green tea, EGCG, Biopolymers, Nanoparticles

Introduction

Cancer remains a major challenge owing to the diversity of affected patients, the multitude of sites involved, and the poor efficacy of many conventional treatments. The complexities of
targeting cancer at the genetic and phenotypic levels represent a challenging task [1]. Among the numerous treatment strategies employed, the use of nutraceuticals such as plant polyphenols and carotenoids holds chemopreventive potential against diverse cancer types [2]. Recent evidence demonstrates that polyphenols such as Curcumin, Resveratrol, Quercetin, etc. found in fruits, vegetables and other plant parts possess cancer-preventive effects and can inhibit growth of cancer cells[3-5]. Similarly, in the past few years the anti-cancer potential of “green miraculous drink” i.e. green tea and its catechins, have been demonstrated in animal models involving multiple cancer types. The probable mechanisms identified that hamper cancer development and/or progression include inhibition of proliferation, initiation of apoptosis, and suppression of critical processes including invasion, migration and angiogenesis[6]. Therefore, there is the potential to reap the benefits of a pleasantly flavoured medicinal drink with multiple health benefits against a myriad of illnesses, including cancer[7].

Next to water, tea is the major consumed beverage worldwide, prepared from the dried leaves of *camellia sinensis*[8]. Alternative forms of tea can be obtained by different preparation techniques, including the major varieties of green, black and oolong tea. Green tea contains polyphenols commonly known as tea catechins, such as (-)Epigallocatechin-3-gallate [EGCG], (-)Epigallocatechin[EGC], (-)Epicatechin-3-gallate[ECG] and (-)Epicatechin[EC] which account for their major biological and pharmacological activities[9]. Catechins present in green tea constitute 33% of the total dry weight, which is significantly higher than red and oolong tea[10]. Among the catechins, EGCG is of high importance due to its abundance, constituting 50-80% of total catechin content in green tea, which is equivalent to 200-300 mg/brewed cup. In addition its safety profile, easy availability and simple extraction procedure set it apart from other polyphenols[11]. EGCG possesses several therapeutic properties including anti-oxidant, anti-proliferative, anti-angiogenic and anti-carcinogenic
effects[12]. EGCG is an example of a class of monomeric flavan-3-ols which are characterized by hydroxylated aromatic rings. The presence of a pyrogallol-type structure and gallolyl moiety on separate rings of EGCG are strongly associated with induction of apoptosis, formation of reactive oxygen species and cytotoxic activities against cancer cells[13-15]. A plethora of epidemiological, cell-based studies, animal models and clinical trials have provided convincing data to support EGCG’s anti-cancer effects. EGCG modulates multiple cancer-related pathways, including downregulation of redox-sensitive transcription factors (eg NFκB), triggering of programmed cell death through upregulation of Bcl-2, inhibition of angiogenic molecules including VEGF, induction of cell cycle arrest at G0/G1 phase, and modulation of intracellular signal transduction pathways such as MAPK and PI3K/AKT[16].

Adding to the benefits of EGCG, newer data gleaned from pharmacokinetic studies suggest that after green tea intake a majority of the plasma population (> 75%) represents free EGCG rather than EGC and EC[17-19]. Interestingly no detectable amounts of EGCG or its metabolite 4',4'-DiMeEGCG have been detected in urine samples, despite significant plasma concentrations, suggesting a non-renal mediated clearance[20]. Excretion of EGCG in rats occurs via the hepatic route, which is the presumed route in humans as well[21]. This may explain the longer elimination half-life of EGCG in the body (3.4±0.3 h ) compared to other catechins[22]. Despite low systemic circulation, EGCG was still found in human prostate tissue[23] animal tissues of mice[24], foetuses, placenta[25], and the brain of rats[26] suggesting distribution properties that could be exploited for other medical conditions. Clearly EGCG possess great potential in cancer treatment and prevention but low bioavailability, inappropriate systemic release and bioaccessibility has limited the role EGCG in clinical settings. Although there are several aspects of EGCG biology, pharmacodynamics, and pharmacokinetics to be better understood in order to target cancer effectively (Figure 1),
improving the bioavailability and site specific delivery are presumed to be fundamental to enhancing its efficacy.

Oral bioavailability of EGCG is less than 2% of the administered dose in rats[27] and below 20% in mice[28]. The interpretation of EGCG bioavailability in humans is hampered by a lack of adequate information as most studies examined the pharmacokinetics of tea catechins. Oral bioavailability of EGCG is affected by several factors such as molecular weight, pH, biometabolic conversions, presence of metal ions, temperature and oxygen concentration[29].

High molecular weight catechins, a greater number of hydroxyl groups and presence of a gallolyl moiety in EGCG accounts for its inability to pass through the intestinal epithelium resulting in poor bioavailability[30]. Degradation of EGCG increases with relative humidity and temperature, as demonstrated using spray dried green tea extract, thus lowering its bioavailability[31]. EGCG is also sensitive to the pH range within the human gastrointestinal tract. High pH leads to oxidation of EGCG which results in the formation of the dimerized product the asinensins A and B, hence reducing pure EGCG’s accessibility[32-34].

The various processes occurring in gastrointestinal tract (GIT) including drug metabolism, molecular transformation, and interaction with the colonic microbiota, can impair EGCG absorption. Extensive modification of EGCG to its sulphated, methylated and glucuronidated conjugates in the small intestine, or its metabolism into phenolic acids and valerolactones by gut microbiota impedes EGCG entry into the blood stream[35, 36]. Samples from human plasma demonstrate that EGCG can be found within several hours after green tea consumption but is undetectable after 24h. When EGCG gets ingested with acidic food types such as strawberry sorbet[37] and fruit juices[38], or dietary proteins (casein, soy protein or skimmed milk)[39], systemic absorption and bioavailability can be impaired [40]. Apart from the above issues affecting bioavailability, the administration of pure EGCG through diet or
intraperitoneal (I.P) injection is not well tolerated due to its various side effects. These include hepatotoxicity and upregulation of inflammatory responses with increases in inflammatory markers[41]. Hence it is imperative to consider potential approaches to improve EGCG bioavailability, stability and therapeutic utility for future use.

A successful strategy gaining momentum in pharmaceutical research is nanoencapsulation, as demonstrated by the exponential increase in research articles, patents, FDA approved products with nanoparticles, and investment in the nutraceuticals area. Research from several groups have explored the use of nanomaterials such as Gold Nanoparticles[42], Polymeric Nanoparticles[43], and Liposomes[44] for delivery of EGCG to facilitate drug entry, improve bioavailability, escape biometabolic modification and prolong circulation time.

Among these nanoparticle solutions, biopolymeric nanomaterials have emerged as a spatio-temporal therapeutic drug delivery nanocarrier which has addressed many of the above challenges. Polymeric nanoparticles have many advantageous properties such as enhanced permeation (chitosan and positively charged polymers), escape from hepatic first pass metabolism by delivery of therapeutics via M-cells (PLGA nanoparticles) and providing protection against high pH and enzymes (alginate and chitosan based polymers) in the gut. In addition, polymeric nanoparticles can be easily formulated and scaled up to a variety of sizes yielding steady drug levels compared to ceramic, gold or metal nanoparticles[45]. However, the major challenge for drug nanocarriers in pharmaceutical therapeutics are low drug encapsulation, premature release, poor permeability across epithelial junctions, degradation by digestive enzymes, and instability to the varying pH found throughout the gastrointestinal tract[46, 47].
The source of polymeric nanoparticles used for nanodelivery can be either natural (e.g. Chitosan, Alginate) or synthetic (e.g. polylactides, polyalkylcyanoacrylates). Naturally occurring polymers have advantages over synthetic polymers with respect to accessibility, immunogenicity, patient compliance, biodegradability and toxicity[48].

EGCG has been increasingly researched with each passing year for various applications as shown in Fig 2. Therefore the future of nanoparticle based drug delivery systems for EGCG is an area of great promise.

This review summarizes the potential benefits and drawbacks of EGCG as an anti-cancer nutraceutical and recent research highlighting the importance of natural polymeric nanocarriers in improving the effectiveness and translating EGCG research into human health.

**Biopolymer Nanoparticles: Saviour for EGCG**

Conventional methods of delivering EGCG suffer from multiple drawbacks, therefore novel and potent carriers are needed to tackle the intricacies of EGCG biodelivery. New evidence supports the notion that encapsulation of EGCG inside a matrix of polymers improves its pharmacokinetics and pharmacodynamics in comparison to noncapsulated EGCG. Table I lists the studies carried out with different polymeric nanomaterials, their synthesis methodologies, and the effect of EGCG loaded NPs on cancer cell lines.

To deliver polyphenols such as EGCG, chitosan based nanocarriers have become the ideal choice owing to the availability of amino groups for further functionalisation, uncomplicated
drug loading methods, interaction with the gastrointestinal mucus layer and versatile routes of administration[49]. Several studies have investigated the bioavailability of EGCG encapsulated in chitosan based carriers and found them to be more proficient relative to free EGCG. Oral administration of chitosan tripolyphosphate nanoparticles encapsulating EGCG augmented intestinal absorption and elevated the jejunum and plasma exposure of EGCG by 2.3 and 1.5 fold respectively in mice. The improved oral absorption and enhanced plasma exposure was believed to be due to improved drug stability within the GIT due to CS NPs leading to an increase in steady-state transport of EGCG across the epithilium[50].

Rocha et al. synthesized nanoparticles by dissolving gum arabic in water followed by addition of maltodextrin and EGCG. The main function of this polymeric matrix nanoparticle carrier system is improved drug encapsulation efficiency and improved drug stability via decreased oxidation. Significant degradation of EGCG has been observed within 1h at intestinal pH, however loading into polysaccharide nanoparticles resulted in a sigmoidal drug release profile with 46% of drug discharge in 10 min followed by slow release. Encapsulated EGCG showed significant antiproliferative activity against human prostate cancer cells (Du145), at very low concentrations(1-2 µM) in comparison to free EGCG [51].

One of the methods of increasing the intestinal absorption of soluble but poorly absorbed nutrients is through the direct uptake of nanoparticles. Accordingly a study reported the synthesis of chitosan-casein-phosphopeptide nanoparticles(CPP-CSNPs) through sonication. During this process anionic CPP and cationic CS associate electro statically to form nanostructures. Encapsulation of EGCG takes place due to hydrophobic association between the gallate groups of EGCG and proline groups of CPP. A key finding of this study is the use of a combination of polymer and peptide in order to improve EGCG’s bioavailability. Efficient endocytosis of chitosan nanoparticles and utilization of peptides to lessen the
cytotoxicity makes this nanocomposition an appropriate carrier for drug delivery. An increase in the cellular uptake of nanoparticles and significant increase in $P_{app}$ of EGCG was observed in a time and dose dependent manner, most likely due to the protection of EGCG against intestinal enzymes afforded by the nanoparticles. Upon nanoparticle entry into lysosomes and exposure to acidic pH, EGCG is released after digestion of chitosan nanoparticles. Furthermore, reduction in transepithelial resistance in caco-2 monolayers exposed to chitosan nanoparticles due to compromised tight junctions is consistent with paracellular penetration of EGCG [52].

In another study carried out by the same group nanoformulation of CCP-CS NPs and the natural cross-linking agent from gardenia fruits, Genipin, has been used to overcome the poor stability and limited drug loading capacity. Crosslinking with Genipin stabilizes CPP-CS NPs to the harsh gastrointestinal pH range (1.2-7.4) and the presence of digestive enzymes, as demonstrated by the minimal changes in nanoparticle size, morphology and zeta potential after exposure to simulated gastric conditions. Controlled release of cargo within a preferred pH range was found to be dependent on the degradation of chitosan chains, which could be modulated by adjusting the degree of cross linkage. Cross-linked NPs released drug more efficiently in the intestinal environment compared to the gastric environment, which is a vital requirement for oral delivery. Cross-linked encapsulated CPP-CS NPs inhibited replication in HepG2 and BGC24 cancer cell lines to a similar degree as free EGCG, demonstrating retained anti-cancer properties of EGCG[53].

Using an alternative approach, De Pace et al. synthesized EGCG nanocarrier using nanoliposomes (LIPO-EGCG) made up of the cell membrane components cholesterol and phosphatidylcholine using a thin film hydration method. Additionally 0.2 % coating of chitosan over the void (CSLIPO) and drug encapsulating nanoliposomes (CSLIPO-EGCG)
were added to improve the stability of liposomes and EGCG. Chitosan coated nanoliposomes were found to be resistant to quick degradation at 4°C in 1X PBS showing only 22% breakdown after 6 days. However native EGCG was completely degraded within 5 days. A similar delay of degradation was shown by coating nanoliposome in 10% FBS at room temperature for 1h. Also, nanoencapsulation enabled an increase in cellular EGCG content as demonstrated by higher uptake in MCF-7 cells, revealing a role of chitosan as a cellular uptake enhancer. Sustained release of EGCG from CS-LIPO was seen at pH 5, whereas native EGCG showed a rapid drug dissolution rate at this pH. Coated nanoliposomes attenuated cell viability by 40% and induced apoptosis in up to 27% MCF-7 cells at a concentration of 10 µM in contrast with native EGCG[54].

Apart from hydrophilic polymers such as PLA and PEG, bio-nanoparticles including protein and polysaccharide based formulations have also been exploited to increase the biodistribution of EGCG. One approach utilizes the egg white protein ovalbumin and glucan dextran to prepare nanoparticles via the Maillard reaction. Li et al. hypothesized that hydrophobic protein forms a core of the nanoparticles and the shell is formed by hydrophilic dextran. This organization produces drug conjugated nanoparticles by self-assembly with EGCG. Additionally cross-linking of ovalbumin with glutaraldehyde was carried out to maintain NP’s structure.[55]. Crosslinking in NPs also enabled controlled drug release in SGF and SIF in the presence or absence of digestive enzymes when compared to conjugated NPs due to enhanced stability. Interstingly conjugated NPs produced similar transpeithelial resistivity but augmented the permeability coefficient of EGCG on Caco-2 monolayers in comparison to drug alone, consistent with higher cellular uptake of NPs.

An alternative strategy utilises bovine serum albumin(BSA) to ameliorate EGCG absorption. BSA NPs were prepared by desolvation with loading of EGCG accomplished during
synthesis by simple mixing. In this method coating nanoparticles with absorption enhancers such as Chitosan (CBEN) and Poly-ε-lysine (PBEN) gave an added advantage of high cellular uptake[56]. The hydrophilicity of these polymers hinders particle aggregation at pH 1.5-6. BSA-NPs without coating resulted in rapid release of 52.7% and 68.5% in SGF and SIF in one hour respectively. In contrast, coated NPs delayed EGCG release, which was attributed to the enzymatic resistance created by coating them with enzyme responsive polymers, making them well suited for drug delivery. In addition, coating nanoparticles with polymers increased the storage stability of the EGCG at 60 ºC for 6 hrs when compared with uncoated particles. Chitosan coating resulted in marked decrease in the TEER (Transepithelial electric resistance) value of treated monolayers and an increase in the permeability coefficient of EGCG at 30 and 120 minutes when compared with EGCG alone.

In spite of the experimental benefit of using nanoparticles to deliver EGCG in-vitro validation of in-vivo activity is required before being able to utilize nanocarriers in clinical settings. To prepare chitosan nanoparticles for systemic administration, water soluble chitosan was allowed to first interact with EGCG and then with TPP (pentasodium tripolyphosphate hexahydrate) by alternative cycles of stirring and sonication. Chitosan facilitated the stability of NPs in an acidic environment as demonstrated by slow drug release in gastric juices, and enhanced drug release kinetics in simulated intestinal fluid by 5-fold compared to synthetic polymer encapsulation (PLA-PEG) used earlier by the same group[57].

In vivo prostate cancer studies were performed using athymic male nude mice subcutaneously injected with androgen positive 22Rv1 cells resulting in fast tumor growth and secretion of prostate cancer marker PSA in serum[58]. Nanopreparations of EGCG impeded the growth of tumor xenografts with 6-fold less dosage required compared to native EGCG. Furthermore, Chitosan nano-EGCG treatment delayed the emergence of tumor growth by approximately a month relative to control chitosan nanoparticles. Nanoparticle treatment was associated with
decreased secretion of the prostate cancer marker PSA and cleavage of DNA repair protein PARP. Also induction of programmed cell death in conjunction with impaired cell proliferation was observed after NP treatment with markedly augmented Bax levels, reduced Bcl-2 expression, activated caspase-3, 8 and 9, and attenuated expression of both proliferation markers (Ki-67 and PCNA) and angiogenesis markers (CD-31 and VEGF). The same group further investigated the effectiveness of nanoencapsulated EGCG against melanoma tumor xenografts[59]. NPs demonstrated significant inhibitory effects against growth of Mel 928 cells by inducing the apoptotic pathway with modulation in apoptotic protein levels. Treatment with NPs caused melanoma cell-cycle arrest in the G2/M phase in a dose dependent manner. These results were accompanied by increased expression of major cell cycle regulatory proteins (p21 and p27) as well as a decrease in p27 regulated cyclins (cyclin D1 and D3). In vivo data in a mouse model of melanoma supported the in vitro findings with impairment of tumor growth and development observed and a 10-times EGCG dose advantage relative to the native compound.

Building on the benefits ascribed to active targeting of cancer, Lin et al. designed NPs with chitosan fucose (FCS) and PEG conjugated chitosan (PCS) complexes by continuous stirring after adding all components. PCS was prepared by dissolving depolymerised chitosan in DMSO and acetic acid followed by addition of methoxy PEG succinimidyl ester. In case of FCS, chitosan was first dissolved in 1:1 ratio of methanol and acetic acid with further addition of fucose. To increase the encapsulation efficiency of EGCG in chitosan conjugated NPs, a natural polymer gel was used. For site specific targeting fucose was preferred as its receptors are highly overexpressed in gastric cancer cells. These modifications imparted stability to the NPs and prevented protein repulsion while increasing the interactions at biological interphases. Sustained drug release from conjugated nanoparticles was observed at pH 1.2-5.5 due to the protein stabilizing property afforded by PEG agasint proteases present.
in gastric fluids. At higher pH (> 6.5) deprotonation of the NH$_2$ groups in chitosan occurs, resulting in release of 85.5% of EGCG within 6h. Due to the mucoadhesive properties of chitosan, EGCG release was more prominent around and within MKN45 cell monolayers resulting in significant reductions in cell growth. In addition EGCG-NPs exhibited 2.5 times higher apoptosis and decreased VEGF expression compared to EGCG alone. VEGF expression has been decreased to approximate 85, 71 and 53% after treatment with nanoparticles having EGCG concentration of 10, 20 and 40 mg/L respectively. *In vivo* studies using an orthotopic gastric tumor mouse model suggested that 40mg/kg of EGCG in nanoparticles was sufficient to inhibit gastric tumour growth by reducing tumor tissue spread and augmenting inflammatory responses, resulting in an improved survival rate[60].

Nanoparticle assisted combination therapies have been used in cancer treatment to capitalise on the synergistic effects of drugs. In one study nanocarriers were synthesized using the FDA approved polymer poly(lactic-co-glycolic acid) and milk protein casein by emulsion and precipitation to target breast cancer cells using intravenous drug delivery. The hydrophobic anti-cancer drug paclitaxel and hydrophilic EGCG were encapsulated in the PLGA core and casein shell of the nanocarrier respectively. Nanoparticles were also coupled with anti-EGFR and anti-HER2 antibodies that selectively targeted nanoparticles to cancer cells by utilising cell surface receptor expression. This combinatorial strategy utilising two drugs and receptor mediated targeting allowed sequential release of EGCG followed by paclitaxel through controlled means. Interestingly, *in vitro* results demonstrated that the encapsulation of both drugs together increased their cellular uptake, cytotoxic behaviour and apoptotic potential against paclitaxel resistant breast cancer cells (MDA-MB-231) compared to a nanocarrier carrying one drug only. The collective action of the drug combination ameliorated chemotherapy resistance exhibiting an anti-NFkB effect resulting in reduced expression of NFkB dependent genes (MMP9, VEGF1, BIRC5), and lowering efflux pump (P-gp)
expression. Use of these targeted combined nanocarriers circumvents paclitaxel shortcomings and augments its anti-cancer effect in MDA-MB-231 cells and patient-derived cancer cells. These studies demonstrate that combining anti-cancer drugs and polyphenols such as EGCG using polymeric nanocarriers is a worthwhile avenue for ongoing cancer research[61].

A further advance in the field of nanocarrier mediated drug delivery is the development of a ligand-targeted delivery system. Although laborious and costly this strategy has numerous advantages including increased efficacy and reduced side effects[62]. This concept was utilised through an insightful strategy targeting prostate cancer cells with folate functionalised BSA nanoparticles. Folic acid functionalisation led to a significant increase in EGCG-BSANP cellular uptake linked to enhanced PC-3 cell death up to 82.8% compared with EGCG[63].

Taken together the promising studies cited above support the concept of anti-cancer effect of EGCG and empower the idea of utilizing biopolymer nanoparticles as novel and efficient means for protecting EGCG from the harsh gastrointestinal environment and delivering it to target locations. Figure 3 shows the positive effects imparted by polymeric nanomaterials with respect to EGCG in cancer prevention.

**Conclusion and Future Perspective**

There is increasing interest in linking lifestyle, including dietary factors, to cancer prevention and adjuvant treatment. Studies globally have revealed that tea flavonoids are endowed with multiple potential health benefits, and may represent a significant step in anti-cancer therapy. Despite the strong evidence of catechin anti-cancer activity in pre-clinical studies several issues need to be addressed when considering translating these results into human studies. To exploit the full potential of chemopreventive agents from tea, polymeric nanocarriers appear
to be an effective solution. Several nanotechnology-based formulations, some already approved by FDA, and others undergoing clinical trials such as Doxil, DaunoXome, DepoCyt, Abraxane, Genexol-PM, and BIND-014, underscore the progress that this multidisciplinary field has made in developing effective anti-cancer therapies[64].

The concept of nanochemoprevention is a plausible approach, opening up new horizons for preventing carcinogenesis by enhancing the stability bioavailability and targeting of anti-cancer nutrients such as EGCG. However, filling in scientific gaps will be critical in order to translate their use into clinical settings, for example the super-physiologic doses used in various *in vitro* studies of EGCG leading to inconsistent results *in vivo* [65]. Therefore, administration of an appropriate concentration of drug, form of dose (capsule, brewed, tea extract, or accompanying a meal) and frequency of dosage need to be considered before its application *in vivo*. Low oral bioavailability of EGCG is often linked with its poor bioefficacy, therefore it is important to follow systematic approaches to improving bioavailability. Firstly, by utilizing other modes of administration in order to escape metabolic conversion and conjugate formation. Secondly, by selecting and designing specific nanocargo to mask EGCG during its transit from the site of administration to its target tissue, without degradation in the harsh conditions of the GI tract[66].

In order to fully elucidate the role of tea catechins *in vivo*, various metabolites of EGCG, which have been documented in plasma, urine, and faeces after green tea intake, must be comprehensively researched to assess their chemopreventive properties, which are unknown at the moment. Since EGCG is versatile in nature and exerts its diverse effects by acting on multiple intracellular targets within cancer cells, it would be beneficial to concurrently target synergistic pathways through the use of combinatorial therapies. Although studies reported
the adjuvant effect of EGCG when used along with chemo/radio therapies, the dose of EGCG used in these studies limits their interpretation and hence additional efforts are needed[67].

The currently described bipolymeric nanoparticles hold great promise, however they suffer from challenges including low encapsulation efficiency, instability, and limited modification potential. The use of inorganic nanocarriers in combination with biopolymeric materials could alleviate the above disadvantages. Recently, the use of FDA approved bio-protein based nanoparticles and nutraceutical-polymer nanocomposites have gained popularity due to their ability to encapsulate many hydrophobic nutraceuticals such as Curcumin, Resveratrol and Quercetin, however its applicability in loading and delivery of EGCG is not well-studied to date[68-70]. Inorganic nanocarriers like mesoporous silica nanoparticles (MSNs) have shown great promise in delivery of polyphenols [71-74]. We believe that MSNs also hold great promise in encapsulation of EGCG and can be used alone or in combination with biopolymers such as chitosan, alginate and lipidsto create smart nanodelivery systems for better pre-clinical and clinical outcome using EGCG.

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References:


**Figure 1.** Major areas requiring investigation for exploiting the anti-cancer potential of EGCG using drug delivery.
Figure 2. (a) Numbers of SCI-indexed publications searched on PubMed demonstrating increased research interest of EGCG. (b) Graph signifies the continuous increase in publications referencing EGCG encapsulated nanoparticles and significant increase in number of publications.
Figure 3. Multiple beneficial effects of biopolymeric nanomaterials encapsulating anti-cancer drug EGCG. EGCG: Epigallocatechin-3-gallate, SI: Small intestine.
Table I. Different biopolymeric nanomaterials utilized for delivery of EGCG (NPs: Nanoparticles)

<table>
<thead>
<tr>
<th>Polymer nanomaterials</th>
<th>Size (in nm)</th>
<th>Synthesis method</th>
<th>Loading Efficiency (%)</th>
<th>Cancer cells/Model s used</th>
<th>Concentration of nanomaterials used in assays</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltodextrin and gum arabic NPs</td>
<td>120 ± 28</td>
<td>Dissolve followed by constant homogenization</td>
<td>approx 85%</td>
<td>Du145 (Prostate)</td>
<td>100µM: MTT assay, Caspase assay 1-100 µM: Clonogenic assay</td>
<td>51</td>
</tr>
<tr>
<td>Chitosan-caseinophosphopeptides</td>
<td>150± 4.3</td>
<td>Sonication, Stirring</td>
<td>24-53%</td>
<td>Caco-2 (Colon)</td>
<td>0.002-0.5mg/mL: MTT assay 0-500 µg/mL: Cellular uptake studies</td>
<td>52</td>
</tr>
<tr>
<td>Genipin-crosslinked caseinophosphopeptides-chitosan NPs</td>
<td>236± 26.3</td>
<td>Sonication, Stirring, Nucleophilic attack</td>
<td>71%</td>
<td>BGC823 (Gastric) HepG2 (Liver)</td>
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<td>Caco-2 (Colon)</td>
<td>0.025 mg/mL: EGCG concentration maintained for TEER measurement</td>
<td>55</td>
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<tr>
<td>Glutaraldehyde ovalbumin-dextran conjugate NPs</td>
<td>339</td>
<td></td>
<td>30%</td>
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<tr>
<td>Bovine serum albumin NPs coated with chitosan</td>
<td>300</td>
<td>Desolvation</td>
<td>32.7%</td>
<td>Caco-2 (Colon)</td>
<td>0.023 mg/mL: EGCG concentration for TEER measurement</td>
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<tr>
<td>Chitosan NPs</td>
<td>150-200</td>
<td>Stirring, Sonication, Dialysis</td>
<td>10% w/w</td>
<td>22Rv1 (Prostate cancer xenograft model)</td>
<td>3mg, 6mg (Chitosan nanoparticle encapsulated EGCG)/kg body weight: Invivo studies</td>
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<tr>
<td>Chitosan NPs</td>
<td>150-200</td>
<td>Stirring</td>
<td>10% w/w</td>
<td>Mel-928</td>
<td>0-80 µM: MTT assay</td>
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<td>Material Description</td>
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<td>Sonication, Dialysis</td>
<td>(Melanoma cancer xenograft model)</td>
<td>1-4 µM: Apoptotic studies, Cell cycle studies 100µg/mice: Invivo studies</td>
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<tr>
<td>Fucose-chitosan/polyethylene glycol-chitosan/gelatin NPs</td>
<td>Gelation of EGCG aqueous solution mixed with FCS/PCS/Gel</td>
<td>53.7±1.8%</td>
<td>MKN45 (Gastric) Luc MKN45 Orthotopic Gastric tumor model 0-40mg/L: MTT assay 10,20, 40 mg/L: Apoptotic studies 40mg EGCG/kg body weight: Invivo studies</td>
<td>60</td>
<td></td>
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<tr>
<td>Poly(lactic-co-glycolic acid) and Casein Nanocarrier</td>
<td>Emulsion and Precipitation</td>
<td>76.8±9.1%</td>
<td>MDA-MB-231 MCF-7 (Breast) 40-120µM: Cytotoxic studies 100 µM: Apoptotic studies</td>
<td>61</td>
<td></td>
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