

*Review Article***Nano-pharmaceuticals in Medicine**YOGINDER K GUPTA^{*,1} and A K DINDA²¹Dept. of Pharmacology, All India Institute of Medical Science, New Delhi 110 029, India²Dept. of Pathology, All India Institute of Medical Science, New Delhi 110 029, India

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Nanotechnology is an emerging field with a major goal to target drug delivery to improve the efficacy and safety profile. The application of nanotechnology to medicine, known as nano-medicine, concerns the use of precisely engineered materials to develop novel therapeutic and diagnostic modalities. Nanomaterials possess various unique physiochemical properties, such as small size, large surface area to mass ratio, and high reactivity, which are different from bulk materials. These properties can be used to overcome limitations found associated with traditional therapeutic and diagnostic agents. This review focuses on recent progress and development in the field nano-pharmaceuticals for various biomedical applications.

Keywords: Nano-pharmaceuticals; Nano-particles; Nano-medicine; Nanomaterials

Introduction

Application of nanotechnology in medicine or Nanomedicine is revolutionising the medical practice both in the areas of diagnostics and therapy. One of the major areas of focus in nanomedicine is the area of nanoparticle based drug delivery systems (DDS). These nano DDS can be broadly grouped as nano-pharmaceuticals which may have a potential to replace the market of conventional drugs (pharmaceuticals). The drug or other biomolecules can be loaded inside nanoparticles (NPs) which can be targeted to disease site leading to increased bioavailability, therapeutic efficacy simultaneously reducing the off-target effect and toxicity. Over the last several decades, numerous nanocarriers have been developed that include liposomes, polymeric particles, drug conjugates, dendrimers, solid lipid nanoparticle, protein and carbohydrate as well as inorganic system like iron, carbon, silica and so on. The biocompatible and biodegradable carriers hold great potential to be used in drug delivery applications.

In the past few decades, several therapeutics based on nanoparticles, which are particles in the size range of 1-1000 nm, has been successfully introduced for the treatment of various diseases. These

therapeutics harness the potential of various opportunities provided by nanomaterials to target the delivery of drugs more precisely, in order to improve their solubility, to extend their half-life, to improve their therapeutic index and to reduce their immunogenicity. The developments made in the field of NPs-based delivery systems have opened up new vistas to engineer non-immunogenic and targeted systems for delivery of biomolecules. Hence, in this report, we focus on the advances made during last five years in India.

Nanoformulations for Vaccines Delivery

Vaccination has a major impact on the control of infectious diseases. However, there are still many infectious diseases for which the development of an effective vaccine has been elusive. In many cases, the failure to vaccines is a consequence of the inability of vaccine candidates to evoke appropriate immune responses. Over the past decade nanoscale (size<1000 nm) materials, such as virus-like particles, liposomes, polymeric, nanospheres have received attention as potential delivery vehicles for vaccine antigens which can both stabilize vaccine antigens and act as adjuvants. Importantly, some of these NPs are able to enter antigen-presenting cells by different

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pathways, thereby modulating the immune response to the antigen.

The researchers at AIIMS have developed a novel nanoadjuvant or NPs which can deliver different protein antigens as vaccine. The NPs are made of low molecular weight biocompatible, biodegradable polymer Polycaprolactone coated and stabilized with Pluronic (Prashant *et al.*, 2014). The average size of the NPs is around 60 nm. The slow degradable polymers stay inside the antigen presenting cell *in vivo* for 4 to 6 months and produce sustained high antibody response with single dose. They have demonstrated the antibody response with Tetanus antigen. The major breakthrough is the capability of the NPs for lymphatic targeting following administration through oral route with higher disseminated antigen presentation as well as higher antibody response than conventional injectable vaccine. Recently, a study by Dinda *et al.* (2016) reported NPs with 14KDa polycaprolactone entrapping hepatitis B surface antigen (HBsAg) stabilized with Pluronic® F127 and used it as oral delivery vehicle (Dinda *et al.*, 2016). The efficacy for specific antibody production was evaluated and compared with parenteral routes of immunization in mice. A superior antibody response with a higher titer of anti-HBsAg antibody till 2 months following single oral administration compared to other routes of immunization and conventional alum-based HBsAg vaccine was found. The NPs with antigen were found in the macrophages residing in small intestinal villi, peripheral lymph nodes and other reticulo-endothelial organs 2 months after oral administration. This study suggests the efficacy of current nanocarrier system for efficient antigen presentation disseminated in peripheral lymphoid tissues, following oral administration with a prolonged antibody response, which can minimize the requirement of booster dose.

In a study by Harde *et al.* development of a novel NPs, comprising of stable glucomannosylated chitosan, for oral immunization was demonstrated (Harshad *et al.*, 2014). Chitosan nanoparticles were stabilized by tandem cross-linking using tripolyphosphate followed by glutaraldehyde. The *in vitro* and *in vivo* performances were established in RAW 264.7 and BALB/c mice, respectively. Results illustrated that the lyophilized formulation was exceptionally stable in simulated biological media and

the enclosed antigen was conformationally stable. The mechanistic understanding of glucomannosylated chitosan NPs in RAW 264.7 revealed trans-cellular uptake via both mannose and glucose transporter-mediated endocytosis. Glucomannan modification resulted in significantly higher systemic (serum IgG titer), mucosal (secretory IgA) and cell-mediated (IL-2 and IFN- γ) immune responses in comparison with non-modified chitosan NPs. As a result, the present strategy is expected to contribute some novel tools for the oral delivery of numerous bio-macromolecules.

In another study by Smitha *et al.* (2014), the development of amidase, an effective vaccine to prevent infections caused by *S. aureus*, encapsulated O-carboxymethyl chitosan NPs (Ami-O-CMC NPs) of 300 ± 50 nm was reported. The particles were prepared by ionic cross-linking method. The prepared Ami-O-CMC NPs had an encapsulation efficiency of 55.39%. Haemolysis assay and cytotoxicity studies proved the hemo-compatibility and cyto-compatibility of the prepared NPs. The sustained release of Ami from the NPs is expected to prolong its immunogenicity and in turn leads to development of better protective immunity against *Staphylococcus aureus* infections.

Nanoformulations for Anti-Tuberculosis Drugs

Tuberculosis, a highly contagious persistent infection caused by *Mycobacterium tuberculosis* and *Mycobacterium bovis*. Tuberculosis is the world's second most common cause of death after HIV/AIDS. In 1993, World Health Organization declared as a global emergency. The disease typically attacks lungs but can also develop as extra pulmonary TB in the central nervous or circulatory systems or elsewhere in the body. Despite potential curative pharmacotherapies being available for over 50 years, the length of treatment and pill burden can hamper patient lifestyle. Thus, low compliance and adherence to administration schedules remain the major reasons for therapeutic failure and contribute to the development of multi-drug-resistant strains. NPs based delivery systems are suitable for targeting chronic diseases such as tuberculosis as these cross the biological barriers and target cellular reservoirs of *Mycobacterium tuberculosis*. Experimental data supports the possibility of intermittent chemotherapy with key first-line as well as second-line anti-

tubercular drugs by employing synthetic or natural carriers, chiefly polymers.

AIIMS group has developed a novel nano-composite with low molecular weight Polycaprolactone and gelatin using a layer by layer technology, wherein four anti tubercular drugs (INH, Rifampicin, Ethambutol and Pyrizinamide) can be loaded in different compartments in one NP. This system is being optimised for respiratory and oral delivery systems (Indian Patent Application #1141/DEL/2014).

Varma *et al.* (2015) used pyrazinamide, which is a highly specific agent against *Mycobacterium tuberculosis*, to formulate polymeric NPs to sustain the release and reduction of the dosing frequency of pyrazinamide (Ravi *et al.*, 2015). The formulated polymeric NPs were in the particle size range of 45-300 nm with a maximum drug entrapment efficiency of 80.9%. The stability study of optimized batch conducted at $40\pm 2^\circ\text{C}/75\pm 5\%$ relative humidity showed no significant changes up to 90 days. X-Ray Diffraction spectrum exhibits the transformation of crystalline form of drug to amorphous in the formulation. Scanning Electron Microscope image showed that NPs were spherical in shape with smooth surface. *In vitro* release profiles were biphasic in nature with burst release followed by controlled release over a period of 24 hr, obeying the diffusion mechanism. *In vivo* and *ex vivo* studies results of this study showed significant uptake of the NPs by alveolar macrophages through fluorescent micrograph. Polymeric NPs formulation of pyrazinamide could encompass significant uptake by alveolar macrophages, the high first-pass metabolism, sustain the release of drug leading to reduction in dose, toxicity and improvement of patient compliance.

Though there are promising results seen in several preclinical animal model of tuberculosis, the greatest challenge is to deliver 4 anti-tubercular drugs in correct therapeutic ratio (Bacteriostatic: Bactericidal) at the disease site. In this regard Prof. Dinda's group in collaboration with Prof. Y.K. Gupta has fabricated a novel nanocarrier with polycaprolactone and gelatin layer by layer loading 4 drugs in one NP with each drug in separate compartment. It showed a high bactericidal activity (Indian Patent Application #1141/DEL/2014).

Nano-formulations for Cancer Therapy

Cancer is a rapidly emerging disease nowadays. The use of chemotherapeutic drugs is, however, limited due to the random and non-targeted distribution of these drugs in the body leading to undesirable side effects with subsequent decrease in their therapeutic efficacy. Consequently, site specific delivery of anti-cancer drugs is the most important requirement for effective chemotherapy. In this regard, scientists have generated great interest in the NPs based delivery systems, owing to their ability to have enhanced penetration and retention effect at the tumor site. Also, targeting the specific cells of the body can be achieved by surface modifications. Several reports have demonstrated the enhanced efficacy of drug loaded NPs in cancer therapeutics.

A study by Kumar *et al.* (2014), used iron oxide nanoparticles to deliver an oncopeptide, NuBCP-9, targeting the BCL-2 BH3 domain (Kumar *et al.*, 2014). Citric acid/2-bromo 2-methylpropanoic acid (CA/BMPA)-capped super-paramagnetic iron oxide NPs were used to immobilize and deliver the NuBCP-9 peptide to the cancer cells without any noticeable off-target effects. Results demonstrated that NuBCP-9- superparamagnetic iron oxide NPs efficiently penetrate into cancer cells and bind to its intracellular target protein BCL-2. Moreover, significant inhibition of proliferation and substantial induction of cell death were observed when cancer cells were treated with NuBCP-9- superparamagnetic iron oxide NPs at different time intervals. Importantly, the IC_{50} values for killing of breast cancer cells with NuBCP-9- superparamagnetic iron oxide NPs were much lower compared to cells treated with the NuBCP-9 peptide linked with a CPP (Arg-8; NuBCP-9-R8). Molecular and biochemical analyses further supported that NuBCP-9-SPIONs killed breast cancer cells by apoptosis-mediated mechanisms. Furthermore, the data demonstrated that administration of NuBCP-9-SPIONs to mice bearing Ehrlich ascites tumors was associated with loss of tumorigenicity and extensive apoptosis in tumor tissues. Taken together, these findings showed that a non-CPP-tagged peptide can be successfully delivered to undruggable intracellular onco-targets using superparamagnetic iron oxide NPs.

Researchers at Amrita University, Kerala demonstrated unique of nanofiber for the controlled

delivery of anti-cancer drug, Temozolomide, in orthotopic brain-tumor for one month using flexible polymeric nano-implant (Ramachandran *et al.*, 2017). A library of drug loaded (20 wt%) electrospun nanofiber of PLGA-PLA-PCL blends with distinct *in vivo* brain-release kinetics (hours to months) were numerically selected. Orthotopic rat glioma implanted wafers showed constant drug release with negligible leakage into the peripheral blood rendering ~1000 fold differential drug dosage in tumor versus peripheral blood. Most importantly, implant with one month release profile resulted in long-term (>4 month) survival of 85.7% animals, whereas 07 day releasing implant showed tumor recurrence in 54.6% animals, rendering a median survival of only 74 days. In conclusion, highly controlled drug delivery is possible for prolonged periods in orthotopic brain-tumor using combinatorial nanofibre libraries of bulk-eroding polymers, thereby controlling glioma recurrence.

Transferrin-targeted core-shell Nanomedicine encapsulating two clinically used drugs, doxorubicin and sorafenib were formulated by researchers at Amrita University against liver cancer (Malarvizhi *et al.*, 2014). Doxorubicin loaded Poly(vinyl alcohol) nano-core and sorafenib loaded albumin nano-shells were prepared. Doxorubicin loaded in nano-shell intercalates in DNA killing cancer cells. Whereas, nano-shells release sorafenib inhibited aberrant oncogenic signaling involved in cell proliferation. They showed enhanced cellular uptake synergistic cytotoxicity in ~92% of cells when iron-deficient microenvironment was present via transferrin ligands targeting. Efficient penetration of targeted core-shell NPs in 3D spheroids of liver tumor causes uniform cell killing. Hence, designed core-shell NPs can be effectively combined with clinically relevant single-agent drugs for exerting synergistic activity against liver cancer.

Bhatnagar *et al.* (2015) used bromelain (BL), a proteolytic enzyme, an anti-tumor agent which however has been limited due to the requirement of high doses at the tumor site (Bhatnagar *et al.*, 2015). They developed the BL loaded poly (lactic-co-glycolic acid) NPs that show enhanced anti-tumor effects compared to free BL. The formulated NPs with a mean particle size of 130.4 ± 8.81 nm exhibited sustained release of BL. Subsequent investigation revealed enhanced anti-tumor ability of NPs in 2-stage

skin tumorigenesis mice model. Reduction in average number of tumors (~2.3 folds), delay in tumorigenesis (~2 weeks), percent tumorigenesis (~4 folds), and percent mortality rate, as well as a reduction in the average tumor volume (~2.5 folds) in mice as compared to free BL were observed. NPs were also capable of modulating the expression of pro-apoptotic (P53, Bax) and anti-apoptotic (Bcl2) proteins. Therefore, our findings demonstrate that developed NPs formulation could be used to improve the efficacy of chemotherapy by exerting chemo-preventive effects against induced carcinogenesis at lower doses.

Thimmaraju *et al.* (2017) demonstrated poloxamer based formulations of meloxicam. They evaluated various parameters like pH stability, drug release and *in vitro* anti-cancer activities in cell lines with an intention to formulate injectable sustained biodegradable drug delivery system. Various strengths of meloxicam formulations were prepared by using poloxamer 407 and were analyzed for drug content and pH stability by using HPLC. Drug release studies were tested by using USP dissolution testing apparatus. Further, they evaluated *in vitro* anti-cancer activity among these formulations by using sulphorhodamine-B assay in two leukemia cell lines such as HL-60 and K-562 cell lines. It also showed 60% drug release and exhibited good anti-cancer activity in HL-60 cell line with $GI_{50} \leq 10 \mu\text{g/ml}$, similar to adriamycin. Thus, the formulation has a potential to be a sustained release, passive targeted delivery system for meloxicam, with reduced side effects associated with the drug.

Researchers at IIT Delhi demonstrated poly (styrene-co-maleic anhydride), a biocompatible copolymer that was further conjugated with paclitaxel (PTX) via ester linkage and self-assembled to form poly (styrene-co-maleic acid)-paclitaxel (PSMAC-PTX) NPs (Dalela *et al.*, 2015). The *in vitro* release of PTX from PSMAC-PTX NPs showed a higher release at lower pH than at the physiological pH of 7.4, confirming its pH-dependent release. IC_{50} values of 9.05-18.43ng/mL of PTX equivalent were observed in various cancer cell lines after 72 hr of incubation. Pharmacokinetic studies revealed that the conjugation of PTX to the PSMAC co-polymer not only increased the plasma and tumor C(max) of PTX but also prolonged its plasma half-life and retention in tumor via enhanced permeability and retention effect.

Administration of PSMAC-PTX NPs showed significant tumor growth inhibition with improved apoptosis effects *in vivo* on Ehrlich Ascites Tumor (EAT)-bearing BALB/c syngeneic mice in comparison with Taxol, without showing any cytotoxicity. Therefore, PSMAC-PTX NPs may be considered as an alternative nanodrug delivery system for the delivery of PTX in solid tumors.

PLGA nanoparticles loaded with 2-Methoxyestradiol were prepared and stabilized with casein or poly(ethylene glycol) by Gopikrishna *et al.*, 2017 *et al.* These particles were evaluated for cellular interactions, pharmacokinetics and tumour accumulation. It was found that particle-cell interactions and the anti-angiogenesis activity were similar for both nanoformulations *in vitro*. But in case of PEGylated nanoformulation, the *in vivo* pharmacokinetics and tumour accumulation of the drug were substantially improved. Reduced protein binding was observed for PEG stabilized PLGA NPs. Hence, 2-Methoxyestradiol nanoencapsulated with PEGylated PLGA nanocarrier could improve its half-life and plasma concentration and thereby increase the tumour accumulation.

Nanoformulations for Ocular Delivery

The unique anatomy and physiology of eye poses a huge challenge to the delivery of drugs in the eye. The blinking reflex and tear drainage prevents drug retention on ocular surface which necessitates repeated drug dosing; this affects patient compliance, causes blurring of vision etc. Moreover, the different anatomical layers of the eye prevent topical drugs from reaching the posterior segment of the eye. Over the last decade, the benefits of nanotechnology in encapsulating drugs in NPs for sustained release and improved availability has made remarkable contribution towards ocular drug delivery.

Posterior capsular opacification (PCO) is a major post-surgical complication following cataract surgery. Different approaches to prevent PCO have been attempted by targeting the cells involved in the pathology or by targeting molecular pathways involved in this fibrotic change. Major cause of failure with intraocular drugs was either toxicity or inadequate drug availability. Sustained delivery of Doxorubicin through Methyl polyethylene glycol-polycaprolactone NPs was designed to selectively destroy lens epithelial

cells after cataract surgery to avoid toxicity induced by Doxorubicin on the healthy cells of the eye (Guha *et al.*, 2013). *In vitro* evaluation of cellular entry of Doxorubicin NPs and free drug and arrest of cellular proliferation was evaluated in lens epithelial cells. *In vivo* evaluation of efficacy of Doxorubicin NPs in prevention of PCO and its safety for ocular use was reported in rabbit model.

Curcumin has been established for its anti-inflammatory, anti-angiogenic and anti-fibrotic property, but unfortunately major hindrance in exploiting the properties of curcumin for clinical use is its insolubility and poor bioavailability. To improve the retention time and bioavailability of Curcumin, curcumin loaded NPs were designed and evaluated in animal model of corneal neovascularisation (CNV) (Nirparaj *et al.*, 2015). CNV is a leading cause for compromised vision. Therapeutic prevention of CNV is a major clinical challenge and there is a compelling need to seek effective and safe therapy for this pathology. MePEG-PCL NPs of curcumin has shown increased efficiency in preventing angiogenic sprouting *in vitro*. Topical delivery of curcumin NPs in the eye showed enhanced retention of curcumin in the cornea, significant improvement in prevention of CNV over free curcumin as graded clinically and was supported by data showing suppression of VEGF, inflammatory cytokines and MMP expression. Anti-inflammatory activity of curcumin was demonstrated by suppression of NFκB. Histopathology and scanning electron microscopy shows absence of any adverse change in the corneal structure following application of curcumin NPs.

Nanoformulations for Targeting Central Nervous System

Alzheimer's Disease

Neurogenesis is a process of generation of new neurons which is negatively regulated by age, stress, sleep deprivation and increased by caloric restriction, exercise, and physiological activation. Several animal and clinical studies suggest reduced neurogenesis in neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, and Huntington's disease. Hence, nanoparticles mediated induction of neurogenesis via targeting endogenous neural stem could be a promising therapeutic approach to such diseases.

Tiwari *et al.* (2014), working at IITR, Lucknow, loaded curcumin, a neuroprotective agent with limited brain bio-availability, in PLGA formulating NPs. Better induction of neural stem cell proliferation and neuronal differentiation *in vitro* and in the hippocampus and subventricular zone of adult rats was observed as compared to bulk curcumin. The NPs were also found to significantly increase the expression of genes involved in cell proliferation (reelin, nestin, and Pax6) and neuronal differentiation (neurogenin, neuroD1, neuregulin, neuroligin, and Stat3). Reverse learning and memory impairments in an amyloid beta induced rat model of AD-like phenotypes, by inducing neurogenesis by these NPs was also observed. Molecular docking studies suggest curcumin interaction with Wif-1, Dkk, and GSK-3 β . Hence, curcumin NPs could provide an interesting therapeutic approach to neurodegenerative diseases such as Alzheimer's disease by inducing brain self-repair mechanism.

Parkinson's Disease

Pahuja *et al.* (2015) demonstrated sustained and safe delivery of dopamine across the blood brain barrier for successful therapy in Parkinson's disease was. Dopamine-loaded PLGA NPs (DA NPs) were synthesized to deliver dopamine to the brain. These NPs slowly and constantly released dopamine, showed reduced clearance of dopamine in plasma, reduced quinone adduct formation, and decreased dopamine autoxidation. DA NPs were internalized in dopaminergic SH-SY5Y cells and dopaminergic neurons in the substantia nigra and striatum, regions affected in Parkinson's disease. NPs were able to cross the BBB and capillary endothelium in the striatum and substantia nigra in a 6-hydroxydopamine induced rat model of Parkinson's disease. Systemic intravenous administration of DA NPs caused significantly increased levels of dopamine and its metabolites and reduced dopamine-D2 receptor supersensitivity in the striatum of parkinsonian rats. These results suggest that NPs delivered dopamine into the brain, reduced dopamine autoxidation-mediated toxicity, and ultimately reversed neurochemical and neurobehavioral deficits in parkinsonian rats.

Epilepsy

Benzodiazepine class of drugs are widely used as

antiepileptic drugs. Diazepam, lorazepam and midazolam are given via intravenous route as first line of treatment in the emergency management of Status Epilepticus. In a study by Sharma *et al.* a pre-hospital treatment in the form of a delivery system which could be given via non-invasive route (nasal route) to the patients suffering from SE in emergency conditions was demonstrated. PLGA NPs of Diazepam, lorazepam and midazolam were prepared via nano-precipitation method for nose to brain delivery (Sharma *et al.*, 2016, Sharma *et al.*, 2015). The developed NPs were found to be in nanometric range with high percentage drug entrapment, which was further confirmed by TEM imaging. *In vitro* and *ex vivo* drug release supported the hypothesis of fast onset of action from PLGA NPs by showing initial burst release and further controlled drug release. Cell viability assay on Vero cell line showed that developed drug loaded NPs were not cytotoxic. The bio-distribution studies on Sprague-Dawley rats with technetium-99m radiolabelled drugs supported the hypothesis of nose to brain pathway for drug delivery of developed polymeric NPs. The results showed that drug loaded polymeric NPs showed higher brain uptake of drug when administered intra-nasally compared to intranasal drug solution and intravenous drug solution. Pharmacokinetic studies suggested prolonged action of diazepam/midazolam/lorazepam concentrations in brain (higher AUC_{Brain}) due to slow erosion of PLGA from NPs. Brain/blood distribution studies showed that drug loaded polymeric NPs provided higher brain/blood concentration of drug after NPs intranasal administration, compared with drug solution intranasal and intravenous administration. Moreover, Gamma Scintigraphic images were in agreement with the bio-distribution results in Sprague-Dawley rats. The investigation demonstrated that intranasal drug loaded NPs can potentially transport diazepam, midazolam and lorazepam via nose-to-brain and can serve as a non-invasive alternative for pre-hospital treatment of SE.

Nanoformulations for Wound Healing

Wound healing is an innate physiological response that helps restore cellular and anatomic continuity of a tissue. Selective biodegradable and biocompatible polymer materials have provided useful scaffolds for wound healing and assisted cellular messaging. Nanotechnology is on the threshold of providing a host

of new materials and approaches, revolutionizing the medical and pharmaceutical fields. Recently, silver NPs have attracted interest for a clinical application because of its potential biological properties such as anti-bacterial, anti-inflammatory, and also wound healing efficacy, which could be exploited in developing better dressings for wounds and ulcers.

Researchers at AIIMS developed Silver nanoparticles (20-30nm in size) loaded inside Glutathione Collagen NPs which showed a higher anti-infective and wound healing property in comparison to silver sulphadiazene (Indian Patent Application #201711007245). The silver NPs embedded inside the collagen NPs are less toxic and the anti-microbiocidal activity is due to silver ions released at the wound area.

Chitosan alginate mucoadhesive NPs were fabricated for treatment of pemphigous ulcers and other ulcers in mouth. These particles were loaded with different immunosuppressive and anti-inflammatory agents for local sustained release at the mucosal ulcer bed. Both the systems can be modified for application to periodontal infection and other inflammatory diseases.

Dhapse *et al.* (2014) developed biogenic silver NPs, using a single-step green synthesis of silver NPs (AgNPs) to investigate *in vitro* and *in vivo* behaviour as wound-healing agents. Stable AgNPs with characteristic absorption at 408nm and 15 ± 3 nm particle size were generated via the active involvement of *Bryonia laciniosa*. No loss of stability was detected after 6 months at room temperature. Anti-bacterial activity was observed against both Gram-negative and Gram-positive bacteria with no cytotoxicity observed *in vitro* at a concentration of 200 $\mu\text{g/mL}$ and effective cytokine modulation. *In vivo* wound healing experiments showed improved wound contracting ability in rats where, after 14 days, wound alleviation was $47.1\pm 2.2\%$ in the control groups, compared with $78.1\pm 1.4\%$ and $92.6\pm 6.7\%$ for a silver-based marketed cream and the AgNPs, respectively. As a result, the developed AgNPs proved to be superior wound healing agents owing to scarless healing with insignificant inflammation and toxicity.

Tiwari *et al.* (2014) illustrated the synthesis of copper NPs by *Pseudomonas aeruginosa* and testing their efficacy in enhancing the pace of wound healing.

Culture supernatant was used to synthesise copper NPs. Optimum conditions were selected to maximise the biosynthesis of NPs. Biosynthesised copper NPs (BNCs) were characterised by Malvern zeta sizer and scanning electron microscopy. Average particle size, polydispersity index and zeta potential of BNCs were found to be 110.9 nm, 0.312 and (-) 18.3 mV, respectively. BNCs were evaluated for its wound healing activity by excision wound model in rat. The pace of wound healing was enhanced by BNCs compared with copper in native form.

Nanoformulations for Anti-leishmaniasis Drugs

Visceral leishmaniasis is a fatal vector-borne parasitic syndrome attributable to the protozoa of the *Leishmania donovani* complex. The available chemotherapeutic options are not ideal due to their potential toxicity, high cost and prolonged treatment schedule.

Want *et al.* (2015) used nano drug delivery systems for plant-derived secondary metabolite, artemisinin, as an alternative strategy for the treatment of experimental visceral leishmaniasis (Want *et al.*, 2015). Artemisinin-loaded poly (lactic-co-glycolic acid) (ALPLGA) NPs prepared were spherical in shape with a particle size of 220.0 ± 15.0 nm, $29.2\pm 2.0\%$ drug loading and $69.0\pm 3.3\%$ encapsulation efficiency. ALPLGA NPs administered showed superior antileishmanial efficacy compared with free artemisinin in BALB/c model of visceral leishmaniasis. There was a significant reduction in hepatosplenomegaly as well as in parasite load in the liver ($85.0\pm 5.4\%$) and spleen ($82.0\pm 2.4\%$) with ALPLGA nanoparticles treatment at 20mg/kg body weight compared to free artemisinin ($70.3\pm 0.6\%$ in liver and $62.7\pm 3.7\%$ in spleen). In addition, ALPLGA NPs treatment restored the defective host immune response in mice with established visceral leishmaniasis infection. In conclusion, results advocate superior efficacy of ALPLGA NPs over free artemisinin, which was coupled with restoration of suppressed cell-mediated immunity in animal models of visceral leishmaniasis.

Dr. Dinda's group in collaboration with Prof. Y.K.Gupta, at AIIMS, New Delhi, fabricated a low cost cholesterol grafted chitosan NPs stabilised by Pluronic with a high (>65%) Amphotericin B entrapment efficiency. This system showed 10 fold higher efficacy than Ambisome and also effective

through oral administration in rodent model (Dinda *et al.*, 2016).

Nanoformulations for Anti-fungal Drugs

AIIMS group has fabricated Chitosan grafted cholesterol NPs with average size of 220nm with a high (63%) loading efficiency of Amphotericin B. It has an unique property of endosomal escape in parasite containing cells leading to sustained cytosolic release of drug leading to higher parasitocidal activity in 1/10 dose of Amphotericin B when compared with the liposomal preparation Ambisome. This nanocarrier can deliver Amphotericin B orally as documented in rodent model. This novel low cost nanocarrier for Amphotericin B with higher efficacy and low toxicity has a high translational potential (Indian Patent Application #201611009768).

Nanoformulation for Cosmeceuticals

Researchers at AIIMS have fabricated a novel Glutathione and goat Collagen NPs which has high antioxidant and pro-healing property (Indian Patent Application #201711007245). It can reduce melanin synthesis following sun exposure (UV irradiation). The average size of NPs was found out to be 30nm. It can be loaded with curcumin and other agents with a high potential use for nano-cosmeceuticals.

Nanocarriers for Oral Gene Delivery

Gene or any other nucleic acid (Micro RNA/ SiRNA) cannot be delivered orally due to acid peptic digestion. A group at AIIMS, New Delhi have loaded the DNA (gene) in calcium phosphate NPs and sequentially coated it with Poly acrylic acid (PAA), Chitosan and PAA. This novel layer by layer technology makes particle acid resistant resulting in successful oral gene delivery system as demonstrated in murine model (Indian Patent application # 3256/DEL/2012).

Nanoformulations for Anti-Bacterial Drugs

Blood borne bacterial infections continue to be a major cause of mortality despite significant advances in modern medicine. Most bacterial infection causes fever of unknown origin without any unique clinical symptoms; these cases require diagnostic confirmation and appropriate antibiotic therapy should be administered. Culture diagnosis has been the gold standard in bacterial infection diagnosis for last 75

years. In many of the infection episodes, the time lost in diagnosis can be a major cause of mortality. Requirement for a rapid diagnosis has been stressed by many health/funding agencies across the world. Hence with nanotechnology components/devices, many interesting technical advances in molecular diagnostics, nucleic acid amplification assays have been developed.

Researchers at IIT have developed a novel typhoid diagnostic system called iMC2 (Singh *et al.*, 2016) with a reduced turnaround time of 7 hr. Antibody coated NPs to specifically isolate target cells from a large sample volume by using a unique combination of magnetic field was used. Blood sample was collected in a capture bottle with prefilled reagents and incubated for 6 hr. After the incubation period, target cells were isolated from bulk sample and enriched by 200 folds. This enrichment allows detection of Salmonella typhi cells using lateral flow assay strip with reduced time.

Further, reduced the turnaround time for bacterial detection to 60 minutes using SiMED (Spot immunomagnetic enrichment device) (Singh *et al.*, 2016). Magnetic NPs were used to tag the target pathogens and captured into a 100ml volume chamber using moving magnetic field. By combing immuno-magnetic enrichment with fluorescent detection, they have been able to identify ultralow concentrations of typhi cells with a turnaround time of an hr. These novel applications of nanomaterial will continue to improve the performance of diagnostics, healthcare applications.

Nanoformulations as Biomarker

One of the most important factors in electrochemical immunosensing is the quality of sensing electrode. During the development of highly sensitive and stable immunosensors, it is a crucial requisite to create new types of electrodes that allow fast and simple measurements of specific biological interactions. Organic self-assembled monolayers are of great significance in surface technology since the presence of chemically bound molecules render the properties of an interface entirely different compared to those that are unmodified. The most commonly used biomarker for Prostate cancer detection is Prostate specific antigen. In a study done at MGPG College, Gorakhpur, gold screen printed electrode were

modified and functionalized by Self Assembled Monolayer of cysteamine and Single Walled Carbon nanotubes (SWNTs) for detection of Prostate specific antigen (Shukla *et al.*, 2017, Guiseppi *et al.*, 2010). The Prostate specific antigen immobilized electrode was placed in a three electrode cell as working electrode. DPV measurements were used to determine the linear range and to estimate the detection limit of Prostate specific antigen as the routine biosensor. The voltammograms clearly showed the plot of the peak current versus Prostate specific antigen concentration of cysteamine, cysteamine/SWNTs, cysteamine/SWNTs/Anti-Prostate specific antigen modified SPE. By modification of cysteamine/SWNTs, a well-defined response was observed as compared to cysteamine modified SPE. The cysteamine and SWNTs modified gold screen printed electrode will propound new area in the field of Biosensors used for Prostate cancer detection.

Nanoformulations for Multiple Release

To achieve multiple release profiles, NPs with complex architectures, such as those with multiple shells responding to the trigger to different extents or those which respond to different triggers are engineered. Though promising, the complex chemistries involved in their synthesis is challenging. Towards this, group of researchers at IIT, Delhi developed a simple formulation using a mixture of NPs, responding to different extents for a single trigger, temperature (Deshpande *et al.*, 2017). The formulation consists of Gold NPs based core with a thermoresponsive polymeric shell composed of pNIPAm or pNIPMAm, able to deliver the drugs using capacitive radiofrequency generated hyperthermia. Exposing the system to radiofrequency for shorter time resulted into increase of the temperature, consequently resulting into release of drug primarily from pNIPAm shells, while the second exposure, for longer time, resulted into release from pNIPMAm shells. Thus, it provides a simple alternative for developing a sustained-triggered drug delivery system.

Nanoformulations from Herbal extracts

Researchers at Delhi University have developed silver NPs (AgNPs) using plant extract of various medicinal plants and conducted bioassay against bacteria, cancer and various mosquito vectors. The NPs were synthesized using tannic acid and determined their

anti-bacterial activity against both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. The mean size of AgNPs was 12 nm and stable up to seven months. It was reported that AgNPs at low concentration exhibited strong anti-bacterial activity (Gupta *et al.*, 2013).

Also, AgNPs were synthesized using bark extract of *Holarrhena antidysenterica* and evaluated larvicidal activity against third instar larvae of *A. stephens*. It has been reported that green synthesized AgNPs exhibited strong larvicidal activity against *A. stephens* having LC_{50} and LC_{90} value of 2.672 ppm and 4.482 ppm only, respectively (Kumar *et al.*, 2017).

Nanoformulations for Peptide Delivery

Consistent efforts in developing effective nanopharmaceuticals in the Srivastava lab at IISER Bhopal, leads to development of biocompatible and biodegradable polyelectrolytes derived from polyaspartic acid framework (Sharma *et al.*, 2013). The muco-adhesive nature of the polyamide backbone of these polyelectrolytes is noteworthy. Electrostatic interactions between oppositely-charged polyelectrolytes yielded nano sized complexes that were stabilised through *in-situ* disulphide crosslinking. The disulphide cross linking stabilised these nanocomplexes under physiological conditions but made them susceptible to reducing conditions such as those found intra-cellularly. These redox-responsive nano complexes were utilised for facile encapsulation of the peptide therapeutics such as Insulin and its delivery to lung cancer cells. Once internalised, these nano complexes unravelled to release the entrapped peptide cargo intra-cellularly.

This achievement was further elaborated by developing amphiphilic polymers towards delivering hydrophobic drugs such as Curcumin (Fatima *et al.*, 2016). Facile solubilisation of commercial Curcumin powder in aqueous medium was achieved by utilising these polymers. Very high drug loading (upto 40 wt.%) was also possible in these nano-formulations. The inherent hemotoxicity of the polymers was significantly reduced by polyelectrolyte complexation with sodium polyaspartate. The resulting nano-formulations had a favourable size and could be dehydrated to yield reconstitutable powders.

Nanoformulations for Gene Delivery

Researchers at IIT Guwahati demonstrated cationic BSA nanoparticle system incorporating bimetallic Au–Ag luminescent nanoclusters (NCs) for the delivery of therapeutic suicide gene in HeLa cancer cells (Deepanjalee *et al.*, 2016). Combinatorial therapy is achieved with cationic BSA Au–Ag NCs composite NPs loaded with pDNA, which binds efficiently with pDNA due to its positive nature. While successful delivery of a suicide gene (CD-UPRT) into cells by pDNA loaded composite NPs initiates a therapeutic response cascade by converting prodrug 5-FC to 5-FU, Au–Ag NCs results in ROS (reactive oxygen species) triggered apoptosis mediated cell death. Further, luminescence of Au–Ag NCs serves to track the gene delivery into cells. The detailed mechanism of uptake and manner of cell death has been demonstrated to comprehend the combinatorial therapeutic efficacy of the composite system.

Researchers at JNCASR, Bangalore, demonstrated glucose-derived carbon nanospheres nuclear targeting vector (Selvi *et al.*, 2008). Besides, the intrinsic fluorescence property of carbon nanospheres helps in tracking their cellular localization without any additional fluorescent tags. The spheres are found to target the nucleus of the mammalian cells, causing no toxicity. Interestingly, the *in vivo* experiments show that these nanospheres have an important ability to cross the blood-brain barrier and localize in the brain besides getting localized in the liver and the spleen. There is also evidence to show that they are continuously being removed from these tissues over time. Furthermore, these nanospheres were used as a carrier for the membrane-impermeable molecule CTPB (N-(4-chloro-3-trifluoromethylphenyl)-2-ethoxybenzamide), the only known small-molecule activator of histone acetyltransferase (HAT) p300. Biochemical analyses

such as Western blotting, immunohistochemistry, and gene expression analysis show the induction of hyperacetylation of histone acetyltransferase (HAT) p300 (autoacetylation) as well as histones both *in vitro* and *in vivo* and the activation of HAT-dependent transcription upon CTPB delivery. These results establish an alternative path for the activation of gene expression mediated by the induction of HAT activity instead of histone deacetylase (HDAC) inhibition.

Nanoformulations as Antimicrobial Scaffold

Researchers at IIT, Delhi, presented a novel, elastic, non-adhesive and antimicrobial hydrogel PVA scaffold (loaded with AgNPs) that were synthesized using freeze-thaw method (Bhowmick *et al.*, 2016). The direct visualization of the synthesized (one-pot green synthesis methodology) AgNPs using TEM shows particle size in the range of 7 ± 3 nm. The minimum inhibitory concentration (MIC) of AgNPs for *Staphylococcus aureus* and *Escherichia coli* was estimated to be 7.81 $\mu\text{g/mL}$, whereas for *Pseudomonas aeruginosa* (gram negative) it was around 3.90 $\mu\text{g/mL}$. The antimicrobial efficacy of AgNPs was further studied by protein leakage, ROS and LDH activity assay. The quantitative elemental analysis of silver was calculated before and after release in phosphate buffer (pH-7.4) by atomic absorption spectroscopy. The antimicrobial efficacy of the scaffold was retained even after 96 hr of release of AgNPs which suggests that the scaffold can be used as a reservoir for AgNPs to maintain a moist and sterile environment for a long period of time.

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