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Nanoscale drug delivery systems: An updated view

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Introduction

Nanomaterials are the cornerstone of the rapidly advancing field of nanotechnology having potentials to revolutionise diagnostics and therapeutics. The National Nanotechnology Initiative defines nanoscale materials as particle approximately in the 1 -100 nm size regime in at least one dimension. Generally these structures are up to several hundred nanometers in size being fabricated by top-down or bottom-up approaches [1]. Compared to their conventional counterpart, nanoparticulate entities have distinctive physicochemical and biological properties [2]. Many of their properties such as size, shape, chemical composition, surface structure, charge, aggregation and solubility influence their interactions with biomolecules and cells; concomitantly influence the way the encapsulated/attached entity (drugs) behave in the biological system [2]. Owing to their nanoscale effects, increased surface area and other desirable attributes, they are promising tools for the advancement of diagnostic biosensors, drug and gene delivery, and biomedical imaging [3].

In the recent scenario, developments of newer drugs are high on pharma agenda; however, widespread clinical applications of these efficacious drugs are limited. All drugs face several enroute barriers during their journey from their site of introduction to their molecular site of action. Important amongst them includes rapid filtration by the renal system, premature clearance via the reticulo-endothelial system (RES) and their tortuous transport from the bloodstream to target cells within tissues. Basically, at the tissue or cellular target, the drug must overcome the selectively permeable membrane barrier. Within the cell, it must escape the harsh acidic environment of endolysosomes within which biomolecular drugs may be inactivated or degraded and they must also overcome the nuclear membrane barrier and blood-brain barrier (BBB) (in circumstances viz. nuclear acting and CNS drugs). Further, the poor solubilities and poor stabilities of various drugs in the biological milieu represent another daunting challenges [4]. Not surprisingly, recent studies have illustrated particularly promising ways by which nanomaterials can assist in navigating these formidable barriers. In fact, the application of nanomaterials to drug delivery is broadly expected to change the panorama of pharmaceutical and biotechnological industries [5].

In the present chapter, we have highlighted the prospects of nanomaterials for drug and gene delivery. The current state of the art nanomaterial based platforms for drugs and gene delivery has also been discussed. Moreover, at the end of the chapter, a great deal of discussion describing toxicity issues related with various existing nanoparticles have been collated.

Nanocarriers: Potentials for drug/gene delivery

Nanomaterials have gained great impetus particularly in medicine; in fact the practice of supplanting conventional medical procedures has been set into motion. Formulating therapeutic agents with biocompatible nanocarriers (liposomes, polymers, Inorganic Nanoparticles (NPs) and carbon nanotubes etc.) can subdue many of the associated stumbling blocks. Infact, Nanotechnology enables the innovative utilization of drugs under practices or that has been stalled under various issues. Employing nanomaterials as drug delivery platforms, it may be possible to achieve improved delivery of poorly water-soluble drugs thereby increasing their bioavailability in the biological systems [6,7]. Another unique feature of nanomaterial based drug delivery is their ability to achieve targeted delivery of drugs in cell or tissue specific manner [8]. Generally this is achieved either through passive targeting of drugs to the site of action or by active targeting of the drug employing tailored systems sensitive to different stimuli (e.g. pH, temperature, light, etc.) or systems harbouring tissue/cell specific ligands as detailed in

the text. Basically, owing to their miniature size they can efficiently penetrate through small capillaries to tumors and inflamed tissues, and accumulate at the target site, this indirectly leads to reduction in the unwanted side effects and the toxicity of the therapeutic agent; in parallel enhancing their therapeutic efficacy. They also embody features such as delivery of macromolecular drugs to intracellular sites of action [9,10]. Moreover, they could also mediate controlled release of drugs which not only prolongs action but also attempts to maintain drug levels within the therapeutic window to avoid potentially hazardous peaks in drug concentration following administration of the drugs and thereby maximizes therapeutic efficiency [6,7]. Further, they also provide avenues for co-delivery of two or more drugs or therapeutic modality for combination therapy or systems for simultaneous therapeutic and diagnostic applications [11, 12]; cumulatively imparting several potential advantages including synergistic effects, suppressed drug resistance, and the ability to tune the relative dosage of various drugs to the level of a single nanoparticle (NP) carrier and also leads towards newer therapeutic regimen such as hyperthermia and photodynamic therapy (Figure 8.1). Seemingly, these are just a few of the many compelling reasons that nanomaterials hold enormous promise for improving therapeutic efficacies of drugs by subduing their enroute barriers.

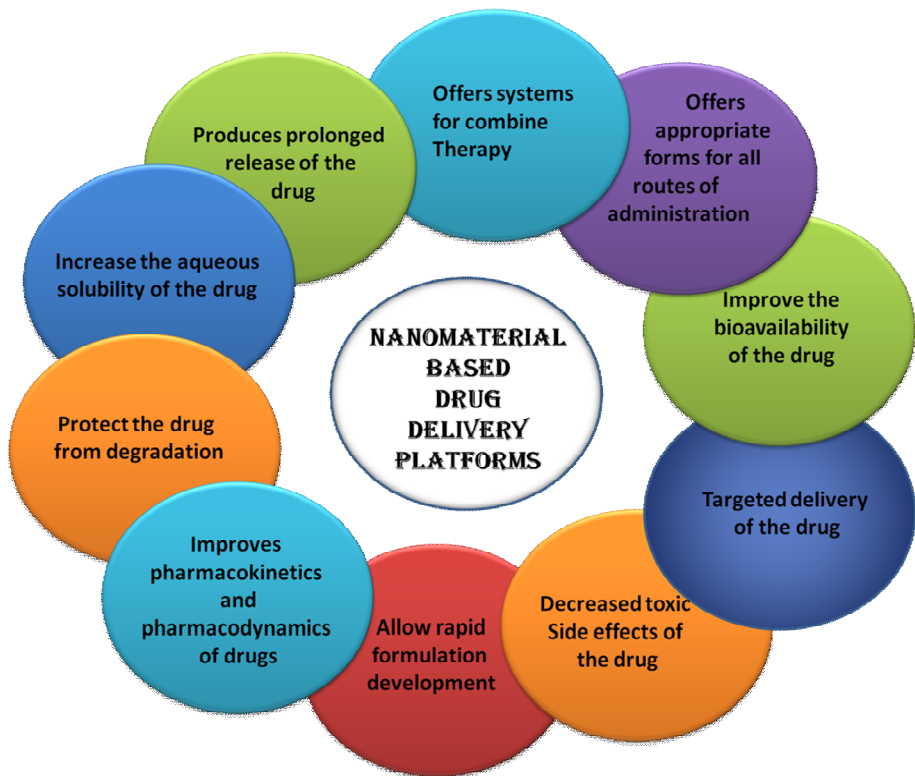


FIGURE 8.1
Advantages of nanomaterial based drug delivery platforms as detailed in the text.

The emergence of nanotechnology has nurtured new prospects for the field of genetic medicine as well. It is well documented that gene therapy has the potential to benefit many untamed diseases. Despite the curability of diseases by restoring or rectifying missing or altered functionalities, as of yet no technically feasible method for gene therapy has been established. Albeit, viral vectors owes potentials to overcome most of their stumbling blocks; besides high transduction efficiency, one

critically important advantage of viral vectors is their appreciable DNA packaging capability. In spite of these advantages, their usage has been of limited application due to their various associated issues such as activation of unwanted immune responses, extreme risk of insertional mutagenesis, high cost of their preparation, safety concerns and constraints in specific tissue targeting [13]. To overcome these shortcomings, synthetic non-viral nanocarriers (lipids and polymer based systems) despite their low transfection efficiency have emerged as potential safer alternatives due to their various desirable attributes to modify the current gene therapeutic regimen including, such as targeted delivery, ease of synthesis, protection in systemic circulation and intracellular delivery etc.

Nanomaterials voyage for drug and gene delivery

Both organic and inorganic materials have been investigated for drug delivery (Figure 8.2), each with its own set of advantages and disadvantages.

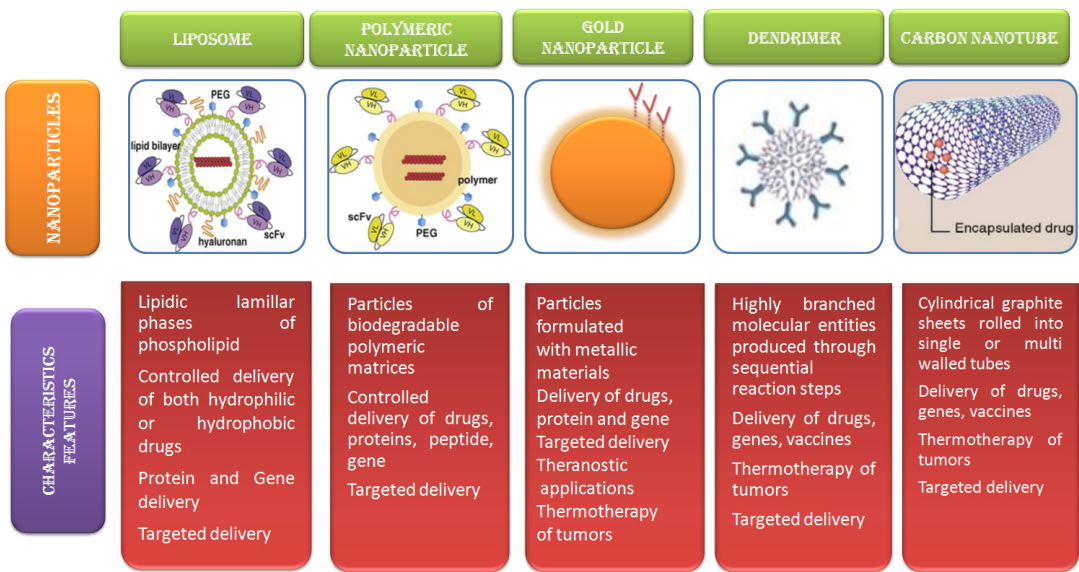


FIGURE 8.2
Representative examples of various types of nanomaterial based drug delivery platforms.

Lipid based nanoparticulate system

The lipid based systems offer diverse delivery platforms comprising of liposomes, micelles, emulsions, solid lipid NPs etc. Apparently, their various features such as biocompatibility, biodegradability, ease of scale-up, capacity to incorporate both lipophilic and hydrophilic drugs, production of fine dispersions of poorly water soluble drugs and cost effective nature compared to polymer based system have enabled them to enjoy the status of the most sought after drug delivery system; moreover, the

application of lipid based systems as drug delivery platforms has been favoured owing to the GRAS (Generally Recognized as Safe) status and their conventional usage in food and pharmaceutical products [14].

Conventional Liposomes

Liposomes are hydrated lipidic lamellar phases comprising of lipid bilayer encapsulating an aqueous core. The tendency of liposomes to deliver the payload (i.e. drugs, antigens, proteins and nucleotides), their relative simplicity, tunable size, charge and their pharmaceutical properties have made these systems the most promising for successful delivery of therapeutic agents. Intriguingly, the first nanomaterial drug delivery systems were lipid vesicles, which were described in the 1960s and later became known as liposomes. Liposomes are one of the most successful delivery systems currently in clinical use for various ailments including cancer, inflammatory, dermatological diseases etc.; of note, Abelcet, Epaxal, Myocet, Doxil represent approved liposomal formulations.

It is in general consensus that the inherent anatomical structures of some tissues especially tumor offers unique advantages for NP targeting; basically, the tumor vasculature is leaky and their lymphatic system is derailed which allows egress of molecular entities of appropriate sizes and their subsequent accumulation inside the tumor, a phenomenon known as Enhanced Permeation Retention (EPR)[15]. To this end, nanoparticulate entities owes competitive advantages while the free drug diffuse non-specifically, they passively accumulate into tumors exploiting EPR effect. Though still exploited in clinics, these passive modes of targeting are not healthier avenues to attain sufficiently desired level of nanoparticle concentration [16]. Although poor lymphatic drainage in the tumors facilitate enrichment of NPs in the tumor interstitium, the EPR phenomenon also induces NPs outflow from the tumor as a result of elevated osmotic pressure in the interstitium and most importantly not all tumors exhibit EPR effect. These issues have motivated the search for active targeting and in the recent scenario targeted drug delivery system (TDDS) are looked upon as more promising strategies. Accordingly, the first example of cell specific targeting of liposomes was described in 1980. Thereafter, there has been flurry of major advancement in the field which simultaneously lead to the developments of various efficacious homing ligands viz. ScFv, Fab, aptamers etc. to be innovatively employed to achieve specific targeting to specific sites which would certainly end up in improved therapeutic outcomes.

Amphotericin B (Amp B), a potent antifungal drug is amongst the one most benefitted by nanomaterial based drug delivery approaches. Researchers have demonstrated that their toxicity issues are considerably reduced upon encapsulating them in nanoparticles especially lipid based system (*AmBisome*, *Fungisome*, *Abelcet*, *Amphotec* represents approved lipid based formulations of AmpB). On this line, we further tried to increase the scope of liposomised Amp B (Lip-Amp B) formulations for the treatment of fungal infections by attaching tuftsin (an immunomodulator) onto their surfaces; the system demonstrated significant improvement over the conventional Lip-AmpB system, as the formulations besides reducing drug toxicity were also anticipated to activate the host's macrophages (important line of host defence against pathogenic fungi) owing to the presence of the tuftsin on their surfaces [16]. Furthermore, we performed elaborative studies to confirm the better efficacy of tuf-Lip-Amp B nanoformulations in enhancing the antifungal activity of amphotericin B [17,18,19,20]; indirectly providing a proof of concept of the better efficacy of the advanced nanoformulations. Moreover, we also demonstrated that tufstin embedded nanoformulations augments the antitumor activity of liposomized etoposide (Lip-ETP) in Swiss albino mice with fibrosarcoma; presumably by nonspecific activation of the host immune system [21].

Further, it is a well known fact that the potential adsorption of antibodies and other immune complex proteins onto these nanoparticulate entities in biological milieu leads to opsonisation and facilitate

their sequestration by the component of the host immune system viz. the reticulo-endothelial system (RES)/mononuclear phagocytic system (MPS). Nevertheless, these innate immunity phenomenon of uptake of nanoparticles (NPs) by the RES may be considered advantageous, providing avenues for targeting macrophages which could be beneficial in the treatment of various ailments including leishmaniasis and candidiasis wherein the pathogen have intracellular abode, residing in the macrophages. There is thus hope for nanotechnology based therapeutic interventions for intracellular pathogens. On the other hand, they also lead to compromise targeted delivery to requisite site. Accordingly, for accomplishing targeted drug delivery, it is enviable to edge uptake by the RES, indirectly interaction with the serum component. Although the surface adaptations of NPs employing hydrophilic and flexible polyethylene glycol (PEG) and other surfactant copolymers (eg poloxamers, polyethylene) have been extensively employed to overcome elimination by MPS [22]. Ironically, they are not free from downsides and there are few limitations that preclude their wide spread application. In case of PEG, issues of polydispersity inside the body and excretion from the body are the main concern in their wide applicability[22]. Though with the commercialisation of advanced purification procedures, PEGs in the market are less polydisperse, but unfortunately the monodisperse PEGs are limited to low molecular weights (< 1000Da only). It is argued that with availability of higher molecular weights analogs, the field of PEGylation chemistry will gain more impetus. Further, there are evidences that the PEG moieties of liposomes maybe immunogenic and evokes antibody responses against second administration. This anticipates that any PEGylated liposomal formulation may display unexpected pharmacological characteristics upon repeated administrations; thereby raising much concern on their utility [23,24, 25].

Furthermore, it is well known that the blood capillaries are lined by a layer of endothelial cells which differ according to the tissue type giving rise to continuous, fenestrated, or discontinuous type of vasculatures. In view of the structural (continuous, fenestrated, and discontinuous) and functional differences (marked by differences in the molecules they express) in the vasculatures of different organ system, NP transport show remarkable differences in various organs and accordingly provides opportunity to aptly design the nanoparticulate formulation to achieved delivery to the requisite organ/tissue. Interestingly, it has been found that liposomes passively accumulate in liver tissues and these phenomena have been exploited for targeting to liver tissues[25]. Furthermore, poly(vinylpyrrolidone-codimethyl maleic anhydride) co-polymer formulations viz. Poly(VP-co-DMMAN) tailored superoxide dismutase has been found to populate kidneys after intravenous administration [26]. This is another compelling illustration of ability of nanomaterials to improve efficacy of drugs by harnessing the advantages offered by the biological system. However, it still remains a daunting challenge for nanotechnologists to satisfactorily harness the opportunities presented by the biological system. Moreover, with advances in the material science, newer drug delivery systems are being introduced embodying attributes to overcome the enroute barriers along with harnessing the benefits offered by the biological system; however, it would be more rationalistic to fine tune the present nanomaterials in voyage to achieve the same. Our laboratory has been extensively working on nanoparticle based drug delivery platforms for the treatment of cancer and a number of infectious diseases. Moreover, we have also deciphered the antimicrobial and anticancerous activity of various natural and synthetic compounds [6-8, 27]. Earlier, we and others have unequivocally demonstrated that several plant based compounds possess strong anti-microbial and anti-cancerous activity. However, their efficacious translation in clinical setting has been hindered due to various aforementioned reasons. Therefore, it is important to address their solubility, palatability, and sustained/controlled release in systemic circulation prior translating the suitability of these potential anti-microbial and anti-cancerous agents in clinical setting. We and others have shown the potentials of one such plant based product viz. garlic as anti-microbial, anti-oxidant and anti-carcinogenic agents. For

skin ailments, topical application is the most desirable approach amongst the avenues available for the administration of medications. Ironically, administration of drugs by this route leads to extensive diffusion particularly of small-sized molecules; thereby leading to low bioavailability and simultaneously reducing efficacy. This advocates development of formulations that can fine tune the bioavailability issues; paving ways towards their effective utilization against skin ailments. In this regards, various drug delivery platforms including micro-emulsion, nano-emulsion, nanoparticles, liposomes and niosomes etc. have been demonstrated to advance delivery of the active drug to the skin. Intriguingly, amongst these, the liposome-based formulations are most promising and leads to enhanced drug penetration, improved pharmacological properties, reduced adverse effects, controlled drug release, and, their biodegradability and non-immunogenicity further adds to their potentials. Keeping into consideration the suitability of lipid vesicles in targeted delivery, we developed pH-sensitive liposomal formulation of the garlic constituent diallylsulphide (DAS) (pH-Lip-DAS) and compared their chemo-preventive potentials with traditional liposomes (Lip-DAS) against dimethyl benz (a) anthracene (DMBA)-induced skin cancer in animal models [28]. In general, both the system viz. Lip-DAS and pH-Lip-DAS were efficacious in suppressing tumor burden compared to the free form of the drug; however, pH-Lip-DAS had an upper edge over the former. Seemingly, the better efficacy of DAS nanoformulations were anticipated to its ability to mediate a depot effect providing sustained release and higher accumulation of the drug at the tumor site and by improving their solubilities issues; and the superiority of pH-Lip-DAS was anticipated to its enhanced ability to deliver the content to the cytosol of the tumor cells. It is well known that DAS mediate its chemotherapeutic effect by altering apoptotic factors populating the cytosolic compartment of the cell; in this regard its association with the cytosolic compartment is desirable for exhibiting its action. To this end, pH sensitive liposomal formulation owing to the presence of dioleoyl phosphatidyl ethanolamine (DOPE) exhibit phosphatidyl-ethanolamine (PE) mediated phase transition at acidic pH thereby mediating cytosolic delivery of the entrapped cargos. Of note, the phospholipid PE not only facilitates the close proximity of approaching bilayers, it is speculated to be directly involved in the merging process. Additionally, the application of liposomes as nanocarrier of anticancer agents including DAS has added benefit as fatty acyl chains of phospholipids may also offer anticancerous effect against various cancers. Recently, oleic acid has been shown to be the key factor responsible for BAMLET/HAMLET mediated killing of cancer cells [29]. Furthermore, to boost their potency as anti-microbial agents; we developed liposomized formulation of DAS for potential application in treating disseminated infection caused by the intracellular opportunistic pathogen *Candida albicans* [30]. The rationale behind the study was to develop a system which could increase their bioavailability in biological system along with providing specific targeting to macrophages wherein intracellular pathogens such as *C. albicans* seek shelter. Interestingly, encapsulating DAS in liposomal formulation would overcome their solubility issues and moreover in doing so they also acquire particulate nature ensuing in avid uptake up by MPS wherein *C. albicans* abode. To translate, these amendments synergistically modulate the activity the molecular drug, inturn increasing their efficacy in treating macrophage resident intracellular opportunistic pathogen *C. albicans*.

With advances in the field of newer generations of drug delivery platforms, liposomal formulation responsive to external or environmental stimuli (e.g., pH, temperature, enzymes) have been fabricated to modulate spatio-temporal release. ThermoDox is a representative example, which are temperature-sensitive nanoliposomal formulation of doxorubicin employed in combination with hyperthermic treatment for cancer therapy [31]; moreover, our next generation immunoliposomes viz. liposomes decorated with infected mouse erythrocyte-specific antibody also represent another non limiting example of advanced TDDS [8]. As it is well documented that the diverse conjugation linkers employed for conjugating antibodies with liposomes not only influence antibody conjugation efficacy but also the

physicochemical behaviour of the formulation; so as to give a deeper sight into the matter Chen et al. provided a glimpse of the influence of different conjugation derivatives on the functionalities of these formulations. They fabricated two variants of anti-EGFR-Fab conjugated immunoliposomal formulations possessing DSPE-PEG-COOH and DSPE-PEG-MAL as conjugation linker and delineated their targeting ability and efficacy in mediating siRNA delivery to SMMC-7721 hepatocarcinoma cells. Both the systems were efficacious in mediating RNAi, with the latter being more effective [32]. This certainly substantiate that the conjugation derivatives should be precisely selected for formulating better targeted drug delivery systems, additionally also corroborates the higher efficacy of targeted systems over nontargeted system.

As mentioned that non-viral vectors are safer and simpler alternatives for gene delivery. Accordingly, liposome and polymer based formulations have been increasingly focused to mediate gene delivery; intriguingly, the lately described liposomal formulations LPD (lipoosomes/protamine/DNA) have displayed superiority over traditional liposomes and DNA polyplexes. Liposome mediated gene delivery was first reported by Felgner in 1987 and as of yet is one of the key method for gene delivery and has been used in human clinical trials. On the contrary, lipid based systems also have various limitations when used for gene delivery as the structures of DNA–lipid complexes are inadequately understood and there arises variations during fabrication step. Cationic liposomal formulation though efficacious and a gold standard for gene delivery in *in vitro* system, their *in vivo* systemic application were rendered inefficient mainly due to their toxicity constraints. With the realization that severe dismal outcomes are associated with the systemic application of cationic liposomes, neutral liposomal formulations revisited their status to mediate systemic delivery of genetic medicines into the cells. Hoffmann and group have innovatively highlighted the feasibility of neutral liposomal formulations to selectively target hair follicles to delivery molecular entities including genes; and their subsequent study illustrated that highly specific targeted and safe gene therapy is indeed viable for hair [33]. Liposomes are still most widely utilized nanomaterial based drug delivery platform in biomedical research endeavours; nevertheless, the system owes several limitations including instability of the carrier, burst release, rapid oxidation of some phospholipids which inturn changes the characteristics of the particular liposomal formulation and non specific uptake by MPS system.

Niosomes

Niosomes are another bilayered vesicular entities composed mainly of non-ionic surfactants being exploited as carrier for lipophilic and hydrophilic drugs. They have the potential to increase the efficacy of the associated drugs [34]. They are biodegradable, biocompatible, non-immunogenic with little toxicity, and structurally and functionally analogous to liposomes [35]. However, unlike liposomes, whose constituents (phospholipids) are more vulnerable to heat and oxidative degradation, they do not impose such reticences. They are formulated on hydration of synthetic non-ionic surfactants with or without incorporation of cholesterol or other lipids which are generally economical than the naturally occurring phospholipids employed in the fabrication of conventional liposomes.

Reckoning with the solubility issues of garlic components and their strong antimicrobial activity; we developed myriads of niosomal formulation of DADS, each differing in their ability to encapsulate DADS to surmount their solubility issues [36]. Interestingly, all niosomal formulations were competent enough to overcome their associated stumbling blocks; albeit those harbouring Span80 were found to be most efficient in encapsulating DADS (size dimensions in the range of 140 ± 30 nm and zeta potential of -30.67 ± 4.5). Furthermore, on evaluating the toxicity of these niosomal formulations, both liver/kidney function tests as well as histopathologic studies suggested that niosome-based DADS

formulations were safe at the dose investigated. Finally, when examined for their efficacy in clearing fungal burden in model animals, it was found that the formulation cleared the fungal burden and increased their survival much efficiently as compared to the free form of the drug. Studies from various groups have provided elaborate overview of niosomes as drug delivery platforms [37].

Further advancements were the introduction of proniosomes which owed attributes to overcome the shortcomings of both liposomes and niosomes. They are basically liquid crystalline compact niosome amalgams which on hydration give rise to niosomes. A recent review by Kuppusamy and group enlighten the various facets of proniosomes [38].

Bacteriosomes

Escheriosomes are lipidic lamellar phases or liposomes being articulated from fusogenic lipids of *Escherichia coli*. Bacteria and yeast have preponderance of unique fusogenic phospholipids within their membranes, presumably to cope with the high multiplication rate. Such lipids seem to facilitate the fusion of the two opposite sites of inner leaflets under physiological conditions. Earlier, we have demonstrated that nanovesicles fabricated from lipid of lower organisms mediate membrane-membrane fusion and thereby offers a novel strategy for effective delivery of the macromolecular drug to the intracellular compartment of the target cells under physiological conditions [39,40]. Considering the potentials of RNAi based therapeutic strategies and the need to achieve safer delivery of RNAi modulators to the cytoplasmic domain of the cell viz. site of their processing and function. In our recent study; we have developed an escheriosomes encapsulated Polo Like Kinase-1-siRNA (PLK-1-SiRNA) nanoformulation and evaluated their efficacy in the treatment of cancer. The nanoformulation delivered the siRNA into the cytosol of the fusing cell; moreover, their near neutral zeta potential and ability to camouflage siRNA inside their bi-layer during the systemic circulation offered safe and efficient intracellular delivery of the intact siRNA cargo with negligible toxicity and widen their therapeutic window, making it more possible to potentialize the effectiveness of siRNA, allowing their usage thereof in various therapeutic arenas in an efficacious manner. The efficacy of whose has been assessed in *in vitro* and *in vivo* models [9]. Furthermore, we also demonstrated that escheriosomes encapsulating DNA vaccine co-expressing Cu-Zn superoxide dismutase and IL-18 conferred protection against *Brucella abortus* [10]; while escheriosomized propofol–linoleic acid (anti-cancer agent) nanoformulation bestowed protection against murine hepatocellular carcinoma [41]. These were some of the non limiting paradigm where escheriosomes have displayed their efficacy in subduing various daunting challenges faced by various treatment stratagems. Whilst their potentials in mediating protection against intracellular pathogens by acting as desirable adjuvant eliciting strong cell mediated and humoral immune responses against encapsulated antigen in analogy with saccharosomes (lipidic lamellar phases or liposomes being articulated from fusogenic lipids of *S. cerevisiae*), leptosomes (lipidic lamellar phases or liposomes being articulated from fusogenic lipids of *Leptospira biflexa*) subtilosomes (lipidic lamellar phases or liposomes being articulated from fusogenic lipids of *Bacillus subtilis*) and archaeosomes (lipidic lamellar phases or liposomes being articulated from lipids of *Archaeobacteria*) are another worth mentioning aspects [39,42,43].

Archaeosomes

As mentioned above, they are liposomes being fabricated from archaeobacterial polar lipids [43]. Extensive efforts have appraised their potentials in drug and vaccine delivery. They possess various advantages over conventional liposomes owing to their attributes of ether lipids. This unique characteristics of archaeal polar lipids viz. ether lipids on contrary to ester lipids present in other

liposomal formulation bestow improved physico-chemical stability including enhanced thermal stability, systemic stability, stability at extremes of pH range, resistance to oxidative stress and action of lipases and bile salts compared to their conventional counterparts. They also display safety profile as revealed by intensive *in vitro* and *in vivo* studies [43].

Generally administration of drug via oral route represents the most promising route of drug delivery. However, the formulations for oral delivery should not only have to overcome the low acidic pH of the stomach but also have to defy the deteriorating effects of lipases and bile salts present in the GI tract. Though, conventional liposomal formulations exhibits stability at neutral and acidic pH; however, they are vulnerable to lipases and bile salts. To this end, archaeosomes with their added virtues offers various advantages over the traditional systems. Interestingly, encapsulation of Coenzyme Q₁₀ in archaeosomes resulted in an increased appearance of the marker in the blood upon oral administration [44]. Moreover, they also exhibit improved thermo-stability over a range of temperature 4–65°C compared to traditional liposomal systems, which could be further enhanced by increasing the ratio of caldarchaeol lipids in the total polar lipids, they open avenues for fabrication of sterile formulations, especially if the encapsulated cargo is also acquiescent to high temperature [45]. Convincingly, they show good prospect for drug delivery applications paving way for their appraisal for actual commercial exploration.

These bacteriosomes technology have advanced rapidly in pre-clinical settings but requires exhaustive scientific evidences to establish their standing as safer and efficacious *in vivo* drug delivery platforms.

Solid lipid nanoparticles

Solid lipid nanoparticles (SLN) were introduced in the 1990s as an alternative to the conventional carrier systems including emulsions, liposomes and polymeric nanoparticles. They are the newer class of drug delivery system with a solid lipid core possessing various competitive advantages over the conventional drug delivery platforms such as better targeting, higher physical stability, lower toxicity, biocompatibility, and ease of scale up [46]. Moreover, being fabricated from lipids present in our system, they could be easily metabolized via the metabolic pathways already present in the system. Owing to their ready metabolism by the body, they do not accumulate in the body thereby ensuing in lower toxic manifestations; infact their lower toxicities issues have been thoroughly validated with various *in vitro* and *in vivo* SLN toxicity studies [47]. Various lipids employed in the preparation of SLN includes triglycerides such as tricaprin, trilaurin, tripalmitin, hard fat types lipids including glycerol behenate and glycerol palmitostearate, and waxes such as cetyl palmitate and different methodology exists for their fabrication such as high pressure homogenisation, microemulsion based methods and solvent emulsifications and more importantly their fabrication methodology avoids usage of harmful organic chemicals. It has been reported that they can be applied through any parenteral route where polymeric systems are tolerable. Intriguingly, SLN based nanoformulation of paclitaxel displayed efficacy equivalent to commercially available Cremophor EL-based paclitaxel formulation against human ovarian and breast cancer cell lines and were physically stable as well. The systems were prepared employing trimyristin (TM), egg phospholipids (ePC) and pegylated phospholipids (PEG2000–PE) through high-pressure homogenization followed by rapid cooling, wherein TM forms the solid core whilst ePC and PEG2000–PE acted as stabilizers [48]. Another important efficacious drug of plant origin is curcumin. Curcumin (difrulyl methane), a constituent of turmeric (*Curcuma longa*) possess strong antioxidant, anti inflammatory and anti cancerous properties. Despite these desirable properties, widespread clinical applicability of this relatively efficacious drug against cancer and other dreadful ailments are limited due to their poor systemic bioavailability. Considerable efforts have been diverted to increase their bioavailability; consequently, myriads of nano material based formulations have been

developed conferring improved bioavailability and efficacy. Wang et al. developed a curcumin-SLN nanoformulation for the treatment of lung cancer. The system was fabricated employing sol-gel method with size range from 20 to 80nm. The preferential lung tumor targeting lead to efficacious tumor inhibition, paving way towards novel method for new anticancer agents development [49]; further to provide a glimpse of their attributes, Qi et al. detailed the pharmacological behaviour of SLNs [50].

Though, SLNs are versatile agent with many desirable features, they also have limitations including low drug loading competence, ambiguity in purity of SLNs; moreover they may undergo transition during storage which may leads to size increment and release of the encapsulated entity [51]. Despite these, they display various competitive advantages over the traditional drug delivery platforms, hence owes merit for future exploration.

Taken together, though lipid based nanoparticulate systems have reputed standing amongst the drug delivery systems, they are susceptible to alteration in temperature and osmotic pressure and other external agents. These issues together with their intrinsic instability (of some lipid based systems) make it necessary to augment stability using hybrid system viz. Lipid–Polymer hybrid nanoparticulate system, which encompasses the unique attributes of both polymeric and liposome systems, while defying some of their limitations.

Polymers

The first controlled release polymer system for delivery of macromolecules was described in 1976. Amongst the largely employed biodegradable and biocompatible polymers, poly(lactic-co-glycolic acid) (PLGA) represent the most sought after material due to their FDA approval. PLGA system comprises of glycolic and lactic acid in various stiochiometric ratio. Their degradation period and release of the encapsulated cargo are dependent on the ratio of glycolic and lactic acid and can be adapted by varying these ratios. In general, system consisting of equal ratio of lactide and glycolide (50:50) degrade much faster than those comprising higher proportions of either of the two monomers [52]. Their hydrolysis products are easily metabolized in the body via the citric acid cycle and are easily eliminated, therefore adverse toxic manifestations with PLGA based drug delivery platforms are low. They have been widely exploited in the niche of efficacious chemotherapeutic drug delivery reservoirs. Taxol are commercially available PLGA nanoformulation of paclitaxel for the treatment advanced prostate cancer whereas Genexol-PM, a polymeric micelle formulation of paclitaxel is approved for the treatment of breast and lung cancers in Korea. Basically, it is composed of block copolymers of PEG and PLGA [53] and the formulation (20-50 nm) is completely soluble having a maximum tolerated dosage (MTD) of 390 mg/m² (50) in phase I clinical trial and exhibited good response rates in subsequent trials.

Apart from their role in improving the efficacy of known chemotherapeutic drugs; increasing interest has emerged to advance the efficacy of biologically active molecular entities that were earlier considered fallow through conventional approaches. In this regard, perillyl alcohol (POH), a monoterpene and constituent of essential oils from a number of plants possess strong anti-cancerous properties against several types of cancer including breast, pancreatic, and liver cancers; however, its therapeutic use is limited due to their various associated challenges. To this end, we developed a PLGA-POH microparticle based systems to address their undesirable issues and evaluated their efficacy against the skin epidermoid cancer cell line (A253) and di-methyl benzo anthracene (DMBA) induced tumors in Swiss albino mice. The formulation when administered to tumor-bearing animals caused greater tumor regression and increased survival rate (~80%) as compared to the free form of POH (survival rate 40%). The superiority of POH-PLGA microparticles over free form of POH could be

attributed to their ability to circumvent the associated stumbling blocks of POH along with bestowing other desirable features [7].

On the same line, we also developed a microcell based system of curcumin. With a view to overcome its solubility, faster degradability and bioavailability constraints; we developed a dual delivery system (810 ± 188 nm dimension and -82.6 ± 2.3 zeta potential) viz. PLGA microparticle encapsulating curcumin co-entrapped in PC liposomes to control release of curcumin in regulated manner. Furthermore, we evaluated the biodistribution of this system and finally assessed their anti-cancerous potentials against di ethyl nitrosamine induced hepatocellular carcinoma in model animals. Intriguingly, the system was efficacious in mediating regulated release of curcumin and displayed time depended release pattern and were free from toxicity issues; in turn the system reduced tumor burden in model animals exemplifying the efficacy of the prepared formulation of the undeveloped drug curcumin [54]. We also developed amoxicillin bearing poly-lactic-glycolic acid (PLGA) microsphere formulation for treatment of experimental listeriosis to boost the potency of the molecular drug "amoxicillin". Interestingly, PLGA microspheres bearing amoxicillin provided a sustained release of encapsulated drug over extended time period, successfully cleared bacterial burdens in vital organs (kidney, spleen, and brain) and also increased survival rate of treated animals in comparison to free form of the drug. The higher efficacy of microsphere based novel formulation of amoxicillin could be accredited to its targeted delivery to infected macrophages as well as to sustained release over an extended period of time [6].

Various important lipid-polymer hybrid nanoparticulate systems viz. lipid-decorated polymeric nanoparticles consisting of a PLGA core, a PEG shell, and a lipid monolayer have also been developed [55]. In this formulation, the PLGA interior incorporates the hydrophobic entities, whilst the PEG coating promotes retention in the systemic circulation and lipid monolayer being present at the interface of polymers promotes sustained release of the entrapped cargo. The system allowed improved drug encapsulation, sustained drug release over an extended period and good systemic stability.

Additionally, Zhang and group developed a biologically inspired system consisting of PLGA NPs surrounded with natural RBCs which owed attributes of long-circulatory half life greater than the gold standard stealth NPs. Besides, in their subsequent studies, they formulated biomimetic nanosponge that functions as a toxin decoy *in vivo*; the system comprising of a PLGA-NPs core encapsulated into RBCs effectively absorbs toxins and in doing so can promisingly address various dismal outcome associated with various toxin secreting dreadful pathogens; and in their proof of concept study in animal model, the system significantly detoxified the staphylococcal alpha-haemolysin (α -toxin). Conclusively, the study highlights the unique feature of nanomaterial based platform i.e. a detoxifying nanobodies that can address issues of toxin mediated toxicities. [56, 57].

Furthermore, SMANCS, a conjugate of the potent chromoprotein neocarzinostatin (NCS) and polymer poly(styrene-maleic acid) (SMA) represents the first practical use of polymer therapeutics as anticancer agents and has been approved in Japan for use in hepatoma treatment in the early 90's. The milestone study, for the first time illustrated the implication of passive tumour targeting through the EPR effect [58]. SMANCS represent the first successful theranostic (field of combine therapy and diagnostics) application, in a sense being administered with a contrast agent lipiodol, they allows X-ray detection of liver tumor nodules as well.

PLGA based formulations have also been exploited for gene therapy with reasonable success. We formulated PLGA-Cox-2 siRNA nanoparticulate system to evaluate their efficacy against experimental skin papilloma (unpublished data). The system with their added virtues displayed efficacy in suppressing tumor burden in experimental animal models. Additionally, reckoning with the fact that cationically modified nanoparticulate entities bind and condense negatively charged oligonucleotides (plasmid, antisense RNA, RNAi modulators etc.) more efficiently and also offers other benefits including

intracellular delivery; various group have connotated the importance of chitosan modified PLGA nanoparticles (CHT-PLGA-NPs). Chitosan, a biodegradable linear polysaccharide comprising of β -(1-4)-linked D-Glucosamine (deacetylated unit) and N-acetylated-D-glucosamine (acetylated unit) embody important advantage to enhance penetration of large molecular entities across mucosal surfaces. Interestingly, Nafee et al. fabricated chitosan coated PLGA nanoparticles with desirable physiochemical features (size in the range 135.95-514.3 nm and surface charges 13.5-60.4 mV) for mediating efficacious gene delivery and for providing proof of concept, the efficacy of the system was evaluated by ensuing efficient delivery of antisense oligonucleotides to lung tumor [59]. Likewise, Yuan et al. highlighted the efficacy of CHT-PLGA NPs for effective and safer siRNA delivery [60].

Dendrimers

Dendrimers are extensively branched molecular entities produced through sequential reaction steps. With virtue of distinct architecture alongwith tunable molecular weight, considerable number of accessible terminal groups as well as capacity to encapsulate cargo molecules, they are forseen as promising delivery platform. Moreover, as the intricacies of dendrimer structure, biocompatibility, retention, and delivery has been increasingly illuminated; novel analogs could be fabricated for better targeting and functionality. It has been unequivocally advocated that an aptly fabricated dendrimer structure can be altered concurrently for desire biocompatibility, bioavailability, and pharmacological properties [61].

Cationic dendrimers including poly(amidoamine) dendrimers and poly(propylamine)(PPI) have been studied not only as an efficient scaffold for the therapeutic drugs but also for delivery of genetic medicines. Cationic groups (specially primary amine) at their surfaces participate in the oligonucleotide (negatively charged) binding, their condensation, cellular uptake and triggering proton sponge in endosomes which enhance their release into the cytoplasm. Zhou and co-worker reported an effective siRNA delivery system based on structure flexible polycationic PAMAM dendrimers; which condenses the siRNA into nanoscale particles, moreover protecting them from enzymatic degradation while mediating substantial release of siRNA over an extended period of time for efficient gene silencing [62]. Studies have also illustrated the potency of various generations of poly(propylenimine) (PPI), carbosilane, polylysine and other dendrimeric analogues for delivery of macromolecular drugs[63,64,65]. McCarroll et al. have fabricated single-walled carbon nanotubes functionalized with polylysine dendrimers for delivery of anti-ApoB siRNA. The formulation demonstrated effective reduction in ApoB mRNA thereby causing reduction in serum cholesterol levels while reducing the toxicity and immunogenicity of SWNTs as detailed below [65]. In the arsenal of dendrimer analogues, PAMAM represent the most widely employed system mainly due to their wide commercial availability. Although, these dendrimers impose toxicity issues, but as more is gleaned about their safety issue along with ease of synthesis they could emerge as versatile drug delivery platforms.

Inorganic NPs

With the development in nanotechnology, inorganic nanostructured materials have been designed/discovered or fabricated with important cooperative physical properties to be utilized in the development of delivery systems with both therapeutic and diagnostic modalities [66]. Among the various inorganic particles explored for improving drug delivery efficiency, due to their credits of good biocompatibility, ease of large scale synthesis, high surface-to-volume ratio, monodispersity, and ready

functionalization, amphiphilicity, safe carrier capabilities, tunable shape and size, gold nanoparticles are anticipated as an enticing scaffold for drug delivery [67-70]. Moreover, as efficient release of the cargoes after reaching to the requisite site is prerequisite for effective therapy; the release of the cargoes from the AuNPs could be triggered by internal (e.g. glutathione (GSH), or pH) or external stimuli (e.g. light, temperature etc.) [71-74] providing avenues for spatio-temporal release. By exploiting the phenomenon of surface plasmon resonance (SPR), their complexation with the materials, delivery and distribution within target tissues can be monitored and provides other benefits as well as highlighted below. These unique properties have drawn great attention across the globe for harnessing them in the development of drug delivery platform. Interestingly, the potential application of AuNPs is investigated in phase I & II clinical trials for cancer therapy [75].

Considering the fact that synthesis process plays an important role in maintaining the unique properties of gold nanoformulations; different preparation procedures yield different AuNPs morphology offering an array of AuNPs including spherical gold nanoparticles, gold nanorods, gold nanocages and gold nanostars among others, each with diverse functionalities. Various methods that have been employed for their synthesis includes chemical reduction producing monodisperse spherical AuNPs in the 10–20 nm diameter range [76]; physical reduction producing hollow Au nanostructures [77]; photochemical reduction method giving rise to cubic AuNPs[78]; biological reduction viz. molecular hydrogels of peptide amphiphiles for producing various shapes of AuNPs [79]; solvent evaporation techniques producing 2D Au super lattices [80]; and biomimetic method yielding diverse AuNPs [81] etc. however, the preferred method particularly depends on the ease of synthesis and application required. Considering the global efforts to revolutionize cancer therapeutics, strategies employing nanomaterial based platforms are increasingly exploited in the recent scenario; El-Sayed et al. developed a tamoxifen-PEG-thiol-AuNP conjugates for displaying efficacy against breast cancer treatment. The system selectively targeted estrogen receptor alpha in human breast cancer cells with up to 2.7-times enhanced potency *in vitro* [82]. Moreover, Rotello and group developed gold nanoformulations to incorporate drug into their hydrophobic pocket to display efficacy in cancer treatment. The system was functionalized with a hydrophobic alkanethiol interior and a tetra(ethylene glycol) (TEG) hydrophilic shell that terminated into a zwitterionic head group which reduced nonspecific binding with cell and macromolecular entities in the biological system [83]; interestingly, the system with miniature size and biocompatibility displayed prolonged circulation and inturn better accumulation in tumor tissues by the EPR effect.

Moreover, Elbakry used monodisperse AuNPs as a scaffold for the implementation of layer-by-layer approach to siRNA-AuNP conjugates, forming a system comprising of (polyethylene-imine) PEI/siRNA/PEI-AuNPs [84]. The inclusion of PEI rendered opportunities to formulate well defined and homogenously distributed nanocarriers and to mediate endosomal escape besides decreasing the net negative charge of the siRNA-AuNP formulation, which facilitated their cellular uptake as a result of decreased repulsion from the cell membrane. Albeit, the researchers reported successful uptake of siRNA-PEI AuNPs; however, the enhanced stability of the nanoparticles were found to decrease the intracellular release of siRNA, necessitating further concern on the theme. Song et al. fabricated an efficient and safe siRNA delivery system of uniform shape and narrow size composed of PEI-capped gold nanoparticles (AuNPs) which were successfully manufactured using PEI as the reductant and stabilizer. Without causing cytotoxicity, the system exhibited efficient knockdown of the oncogene (PLK-1) and induce enhanced cell apoptosis which was not observed when the cells were treated with PLK-1 siRNA using PEI as the carrier; exemplifying the efficacy of PEI-capped AuNPs to be a suitable carrier for intracellular siRNA delivery [85]. Moreover, the system also rendered appreciable intracellular release of siRNA. Although PEI is used as an excipient for imparting various functional attributes to the nanoparticulate systems including AuNPs; however, their toxicity issues has either

lead to the search of other polycationic excipients as a safer platform which additionally also imparts other functional attributes, or modulation of PEI ratio such that it is non toxic to the system or have feeble toxicities. Han et al. developed AuNPs being reduced and stabilized by chitosan (CS) onto which (cis-aconitic anhydride-functionalised poly(allylamine) PAH-Cit/PEI and siRNA were electrostatically deposited; the system owed negligible cytotoxicity against HeLa and MCF-7R cells while mediating their efficient protection against nuclease degradation and triggered release of siRNA as a result of charge reversal mechanism [86]. Moreover, Ghosh et al. developed a simple cysteamine-functionalized AuNPs modified with PEG for the delivery of chemically unmodified miRNAs into living cells [87].

As already mentioned, gold nanoparticles embody unique optical properties owing to strong SPR absorption at visible and NIR wavelengths, thereby exhibits photothermal (PTT) effects which can be exploited to activate myriads of biological manifestations, providing many avenues for future endeavours. Non-spherical AuNPs have some advantages beyond the spherical-nanoparticle as versatile delivery system. Gold nanorods (AuNRds) and gold nanospheres (AuNSs) consisting of a thin gold wall with a hollow interior exhibits strong SPR tunability in the NIR region. Exploiting this property of gold nanoparticulates, Braun et al. developed a formulation comprising of AuNSs that exhibited controlled spatio-temporal release of siRNA cargo upon excitation with NIR laser. The liberation of siRNA from AuNSs upon NIR laser excitation did not show any significant toxicity and exhibited power and time dependence through surface-linker bond cleavage; though decomplexation occurred at low power excitation, but escape from endosome only occurred at high power irradiation; it was foreseen that more advanced transfection methods overcoming the endosomal barrier would have great impetus in the development of more efficacious NIR laser-controlled drug release systems [88]. Hushka et al. developed AuNSs based spatio-temporal nucleic acids (NAs) delivery system comprising of poly-L-lysine peptide (PLL) epilayer covalently attached to the NS surface. They made inclusion of PLL to mediate electrostatic capture of NAs; while on demand liberation of NAs were achieved by excitation with NIR laser. NIR induced delivery of NAs by the NA-PLL formulations resulted in around 50% downregulation of the targeted GFP expression in H1299 lung cancer cells without any significant cytotoxicity [89].

Considering their efficacious nature in mediating improved RNAi regulator delivery in *in-vitro* system, many reports have foreseen their great clinical potential not only for gene therapy but also for drug delivery, biosensing, and bioimaging in *in-vivo* system; yet, the success in clinical settings depends on how these nano-structures behave in the biological system, with the physiological processes and the anatomical structures influencing their behaviours.

As the blood capillaries forms an intimate contact with almost every cells of the body, as a result, any tissue in the body can be accessed through systemic administration of the nanoformulation provided they surpass the anatomical barriers offered by them. Functionalised NPs have been utilised to achieve targeted delivery upon systemic administration. However, functionalized AuNPs as other NPs have strong tendency to associate with the blood proteins such as albumin, fibrinogen, insulin etc. Albumin, the most abundant protein of the blood plasma, besides their role in maintaining the colloidal osmotic pressure of blood and interstitial fluids, is equipped to mediate transport of various molecules (fatty acids, some amino acids, peptides, and steroids and drugs); it also help in the trafficking of Au NPs across the endothelium. With reports suggesting that these albumin-Au-NP conjugates were internalized either by transcytosis (90%) or by fluid phase endocytosis (10%) [90, 91]. Reckoning with the fact that albumin adsorption on gold nano-surfaces facilitate their drainage from blood vessels to the interstitial space by transcytosis [90]; in the recent scenario, this phenomenon is foreseen as a new avenue for the delivery of gold nanoformulations to tissues upon systemic administration. However, on

the contrary, the nonspecific nature of this process will be a major challenge for targeted delivery. Of note, the fate of AuNPs *in vivo* is influenced by the serum proteins on their surfaces, which is been seen as an interesting area of research that will have implications in drug delivery.

Endothelial lining in the brain is completely continuous with endothelial cells firmly adhered to each other by tight junctions, while further strengthened by astrocytes forming blood-brain barrier that allows only highly selective permeability to transverse through, representing tremendous challenge for delivery of various moieties. Any exogenous molecular entity to transverse through brain has to breach through the blood–brain barrier. In this regards, Bonoïu et al. developed an excellent approach for the delivery of siRNA to brain utilizing gold nanoparticles (AuNPs) complexed to siRNA, called nanoplexes, for modulation of the dopaminergic signaling pathway in an *in vitro* model [93]; exemplifying the efficacy of AuNPs for therapies of central nervous system disorders by transmigration across Blood brain barrier. Reckoning with the fact that TDDS are looked upon as more promising strategy; AuNPs were decorated with various homing ligands including alpha-tocopherol, cholesterol, or Hyluronic acid (HA), folic acid, and transferrin and others which ferry them to specific sites [94, 95].

Recent reports illustrate that AuNRds and AuNSs with their unique NIR light absorbing feature, elevate the temperature of their local milieu (45–50°C) upon laser irradiation, eventually has been seen to cause apoptosis of the cancerous while sparing normal cells, as cancerous cells being much more vulnerable to increase in temperature; as a result AuNRds when co-administered with the anticancer drugs along with cognate siRNA would exhibit enhanced suppression of tumour growth as also exemplified by recent reports [93,96]. Moreover, as already mentioned that inorganic NPs possess unique physicochemical and optoelectronic properties, they could themselves translate into a better therapeutic molecule.

Carbon nanotubes (CNTs)

Carbon-nanotubes (CNTs) are fibrilous nano-cylinders comprising of single (single-walled CNTs, SWCNTs) or multiple (multi-walled CNTs, MWCNTs) graphine layer(s) with length and diameter ranging from 50 nm to 100 nm of 1–5 nm or 10–100 nm respectively [97, 118]. The tunability of CNT layers bestow attributes of multi-valent binding to cells alongwith conjugating multiple targeting molecules. Despite their advantages, therapeutic applicability of CNTs is accompanied by concerns about their non-solubility in aqueous milieu and possible adverse effects[98]. It has been argued that proper functionalisation of CNTs (f-CNTs) could stimulate solubility of CNTs; consequently, their proper functionalisation by covalent or non-covalent methodology facilitated their solubility in aqueous solutions and also refrained non-specific interactions in biological milieu thereby minimizing toxicity observed in the case of non-functionalized raw particles inturn increasing biocompatibility and circulating half life [99]. Moreover, functionalization of CNTs with cationic groups serves another purpose of binding with anionic nucleic acid moieties by electrostatic interactions which could have great impact in the arena of gene therapy. The shape characteristics of any NPs including CNT could significantly affect their biodistribution. The length and shape of the NPs should be taken into account when it comes to the well-individualized cylindrical CNTs. SWCNTs display strong absorbance in the NIR region, the region being transparent for the biological systems, as a result providing avenues for optical imaging and PTT. Targeted delivery has been achieved by deploying CNTs by exploiting various homing ligands such as folic acid, epidermal growth factor, herceptin etc. Liu et al. demonstrated that CNTs-drug conjugates could effectively accumulates in tumors exploiting EPR effect and several magnitude higher concentration could be achieved than that of plasma. Furthermore, in their follow up studies,

they demonstrated the higher efficacy of SWCNT–paclitaxel conjugate in reducing tumor growth in a murine 4T1 breast cancer model without any toxic manifestations [100, 101].

Zhang et al. fabricated functionalized SWCNTs (SWCNTs+) conjugated to human telomerase reverse transcriptase (hTERT) siRNA to deliver to tumours *in vivo*. The SWCNT-formulated siRNA was injected intratumourally and induced reduction in hTERT mRNA and protein levels leading to inhibition in tumour cells growth in a xenograft mouse model [102]. Krajcik et al. developed functionalized SWCNTs using hexamethylenediamine (HMDA) and poly(diallyldimethylammonium) chloride (PDDA) generating positively charged PDDA–HMDA–SWCNTs which electrostatically interacts with the negatively charged siRNA (against extracellular signal-regulated kinase ERK). The system bypassed the cellular membrane barrier and suppressed the expression of the ERK target proteins in primary cardiomyocytes (a reduction of 75% was observed) and exhibited negligible cytotoxic effects on isolated rat heart cells at concentrations up to 10 mg/l [103].

Furthermore, McCarroll and group developed SWCNTs functionalized with lysine-based dendrimers covalently attached to lipid chains (Tol 7); basically, inclusion of the lipid moiety was made to mask the hydrophilicity of siRNA and facilitates cell binding, whereas the positively charged dendrimer condensed the siRNA into discrete particles. The system was utilized for systemic delivery of anti-ApoB siRNA and showed effectual reduction in ApoB mRNA, which led to reduction in serum cholesterol levels.

It is in general consensus that owing to their fibrillar structure, they could lead to cytotoxic manifestations, inflammation and DNA damage [104–109, 111]. Generally, SWCNTs and MWCNTs can induce platelet aggregation, mitochondrial dysfunction, ROS generation, lipid peroxidation and oxidative stress resulting in cell death among other manifestations [105, 106, 111]. Evidence suggests that high concentrations of nanotubes demonstrated chronic lung inflammation, including foreign body granuloma formation and interstitial fibrosis leading to toxic effects [104, 107–110, 111]. These adverse side effects can limit the applicability of CNTs in clinical applications. CNTs with desirable features could be exploited for specific application provided their route of administration are aptly considered in which fibrillar structures don't lead to adverse effects. It is too early to establish CNT for clinical settings, these novel carriers are indubitably interesting and deserve further investigation.

Over the decades, the importance of combination therapy for treatments of diseases has been highlighted. In this regard, administration of combined therapeutic modality directed against different targets can enhance therapeutic efficacy or leads to a system with comparable efficacy but with lower side effects. More innovation could involve piling of different therapeutic modalities onto a single system (nano drug delivery platforms) which would lead to simultaneous administration of both; moreover, besides synergism, the nano drug delivery platforms also provide other benefits including reduce toxicity of the free drug and subduing drug resistance [11]. Concurrent with the recent situation, employing nanomaterial based drug delivery platforms for co-delivery of several agents is promising. To translate, it improves rather enhance the action of the therapeutic agents rendering administration of lower concentration of each entity in turn reducing toxicity issues thereby holds tremendous potential for future. On this line, polymer based drug delivery systems were deployed for co-entrapment of conventional chemotherapeutic agent doxorubicin and curcumin. The system displayed improved efficacy on MDR cells, and follow up studies are investigating their efficacy *in vivo* systems [112]. Additionally, combination of RNAi with chemotherapeutics are also promising strategy; based on this, the anthracycline or taxol drugs along with siRNA (VEGF) were encapsulated in cationic micelles to achieve improved therapeutic outcomes [113, 114]. Likewise, the cationic drug mitoxantrone has been complexed with hydrophobic palmitoleic acid to ferry anti-mcl-1 siRNA; the system was found to be a dependable approach [115]. Despite these promising developments, a comprehensive research

is required to advocate the synergistic dose and ratios of siRNA to chemotherapeutics in animal model [11].

Moreover, despite tremendous potential of RNAi based approaches and role of delivery platforms in realising their potentials as of yet, co-delivery of combinational potent synergistic siRNA employing the same platform is in infancy; a group have employed a biodegradable polymer to transport Mdr-1-shRNA and Survivin-shRNA (gene relating to MDR); the polymer with added virtues to compact oligos at neutral pH and liberate them at acidic pH of the endosomal compartment could overcome MDR in tumor cells when delivered with the molecular drugs [116]. More recently, a siRNA combination system viz. a lipid nanoparticulate system encompassing two siRNA viz. VEGF- A and kinesin spindle protein (KSP) paved its way towards clinics. The first-in-human trial of this system illustrated the pharmacokinetics, RNAi mechanism of action, and clinical anti-cancer activity [117] of the same.

More recently, Eldar-Boock et al. have highlighted the developments of nanomaterial based drug delivery systems for combination therapy. Albeit, combination therapy is undoubtedly more complicated than monotherapy; nevertheless, it is certain that apposite drug combinations together with drug delivery platforms can offer important improvements viz. reduced case-fatality, less chances of drug resistance development and in near future efforts would be made to make them cost effective. The selection of appropriate drug delivery platforms plays crucial roles in maintaining the efficacy of combination therapy. Of note, the preference of drug delivery system should be in accordance with physico-chemical attributes of the cargoes [11].

Toxicity of the nanocarrier systems

The potentials of nanomaterial based drug delivery are encouraging. However, they are not free from downside; there are reports indicating that nanomaterials themselves may pose toxicological risk. De Jong and Borm have documented few of the possible adverse toxicological responses observed over the past decade [118]. However, it is intrigued that various amendments in the nanoparticulate entities could lead towards a safer system and it is foreseen that even small changes to the physicochemical characteristics of NPs can have appreciable impact on their behaviour, compelling predictive toxicology impossible. Moreover, considering the toxicity issues of nanomaterials, recently our laboratory has innovatively highlighted the potential of biomimetic synthesis to lead towards novel nanoformulation of the molecular entity itself with the rationale that such advancements would be beneficial to pioneer novel nanoassemblages that will be more efficacious and more importantly free from nanomaterial (excipient) related toxicities. In our follow up studies, we are exploring the efficacies of such novel systems in providing protection against various dreadful ailments including cancer [119]. Interestingly, global efforts have been exploiting computational modelling and screening approaches to explore requisite properties of NPs viz. dimensions, hydrophilicity, stability, density of homing ligands on NP surfaces etc. for safer and efficacious therapeutic applications.

Conclusions

The expansion of nanomaterial mediated drug delivery may play an important role in adding a new armamentarium of therapeutics to the pipelines of existing drugs embodying improved efficacy. Efforts are in practice to revisit the status of suboptimal but biologically active molecular entities that were formerly known to be fallow through conventional approaches with field of nanomedicine moving at a

very rapid pace. It is intriguing that the characteristics of NPs that bestow them their therapeutic properties may also lead to toxicity. Thus it is imperative to fine tune the efficacy and adverse effects to fabricate an efficacious system. More elaborate work from various sectors is needed to lead towards the more “smarter”, “advanced” and yet safer system that could trounce various daunting challenges associated with various pharmacological drugs.

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