Strategic use of nanotechnology in drug targeting and its consequences on human health: A focused review

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Abstract: Since the development of first lipid-based nanocarrier system, about 15% of the present pharmaceutical market uses nanomedicines to achieve medical benefits. Nanotechnology is an advanced area to meliorate the delivery of compounds for improved medical diagnosis and curing disease. Nanomedicines are gaining significant interest due to the ultra small size and large surface area to mass ratio. In this review, we discuss the potential of nanotechnology in delivering of active moieties for the disease therapy including their toxicity evidences. This communication will help the formulation scientists in understanding and exploring the new aspects of nanotechnology in the field of nanomedicine.

Keywords: nanomedicines, nanocarriers, nanotechnology, nanoformulation, toxicity

Introduction

Nanoparticles (NPs) are nanosized colloidal particles, in which a drug is encapsulated, entrapped, or attached to the NPs matrix (Fig. 1). The NP production approaches can be divided into two major categories based on whether the synthesis requires a polymerization reaction or is direct from a preformed polymer or macromolecule [1]. The choice of production approach depends on the nature of polymers as well as on the drug to be encapsulated. NPs have been extensively investigated for oral delivery of drugs, because these tiny particles can protect the drug against enzymatic and hydrolytic degradation in the gastrointestinal tract (GIT), prolong the residence time in the gut by mucoadhesion, and notably improve the bioavailability of drugs [2, 3]. Increased oral bioavailability has been reported in case of the anticancer drugs that are delivered through nanocarrier [4]. The in vivo bioavailability of paclitaxel was reported to be increased by 10 times when compared to orally administered Taxol® [5].

Polymeric materials have the best combination of characteristics as these are stable and allow higher loading of drugs, provide control over drug release kinetics, can be readily modified to display a variety of surface-attached ligands, and are safe for human use [6]. A variety of biodegradable and non-biodegradable polymeric nanocarriers employed for oral drug delivery are presented in Table I. Industries investing in the development of different types of nanoformulations and undergoing clinical trials are presented in Table II.

Poly (lactic-co-glycolic acid) NPs

NPs based on poly (lactic-co-glycolic acid; PLGA) have been extensively studied as therapeutic delivery vehicles...
for many bioactives owing to their biodegradability and biocompatibility. PLGA microparticles have received approval from the US-FDA for establishing sustained release of leuprolide (Lupron Depot®, AbbVie Inc., North Chicago, IL, USA) and triptorelin (Trelstar®, Allergan, Inc., Dublin, Ireland) [22]. PLGA NPs have shown the ability to improve oral bioavailability of drugs with low solubility such as paclitaxel and curcumin [3, 7]. Xie and co-workers synthesized curcumin-loaded PLGA NPs by a solid-in-oil-in-water solvent evaporation method. The results showed 91.96% entrapment and 5.75% loading of curcumin in NPs. The water solubility of curcumin was increased ∼640-fold from the prepared PLGA NPs when compared to that of pure curcumin. The PLGA NPs resulted in an improved oral bioavailability (5.6-fold) and half-life of curcumin compared with that of pure curcumin. The improved oral bioavailability of curcumin might be associated with improved solubility due to the increased effective surface area of NPs [23]. Sustained in vivo release of orally administered cyclosporine from PLGA NPs has been reported to have decreased cyclosporine-associated nephrotoxicity. However, Sandimmune Neoral, a commercially available formulation, failed to achieve both [24, 25].

**Solid lipid nanoparticles**

Solid lipid nanoparticles (SLNs) are lipid-based effective and safe alternate to the existing therapeutics. These tiny particles are free from the potential toxicities of polymeric NPs [26]. These particles have better stability profile in the upper GIT when compared to other lipid nanoparticles. Significant efforts have been made in recent years to improve the bioavailability of various drugs, such as insulin, bufalin, risperidone, and puerarin [8, 17, 26–28]. Luo et al. reported improved oral bioavailability of puerarin loaded in SLNs. The results suggested rapid and improved (>3-fold) oral absorption of puerarin from SLNs when compared to puerarin suspension. The study supported the fact that these lipid-containing tiny particles are promising drug-delivery vehicle for the enhancement of oral bioavailability of BCS class II drugs [29].

**Hydrogel NPs (nanogels)**

Hydrogel NPs (nanogels), a family of nanoscale particulate materials, have shown benefits in oral delivery of drugs over other commercially available drug-delivery...
Interestingly, hydrogel nanoparticulate materials demonstrate the simultaneous characteristics of hydrogels and NPs, such as hydrophilicity, flexibility, versatility, and high water absorptivity [30, 31]. Nanogels with a three-dimensional (3D) network structure and a diameter of 10–100 nm have some smart functional properties such as pH-sensitivity and/or temperature-sensitivity, etc. [32–35]. As a representative for oral drug delivery of nanogels, El-Sherbiny et al. [9] reported a biodegradable pH-responsive silymarin-loaded alginate-PLGA nanogel for oral delivery of silymarin, a hydrophobic natural therapeutic drug. In another study, silymarin-loaded PLGA NPs were prepared using single-emulsion solvent evaporation method. Aqueous solution of sodium alginate containing PLGA NPs was used to make pH-sensitive hydrogel NPs through ionotropic cross-linking. The resultant nanogel was biodegradable that could sustain the release profile and enhance the oral bioavailability of silymarin [36].

**Polymeric micelles (PMs)**

Micelles are amphiphilic polymer and surfactant-based self-assembled particles (~20–100 nm). PMs are the

<table>
<thead>
<tr>
<th>Nanocarriers</th>
<th>Drug used</th>
<th>Pharmacological effect</th>
<th>Size (nm)</th>
<th>Composition</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymeric NPs</td>
<td>Curcumin</td>
<td>Water solubility of curcumin was increased about 640-fold relative to pure curcumin. Oral bioavailability was increased 5.6-fold for longer duration compared with that of pure curcumin</td>
<td>–</td>
<td>PLGA</td>
<td>[7]</td>
</tr>
<tr>
<td>Solid lipid NPs</td>
<td>Puerarin</td>
<td>The relative bioavailability of puerarin from SLN was increased to 43-fold</td>
<td>–</td>
<td>–</td>
<td>[8]</td>
</tr>
<tr>
<td>Hydrogel NPs (nanogels) for oral drug delivery</td>
<td>Silymarin</td>
<td>The developed carrier was biodegradable and enhanced oral bioavailability of silymarin for prolonged time period</td>
<td>10–100</td>
<td>PLGA</td>
<td>[9]</td>
</tr>
<tr>
<td>Polymeric micelles</td>
<td>Paclitaxel</td>
<td>The oral bioavailability of paclitaxel from polymeric micelles was estimated at 12.4%</td>
<td>85–90</td>
<td>PEG-b-P (VBODENA co-AA)y</td>
<td>[10, 11]</td>
</tr>
<tr>
<td>Liposomes in oral drug delivery</td>
<td>Somatostatin analog octreotide</td>
<td>Mild and prolonged hypoglycemic effect was elicited following oral administration. Plasma insulin level was increased for 20 h</td>
<td>–</td>
<td>Tetraether lipids derived from archaea bacteria</td>
<td>[12, 13]</td>
</tr>
<tr>
<td>Nanoeulsion and self-nanoeulumising drug-delivery system for oral drug delivery</td>
<td>Curcumin</td>
<td>Aqueous solubility of curcumin increased to about 6,000,000-fold.</td>
<td>&lt;100</td>
<td>A folate-modified SNEDDS</td>
<td>[14, 15]</td>
</tr>
<tr>
<td>Nanocrystals</td>
<td>Coenzyme Q10 (CoQ10)</td>
<td>CoQ10 dissolution enhanced and the oral bioavailability of CoQ10 was ~2.5-fold higher from nanocrystals than coarse suspensions</td>
<td>&lt;500</td>
<td>–</td>
<td>[16]</td>
</tr>
<tr>
<td>Dendrimers</td>
<td>Short hairpin RNA</td>
<td>RNAi-mediated hTERT gene silencing coupled with dendrimer delivery would be a promising approach for the management of oral cancer</td>
<td>107–315</td>
<td>PAMAM</td>
<td>[17]</td>
</tr>
<tr>
<td>Nanospheres</td>
<td>Cyclosporine A</td>
<td>Eudragit® S100-based nanospheres of cyclosporine A showed improved bioavailability of cyclosporine A compared to the Neoral® microemulsion</td>
<td>100–200</td>
<td>Eudragit®-based nanospheres</td>
<td>[18]</td>
</tr>
<tr>
<td>Carbon nanotubes</td>
<td>Cyclo-D-tryptophan-tyrosine peptide</td>
<td>The developed carbon nanotubes delivered gene to the duodenum, stomach, liver, and kidney via oral route</td>
<td>–</td>
<td>Carbon cylinders composed of benzene rings</td>
<td>[19]</td>
</tr>
</tbody>
</table>
choice of delivery vehicle for the drugs, which are hydrophobic in nature and exhibit poor bioavailability. The stability and bioavailability of poorly water-soluble drugs can be enhanced by incorporating drug into the inner hydrophobic core [25, 37]. Extensive efforts have been made in the development of PMs for intravenous administration of drugs because they avoid hepatic first-pass metabolism and easily reach the lesion sites [38]. Improved oral bioavailability of hydrophobic drugs has been reported using PMs [10, 39]. PMs are the most promising entities for the delivery of paclitaxel. The bioavailability from intravenous route was eightfold higher than oral route [11]; while in another study, 6.5% oral bioavailability of Taxol has been reported through Cremophor EL micelles [40].

Liposomes

Liposomes are microscopic lipid bilayer vesicles that have been studied extensively as potential drug carriers [41]. In 1964, Bangham et al. disclosed the first electron microscopic images of such lipid bilayered spherical vesicles surrounded by an amphiphilic phospholipid membrane [1]. They are composed of phosphatidylcholine and phosphatidyl ethanolamine. Cholesterol is used to modify the lipid membrane rigidity to make it capable of encapsulating hydrophobic and hydrophilic drugs. The membrane permeability and cellular absorption of proteins, peptides, and hydrophilic drugs can be improved through liposomal vesicles. High molecular weight of these drugs leads to their degradation in the GIT and poor absorption [42, 43]. Liposomes have low toxicity, can entrap structurally diverse drugs, and have properties similar to the biological membrane. However, success of orally stable lipid vesicles is limited due to the chemical and enzymatic destabilization. Polymer-coated liposomes have been extensively investigated to defeat the stability issues, extend the residence time in the GIT, and improve oral efficiency of liposomal delivery systems [44].

Nanoemulsion and self-nanoemulsifying system

During the past 2–3 decades, attention has been given on the development of nanoemulsions and self-nanoemulsifying drug-delivery system (SNEDDS) to improve the oral bioavailability of BCS class II drugs [45]. The improved oral bioavailability of drugs from these systems could be due to the increased solubilization and protection of drug from enzymatic and physicochemical degradation. Small droplet size increases the interface between the aqueous gut medium and lipophilic droplets, which leads to homogeneous and wide distribution of drug throughout the GIT [25, 46]. Presence of large amount of surfactants in SNEDDS may alter the membrane permeability, which leads to the GI side effects [47].

Table II | Companies investing in the development of different types of nanoformulations and undergoing clinical trials [20, 21]

<table>
<thead>
<tr>
<th>Drug product</th>
<th>Active constituents/formulation</th>
<th>Therapeutic use</th>
<th>Manufacturer</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEP-ETU</td>
<td>Liposomal paclitaxel</td>
<td>Breast/lung cancers</td>
<td>Neopharma</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>Onco-TCS</td>
<td>Liposomal vincristine</td>
<td>Non-Hodgkin lymphoma</td>
<td>Inex</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>Aroplatin</td>
<td>Liposomal cisplatin analog</td>
<td>Colorectal cancer</td>
<td>Antigenics</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>SPI-77</td>
<td>Stealth liposomal cisplatin</td>
<td>Head and neck cancer</td>
<td>Alza</td>
<td>Phase III</td>
</tr>
<tr>
<td>OSI-211</td>
<td>Liposomal lurtotecan</td>
<td>Lung cancer/recurrent ovarian</td>
<td>OSI</td>
<td>Phase II</td>
</tr>
<tr>
<td>EndoTAG-I</td>
<td>Paclitaxel</td>
<td>Pancreatic cancer</td>
<td>Medigene/SynCore Biototechnology</td>
<td>Phase II</td>
</tr>
<tr>
<td>ThermoDox</td>
<td>Doxorubicin</td>
<td>Hepatocellular carcinoma</td>
<td>Celsion Corporation</td>
<td>Phase III</td>
</tr>
<tr>
<td>Atragen</td>
<td>Liposomal all transretinoic acid</td>
<td>Acute promyelocytic leukemia</td>
<td>Aronex Pharmaceuticals</td>
<td>Phase II</td>
</tr>
<tr>
<td>Auroshell</td>
<td>Gold nanoshells</td>
<td>Aurolace therapy of cancer</td>
<td>Nanustria Bioncs</td>
<td>Phase I</td>
</tr>
<tr>
<td>NKTR-105</td>
<td>PEG–docetaxel</td>
<td>Solid tumors</td>
<td>Nkter Therapeutics</td>
<td>Phase I</td>
</tr>
<tr>
<td>AMG223</td>
<td>Phosphate-binding polymer</td>
<td>Hyperphosphatemia in CKD patients on hemodialysis</td>
<td>Amgen</td>
<td>Phase II</td>
</tr>
<tr>
<td>NKTR-118</td>
<td>PEG-naloxone</td>
<td>Opioid-induced constipation</td>
<td>Nkter</td>
<td>Phase II</td>
</tr>
<tr>
<td>SLIT cisplatin</td>
<td>Liposomes</td>
<td>Lung cancer</td>
<td>Transave</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

CKD: chronic kidney disease; PEG: polyethylene glycol; SLIT: sustained release lipid inhalation targeting
bioavailability of silymarin from oil-in-water (o/w)-based nanocarrier system. The results of in vivo study demonstrated fourfold area under the curve and sixfold $C_{\text{max}}$ of silymarin from orally administered nanomulsion than those of conventional silymarin suspension [49]. Bali et al. constructed pseudoternary phase diagrams to investigate the effect surfactants and co-surfactants in nanomulsion formulation of ezetimibe. The results revealed significant improvement in absorption of ezetimibe from nanomulsion when compared to the conventional tablet (Ezedoc®10, ezetimibe 10 mg) [50].

Self-nanoemulsifying drug-delivery system (SNEDDS)

Emulsion systems are associated with their own complications, including stability and manufacturing constraints associated with their commercial production. Self-emulsifying drug-delivery system (SEDDS) can overcome the problems associated with conventional emulsion systems. The droplet size of SEDDS ranges from 20 to 200 nm. These droplets provide a large surface area for the absorption of dissolved drugs [25, 51, 52]. SNEDDS, globule size range <100 nm, is thermodynamically and isotropically stable solution consisting of oil, surfactant, co-surfactant, and drug, which can spontaneously produce o/w nanomulsion when mixed with water under mild stirring [14, 45, 53]. At present, four SEDDS-based commercial drug products, such as Sandimmune® (cyclosporine A), Sandimmun Neoral® (cyclosporine A), Norvir® (ritonavir), and Fortovase ® (saquinavir), are available in the pharmaceutical market [54]. Although several SEDDS formulations are approved by FDA, in vivo performance of SNEDDS formulations using in vitro data is still in its prediction of infancy [55]. SNEDDS showed multiple absorption mechanisms, such as passive diffusion, active transport, paracellular, endocytosis, and P-glycoprotein-mediated absorption. Thus, a single-mechanism in vitro study (such as dissolution or permeability) might not be appropriate for evaluating the in vivo–in vitro correlation of SNEDDS formulations [56]. Care should be taken when prioritizing SNEDDS to use in humans [57]. Recently, much attention has been given for the development of SNEDDS to improve the solubility, dissolution, and oral absorption of poorly water-soluble drugs, such as apigenin, gemfibrozil, and coenzyme Q10 (CoQ10) and zedoary essential oil [58–61]. Improved oral bioavailability of tamoxifen citrate-loaded SNEDDS has been reported for the treatment of breast cancer. The study revealed complete drug release after 6 h dissolution study in phosphate buffer (pH 7.4) [62].

Nanocrystals

By definition, nanocrystals are <1 μm size particles, but practically their size is ~500 nm. Nanocrystals are prepared by top-down or bottom-up approach with complete drug loading [25, 63]. They do not have any carrier material and may be formulated as suspensions or more frequently as dry dosage forms (tablets and capsules) [64]. A range of oral nanocrystal formulations is available including Emend® (aprepitant), Rapamune® (rapamycin), Megace ES® (Megestrol), Triglide® (fenofibrate), and TriCor® (fenofibrate) [65]. The combination of dose of panzem nanocrystals and temozolomide in patients with recurrent glioblastoma multiforme is under Phase II investigation [66]. Nanocrystal syntheses do not require surfactant such as cremophor EL. Therapeutic relevance of nanocrystals is due to the increased dissolution rates, saturation velocity, and improved adhesion to cell membranes. Piao et al. investigated dissolution profiles of CoQ10 in nanocrystals and its oral absorption in rats and compared with a coarse suspension of CoQ10. In case of nanocrystals, an increase in dissolution profile of CoQ10 was observed and the in vivo study in rats demonstrated that the oral bioavailability of nanocrystals was ~2.5-fold higher than that of coarse suspensions. When the compounds exhibit dissolution rate-limited bioavailability, the nanocrystals can lead to better bioavailability. However, this approach can add tremendous dosing flexibility for other administration routes, especially when highly concentrated formulations are needed [16].

Dendrimers

Dendrimers (<10 nm size) are highly branched 3D tree-like molecules comprising a core, branches, and end groups prepared using monomeric or oligomeric units such as melamine, polyamidoamine (PAMAM), polypropyleneimine (PPI), poly(L-glutamic acid), and polycryl ether [1, 67]. Dendrimer size can progressively increase with an addition of each generation (shell) of branches and can be built from a molecular level to nanoscale size (1–10 nm) [68, 69]. Drug molecules can be attached to the functional groups on dendrimer surface or shielded in the dendritic channels of the interior environment of the sphere. Potential use of PAMAM dendrimers in oral drug delivery has been evaluated by Sadefar and Ghandehari [70]. It has been proved that PAMAM dendrimers are transported by a combination of transcellular and paracellular routes [71]. Depending on the surface chemistry, PAMAM dendrimers can open the tight junctions of epithelial barriers. In 2008, Ke et al. [72] developed drug-PAMAM complex for oral administration and demonstrated a significant increase in oral bioavailability (200-fold) over that of the free drug. However, most of the reports are based on the extent of in vitro drug absorption from dendrimers. No direct in vivo evidence of dendrimer permeability after oral administration has been reported [73].
Nanospheres

Nanospheres are spherical structures composed of a matrix system in which drug is distributed by entrapment, attachment, or encapsulation. These are typically larger than micelles having diameters between 100 and 200 nm, which can be used for oral applications [74]. Wang et al. [18] reported improved bioavailability of cyclosporine A from nanospheres prepared using Eudragit® S100 when compared to the Neoral® microemulsion system. The increase in absorption was attributed to a shorter transit time in the stomach, greater bioadhesiveness, and better protection against degradation.

Carbon nanotubes (CNTs)

CNTs are carbon cylinders composed of benzene rings. These are carbon-structured tubes that can be single or multiwalled. CNTs have attracted tremendous attention of pharmaceutical scientists both from the academia and industries regarding their biomedical applications. Application of CNTs for the oral delivery of drugs has evoked great interest. Hsieh et al. [19] developed cyclo-(D-tryptophan-tyrosine) peptide nanotubes, which can deliver gene to duodenum, stomach, liver, and kidney through oral administration.

Quantum dots

Quantum dots are derived from heterogeneous group of NPs containing 1,000–100,000 molecules and demonstrating uncommon quantum effects [75]. It facilitates the absorption, distribution, metabolism, and excretion. The toxicity of quantum dots has been quantified from environmental conditions and inherent physicochemical properties. Their size depends on coating thickness, which varies from 2.5 up to 100 nm.

General Toxic Behavior of NPs

In the present scientific era, nanotechnology is gaining popularity in biology, chemistry, or any other fields. Since the introduction of first lipid-based nanodrug carrier, the continued success of nanotechnology in the healthcare system has led to the birth of nanomedicine [76]. The nanotechnology has been associated with many novel things, which are highly promising. There are many questions being raised regarding the safety of nanomaterials. To date, scientists do not know how much toxic the nanomaterials are and what is the intensity of toxicity [77]. It has been proved that the NPs are more reactive and toxic in nature [1, 76]. To understand the toxic effects of NPs, different research groups are working on this aspect using cell line studies, incubation, etc. [78]. Many drugs delivered by incorporating into NPs have shown evidence of reduced toxicity of the drug by lowering its dose and targeting the delivery to the site of action [79]. However, this is not sufficient for the nanoformulations to be considered as safe for human use. To access the toxicity caused by nanoformulations, the toxicity study must be carried out for NPs formulated with and without drug. This comparative study will help the formulation scientists to identify the toxicity caused by the blank or unloaded nanoformulation. Various approaches have been reported to investigate the toxicity of the nanoformulations. There are many evidences that quote the solid toxicological behavior of the prepared NPs. The toxicological issues associated with interaction of nanoformulations within the host cell are mainly governed by the particle size, surface area, shape, and agglomeration. Particle size is the major factor that might cause danger to human health [1]. Table III presents particle types and its description about cause of toxicity.

### Table III

<table>
<thead>
<tr>
<th>Particle type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₁₀ and P₂.₅</td>
<td>The mass fraction of the particle with average diameter 10 and 2.5 μm, respectively</td>
</tr>
<tr>
<td>Ultrafine particles</td>
<td>The fraction of P₁₀ in size ranges 0.1 μm and contains primary particle as well as agglomerate of size 100 nm</td>
</tr>
<tr>
<td>Coarse particles</td>
<td>The size of such particle is 2.5 μm and they are mass fraction of particle P₁₀</td>
</tr>
<tr>
<td>PSP</td>
<td>Low specific toxicity and less soluble, it is fine or ultrafine particle</td>
</tr>
<tr>
<td>DEP</td>
<td>Diesel exhaust particles</td>
</tr>
<tr>
<td>CDNP</td>
<td>Diesel exhaust particles are combustion-derived nanoparticles</td>
</tr>
</tbody>
</table>

PSP: polyamide seeding particle

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Evidences regarding the toxicity of nanoformulations

Bahadar et al. [80] reviewed the toxicological data of NPs in human health. The nanosized particles have greater lung deposition and rapid systemic translocation having various inflammatory, oxidative, and cytotoxic effects than larger particles [1]. The process of deposition of inhaled carbon NPs in the lungs is known as lung burden. It is evident that elimination of CNTs from the lungs is slower and causes lung burden (e.g., inflammation and...
granuloma formation) [76, 81]. Toxicity can also be generated by the direct inhalation of the particle P10 (particles of size ranging 10 mm or less than that). Therefore, there is a need to discuss the toxicity behavior of such modified nanoformulation [82]. Generally, most of the mass of P10 is considered to be harmless in nature, but it is evident that the particle P10 is responsible for the pro-inflammatory effects [83]. P10 is also implicated in causing adverse effects, which is supported by the substantial toxicological data [84]. Due to the nanosize, the surface area per unit mass is very high and this is the causative factor for higher toxicity in comparison to particles with the low surface area [85]. There are many constituents found within the P10, which are not much toxic. There are many deaths occurring due to the cardiovascular diseases and respiratory-related diseases [86]. Inflammation is a very common factor causing adverse effects and is an important property of NP toxicity [87]. There are many evidences confirming inflammatory effects of NPs [88].

The mentioned reports elucidating the toxicity behavior of nanomaterials also conclude that most of the adverse health concerns are associated with the particulate matter, and can be measured as exacerbations of respiratory disease [89]. It is through the pulmonary inflammation and distribution of ultrafine ambient particles (Fig. 2).

Statistics showing toxicological effect of nanoformulations

Carbon nanotubes (CNTs)

CNTs were discovered as novel class of multifunctional nano structures. CNTs are potential to act as biopersistent fibers. In vitro cultivation of keratinocytes or bronchial epithelial cells with high doses of single-walled CNTs leads to generation of reactive oxygen species (ROS), oxidative imbalance, mitochondrial dysfunction, and modifications in the morphology of the cell [90, 91]. An increased attachment of platelets has been reported with single and multwall CNTs. Fullerenes (C60) are applied as building blocks for these CNTs [92]. Multi-walled CNTs also provoke a pro-inflammatory effect in keratinocytes [93]. Various studies using intratracheal instillation of high-dose nanotubes in rodents expressed chronic lung infection, including interstitial fibrosis and foreign-body granuloma formation [94, 95]. On the basis of dose per mass, nanotubes are more toxic than quartz particles [94, 96]. A high dose of aggregated nanotubes and metal impurities is responsible for artificial toxicity.

Fullerenes

Fullerene (carbon nanostructure – C60) was discovered in 1985 by Kroto et al. [97]. Over the past several years, great interest has developed in the potential use of fullerenes in biomedicine [98]. Potential applications of fullerenes are due to their unique free radical chemistry and antioxidant properties. Fullerenes may induce toxicity in presence of light due to surface excitation, which leads to singlet oxygen sensitizer state [99]. With fullerenes, various studies are published based on ecotoxicity of nanomaterials [100]. Uncoated and water-soluble colloidal fullerenes had 48 h LC50 in Daphnia magna ranging from 460 to 800 ppb [98, 99]. However, in case of sonicated C60 fullerenes, the LC50 was higher with 7.9 ppm [101]. No mortality has been reported in largemouth bass. However, lipid peroxidation and glutathione depletion were observed in brain and gill, respectively, after exposure to 0.5 ppm C60 fullerenes for 48 h [102].

Dendrimers

Regardless of wide biomedical applications of dendrimers, toxicity associated due to terminal-NH2 groups and surface cationic charge, the evidence for successful dendrimers remains limited for clinical applications. The in vitro cytotoxicity of PPI, PAMAM, and poly-L-lysine dendrimers has been reported due to their surface cationic groups [1, 103]. The cationic terminal groups of dendrimers interact with RBCs and lead to hemolysis [104]. Cationic melamine dendrimers having surface groups like amine, guanidine, carboxylate, sulfonate, or phosphonate are more cytotoxic than anionic or PE Gylated dendrimers [105]. Cytotoxicity of PAMAM dendrimers in Caco-2 cell lines [106] and cytotoxicity of PPI and PAMAM dendrimers in B16F10, CCRF, and HepG2 cancer cell lines [107] have been reported. Numerous studies have examined that cationic dendrimers interact with negatively charged biological membrane to produce nanoscale holes and cell lysis [108–112]. The mechanism of cationic PAMAM dendrimer induced membrane damage using 1,2-dimyristoyl-sn-glycero-3-phosphocholine lipid bilayers, and KB and Rat2 cells in culture have been reported by Hong et al. [113].

Quantum dots

Studies expressed that quantum dots exhibit less toxicity [75]. In vitro studies showed that quantum dots may be toxic [114–116] and the toxicity can spread with surface coating [114, 117]. Choi et al. [118] concluded that quantum dot toxicity was decreased by surface modification with N-acetylcysteine, in comparison to non-modified cadmium telluride quantum dots. Cytotoxicity of naked quantum dots could be due to the impairment of ROS and damage to the plasma membrane, nucleus, and mitochondria [119]. The core substance of cadmium-containing quantum dots has toxic potential due to release of highly toxic-free cadmium ions [120, 121]. Cadmium telluride quantum dots cause cell death via mechanisms involving ROS and Cd2+ accompanied by lysosomal enlargement and intracellular redistribution [122]. Hardman suggested that all quantum dots are not uniform group of substances. Pharmacokinetic profile and toxicity properties of quantum dots depend on inherent physicochemical properties and environmental
conditions. The toxicity of quantum dots depends on their size, surface charge, capping material, and functional groups of outer coating, oxidative, photolytic, and mechanical stability [75].

Gold NP/nanoshells
A joint evaluation of Food and Agriculture Organization of the United Nation and World Health Organization Expert committee on Food Additives (JECFA) suggested that the gold does not show hazard when used as food additive and coloring agent (2001). However, this information was not considered in nanof ormulation of gold. Metallic gold NPs can be synthesized in different forms and in different size ranges [123, 124]. In health science, they are used as potential carriers of drug delivery, imaging of cell structure, and their development [125], and for the design of novel cancer therapy products [126–130]. The cytotoxic effect of gold NP was considered in the presence of stabilizer cetyltrimethylammonium bromide (CTAB). Removing the excess CTAB with polyethylene glycol (PEG)-modiﬁed gold nanorods did not show the toxicity [131]. Gold solution is used in magnetic resonance imaging by preparation of nanoshells composed of gold and copper, or gold and silver to function as contrast media in diagnostic agent [132] and also used gold-silica for photothermal ablation of tumor cells [133, 134]. Positive in vivo results were obtained in mouse treated by intravenous administration of PEG-coated gold nanoshells in presence of photothermal ablation [129].

Silica
The size of 15- and 46-nm silica NP showed similar dose-dependent cytotoxicity in vitro. The toxicity of silica NPs increases with an increase in dose and exposure time [135]. Chang et al. [136] stated that silica NPs are toxic at high dosages due to the reduction in cell viability and by lactate dehydrogenase (LDH) release from the cells indicating membrane damage. It has been observed that the concentration above 0.1 mg/ml signiﬁcantly reduces cell viability. Alveolar macrophage cell line has been reported to be more liable for NP-induced cytotoxicity than a lung epithelial cell line (A549). This could be due to the phagocytic properties of the macrophage cell line [137]. In another process, the contrast for cationic silica NPs using amino-hexyl-amino propyltrimethoxysilane as a surface modiﬁer reduced the cell toxicity [138]. Various marketed nanoformulation are presented in Table IV.

Natural Bioactives Delivered Through Nanocarrier
Green nanotechnology is gaining worldwide importance because of exclusion of harmful chemicals, economical
and use of different eukaryotic and prokaryotic organisms. Among these, the plant extracts act as reducing and capping agents for the NP synthesis, and the resultant prefabricated NPs exhibit better bioactivity [139, 140].

Resveratrol

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) contains phenol and acts as a phytoalexin protector from external source such as bacteria and fungi. Resveratrol is the best antioxidant [141]. The anticancer activity of resveratrol has been reported for the prevention of skin cancer development in mice [140, 142]. Tremendous efforts have been made to use resveratrol as an anticancer agent in various cancer models, including skin cancer [143–145], breast cancer [146–148], fibrosarcoma [149, 150], lung cancer [151, 152], gastric and colorectal cancer [153], prostate cancer [154, 155], hepatoma [156, 157], neuroblastoma [158], pancreatic cancer [159, 160], and leukemia [161, 162]. The first nanoformulation of resveratrol was developed with the help of chitosan. The chitosan nanoformulations have a sustained release rate was observed with an addition of solidifying agents [163]. In another study, the results expressed that 12-h prematuration of resveratrol-loaded NPs defended the human primary ovarian cancer cells SKOV(3) after 3 weeks in nude mice compared to free resveratrol.

Taxol

Taxol, an effective anticancer agent, has stimulated an intense research effort. Taxol is effective against leukemia and various solid tumors in the breast, ovary, brain, and lungs [140, 167–170]. It was first isolated in 1971 from the bark of Pacific Yew tree, and is among the first FDA-approved chemotherapy drugs obtained from natural sources. Paclitaxel was developed by Bristol-Myers Squibb and sold as Taxol™. Paclitaxel PLGA-loaded NPs were synthesized using interfacial deposition method. Biphasic release of paclitaxel was characterized by fast release during the first 24 h of dissolution studies, followed by sustained release. The results suggested that cytotoxic effect of paclitaxel NPs was more as compared to Taxol [171]. Paclitaxel PLGA NPs prepared by a modified solvent extraction technique showed that the natural emulsifiers are better for paclitaxel NP formulation over the conventional macromolecular emulsifiers like polyvinyl alcohol. After 24-h incubation of HT-29 cancer cell line, the cell mortality caused by paclitaxel NPs was 13 times than free paclitaxel [172]. The antiproliferative activity of nanoparticulate paclitaxel in a human prostate cancer cell line and its effect on tumor inhibition in a murine model of prostate cancer has been reported. The NPs released 60% paclitaxel in 60 days. The IC_{50} of the drug with unconjugated NPs was fivefold higher than the transferrin-conjugated NPs [173].

Camptothecin (CPT)

It is a cytotoxic alkaloid obtained from Camptotheca acuminata first reported by Wall and Wani in 1966. CPT and its derivatives target the nuclear enzyme topoisomerase I and inhibit religation of cleaved, single-stranded DNA. This inhibits the process of replication and cell death. CPT and its derivatives exhibit excellent anticancer effect.
activity. Extreme lipophilicity and instability of the lactone ring is the major limitation of CPT [140]. Hycamtin (topotecan) and camptosar are FDA-approved water-soluble derivatives of CPT, which are used to treat ovarian cancer and advanced colorectal carcinoma, respectively [174]. Significant tumor inhibition effect of irinotecan NPs (20 mg/kg) in mouse models with subcutaneous sarcoma 180 has been reported. Higher concentration of the drug was maintained for a longer residence time and had better tumor suppression effect [175]. The in vitro studies of CPT-PLGA NPs coated with antibodies against colorectal cancer were carried out by McCarron et al. Fluorescently labeled NPs cleared the high uptake of the antibody NPs compared to the NPs without the antibody [176].

Min and co-workers synthesized hydrophobically modified glycol chitosan (HGC) NPs encapsulating CPT. The size of HGC-CPT nanoparticles was around 280–330 nm and had 80% loading efficiency. The anticancer activity of HGC-CPT NP was observed in mice having subcutaneously implanted MDA-MB231 xenograft. The intravenous injection of the nanoformulation seemed to suppress tumor growth when compared to the free dose. In vivo studies of the dye-labeled NPs cleared that large amount of NPs was accumulated in tumor spot. Approximately, increase of the therapeutic effect of CPT when loaded in NPs might be because of the higher accumulation of CPT at the tumor site [177].

Curcumin

Curcumin, obtained from Curcuma longa, is used for the treatment of various cancers including multiple myeloma, pancreatic, myelodysplastic syndromes, colon, and psoriasis. It inhibits cell proliferation and promotes apoptosis [140]. Low solubility, bioavailability, and stability are the major limitations of curcumin. It is unstable in the gut [178], and a small amount of curcumin that passes through the GI tract is degraded rapidly. Application of nanotechnology has solved these reported limitations of curcumin in a very effective way. Bish et al. [179] reported cross-linked and random copolymers of N-isopropylacrylamide, with N-vinyl-2-pyrrolidone and PEG monoacrylate NPs, loaded with curcumin. The size range of these NPs was within 50 nm. Sahu and co-workers synthesized polymeric amphiphile with the help of methoxy PEG and palmitic acid as the hydrophilic and hydrophobic segment, respectively. The conjugate step carried out in a single-step reaction showed minimal toxicity on HeLa cells [180]. Thangazhatham and co-workers explained merits of nanoformulated curcumin over the free curcumin. They stimulate the targeted delivery of curcumin for prostate cancer using curcumin-loaded liposomes coated with prostate-specific membrane antigen antibodies [181]. The antiproliferative activity of liposomal curcumin was studied using LNCaP and C4-2B human prostate cancer cell lines. The results showed 75%–85% decrease in the cellular proliferation. Curcumin has been encapsulated in nanosized carriers using chitosan, alginate, and pluronic. The product was synthesized by ionotropic pre-gelation method followed by polycationic cross-linking. In this process, pluronic F127 enhanced the solubility of curcumin. The NPs were successful in cellular internalization of curcumin [182]. Curcumin-loaded poly (caprolactone) nano-fiber matrix has potential as a wound dressing with reduced inflammation and an increased wound closure rate [183]. PEGylated curcumin conjugates are more effective in pancreatic cancer cell growth inhibition than free curcumin [184]. In a study, Shaikh et al. reported diffusion-controlled in vitro release profile of curcumin followed Higuchi’s release pattern. The pharmacokinetics revealed that curcumin-entrapped NPs demonstrate ninefold higher oral bioavailability when compared to curcumin administered with piperine as an absorption enhancer [185].

Patents Based on Nanoformulations

Development of effective and safe nanoformulation is still challenging. The developed formulation should be safe for the biological environment. During the past 2–3 decades, considerable attention has been made to provide improved therapeutic benefits of drugs using nanotechnology. The interest of formulation scientists in the development of nanomedicines for the treatment and diagnosis is clearly reflected from the increasing number of issued patents. The patented nanoformulations are based on overall knowledge of the synthetic approach and good expertise in physical and chemical properties. There are some patented products discussed in this study based on nanodevelopments, which bring a wave in the drug-delivery field with less expense on developed product and also have excellent response paving the way to the researchers to fill the drug pipeline with innovative developments (Table V). Recently, Bhatia et al. [206] published an interesting article reporting regulatory implications and current status of nanomedicine in India.

Conclusions

The present review recapitulates various nanoformulations used in drug delivery, their toxicological effects, and significance of nanotechnology in delivering natural bioactives. However, there is an essential need to develop rapid whole animal-based testing methods to assess the potential toxicity of nanomaterials in biomedical science. Mathematical modeling might be promising tool for technological revolution of nanotechnology in biomedicine.
<table>
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PEG: polyethylene glycol
Overall, nanotechnology is a new path in the development of effective carrier systems for site-specific delivery of bioactives. This will introduce new vistas in clinically considering their various merits like maximum efficacy, targeted effects, and improved patient compliance. 

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