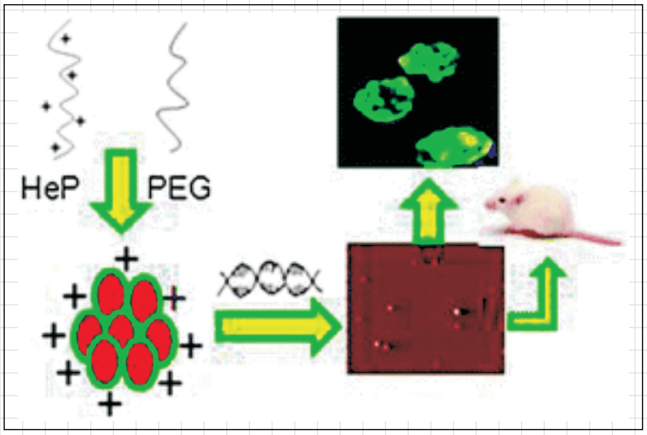
Nanotherapeutics & Nanomaterial Toxicology

Research Highlight

Polyethyleneglycol crosslinked N-(2-hydroxyethyl)-polyethylenimine nanoparticles as efficient non-viral vectors for DNA and siRNA delivery *in vitro* and *in vivo*

A series of electrostatically crosslinked nanoparticles, N-(2-hydroxyethyl)-polyethylenimine-PEG600 (HePP), were prepared by allowing N-(2-hydroxyethyl)-polyethylenimine (HeP) to interact with polyethyleneglycol (600) dicarboxylic acid (HOOC-PEG_{600}-COOH, PEG_{600}dc) and were then evaluated for their capability to transfect cells *in vitro* and *in vivo*. DLS studies revealed the size of the HePP nanoparticles in the range 106-170 nm, which efficiently condensed nucleic acids and provided sufficient protection against nuclease degradation. HePP-pDNA complexes exhibited a considerably higher transfection efficiency



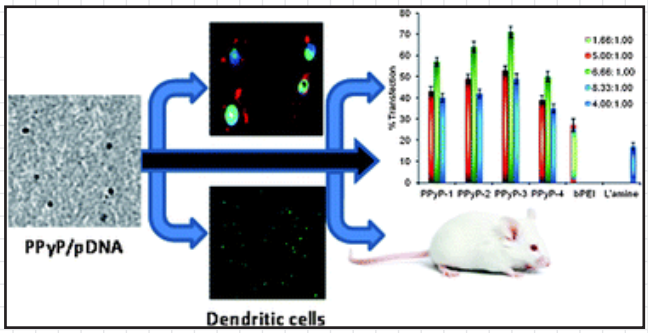
Polyethyleneglycol crosslinked N-(2-hydroxyethyl)-polyethylenimine nanocomposites for efficient delivery of DNA and siRNA, in vitro and in vivo

and cell viability in various mammalian cell lines, with HePP-3-pDNA displaying the highest gene expression, which outperformed HeP and the commercially available transfection reagent, Lipofectamine™. Also, HePP-3 mediated sequential delivery of GFP specific siRNA resulted in ~76% suppression of the target gene. Intravenous administration of HePP-3-pDNA complex to mice, followed by monitoring of the reporter gene analysis post 7d, revealed the highest gene expression occurred in the spleen. Together, these results advocate the potential of HePP nanoparticles as efficient vectors for gene delivery *in vitro* and *in vivo*.

Tripathi et al.; Molecular Biosystem; 2013; doi: 10.1039/C3MB70150F

Self-assembled amphilic phosphopyridoxylpoly-ethyleneimine polymers exhibit high cell viability and gene transfection efficiency *in vitro* and *in vivo*

Branched polyethylenimine (bPEI) was conjugated with hydrophobic pyridoxal phosphate (PLP) in the side chain via reaction with primary amines to obtain amphiphilic phosphopyridoxyl-polyethylenimine (PPyP) polymers. These polymeric amphiphiles with a defined degree of hydrophobicity self-assembled into nanostructures, which were characterized by DLS and evaluated for their capability to condense nucleic acids and carry them into cells. Further condensation of pDNA compacted the size of the self-assembled nanostructures from 421–559 nm to 134–210 nm with zeta potentials from +20–32 mV to +18–28 mV. Conjugation of PLP with bPEI not only reduced the



Enhanced gene transfection efficiency of phosphopyridoxylpoly-ethylenimine polymer *in vitro* and *in vivo*

density of the primary amines (i.e. charge density) but also improved the cell viability of the modified polymers considerably and weakened the binding of pDNA with these polymers. Efficient unpackaging of the pDNA complexes inside the cells led to a several fold enhancement in the transfection efficiency with one of the formulations, PPyP-3/pDNA complex, among the series, exhibiting 4.9 to 8.2 folds higher gene delivery activity than pDNA complexes of bPEI and Lipofectamine™ in HeLa and MCF-7 cells. Flow cytometry analysis revealed a very high percentage of transfected cells by PPyP/pDNA complexes compared to pDNA complexes of bPEI and Lipofectamine™. Further, GFP-specific siRNA delivery using PPyP-3 as a vector resulted in 84% knockdown of the target gene expression (cf. 54% by Lipofectamine™/pDNA/siRNA complex). Moreover, the PPyP-3/pDNA complex displayed 6.7 fold higher transfection efficiency than the

bPEI/pDNA complex in human peripheral blood dendritic cells. Intravenous administration of PPyP-3/pGL3 complex showed the highest gene expression in spleen tissue, advocating the potential of these vectors in future gene delivery applications.
Arif et al.; Journal of Material Chemistry B; 2013; 1; 4020-4031

The activity against Ehrlich's ascites tumors of doxorubicin contained in self assembled, cell receptor targeted nanoparticle with simultaneous oral delivery of the green tea polyphenol epigallocatechin-3-gallate

Doxorubicin (DOX) is a well-known anticancer drug used for the treatment of a wide variety of cancers. However, undesired toxicity of DOX limits its uses. To address the issue of minimizing toxicity of DOX by making it targeted towards cancer cells, DOX was entrapped in self-assembled 6-O-(3-hexadecyloxy-2-hydroxypropyl)-hyaluronic acid (HDHA) nanoparticles. We hypothesized that by encapsulating the drug in biodegradable nanoparticles, its therapeutic efficacy would improve, if targeted against cancer cells. We synthesized cell receptor targeted, DOX loaded HDHA nanoparticles (NPs) and non-targeted DOX loaded O-hexadecylated dextran (HDD) nanoparticles (NPs) and characterized them for their entrapment efficiency, percent yield, drug load, surface morphology, particle size and *in vitro* drug release. The anticancer efficacy of DOX loaded HDHA-NPs was evaluated by measuring the changes in tumor volumes, tumor weights, and mean survival rate of Swiss albino mice grafted with Ehrlich's ascites carcinoma (EAC) cells. For this, the animals were given HDHA-DOX-NPs (1.5 mg/kg b.wt.) intravenously and a green tea polyphenol, Epigallocatechin-3-gallate (EGCG) (20 mg/kg b.wt.), orally through gavage. The targeted NP dose with EGCG significantly increased mean survival time of the animals and enhanced the therapeutic efficacy of the drug compared to the non-targeted NPs and free DOX. Further, we showed that these NPs (HDD and HDHA) were more active in the presence of EGCG than DOX alone in inducing apoptosis in EAC cells as evident by an increase in sub-G1 cells (percent), Annexin V positive cells and chromatin condensation along with the reduction in mitochondrial membrane potential (MMP). The study demonstrates that DOX loaded HDHA-NPs along with EGCG significantly inhibit the growth of EAC cells with ~ 38-fold dose advantage compared to DOX alone and thus opens a new dimension in cancer chemotherapy.
Ray et al.; Biomaterials; 2013; 34; 3064-3076

Synthesis of PLGA nanoparticles of tea polyphenols and their strong *in vivo* protective effect against chemically induced DNA damage

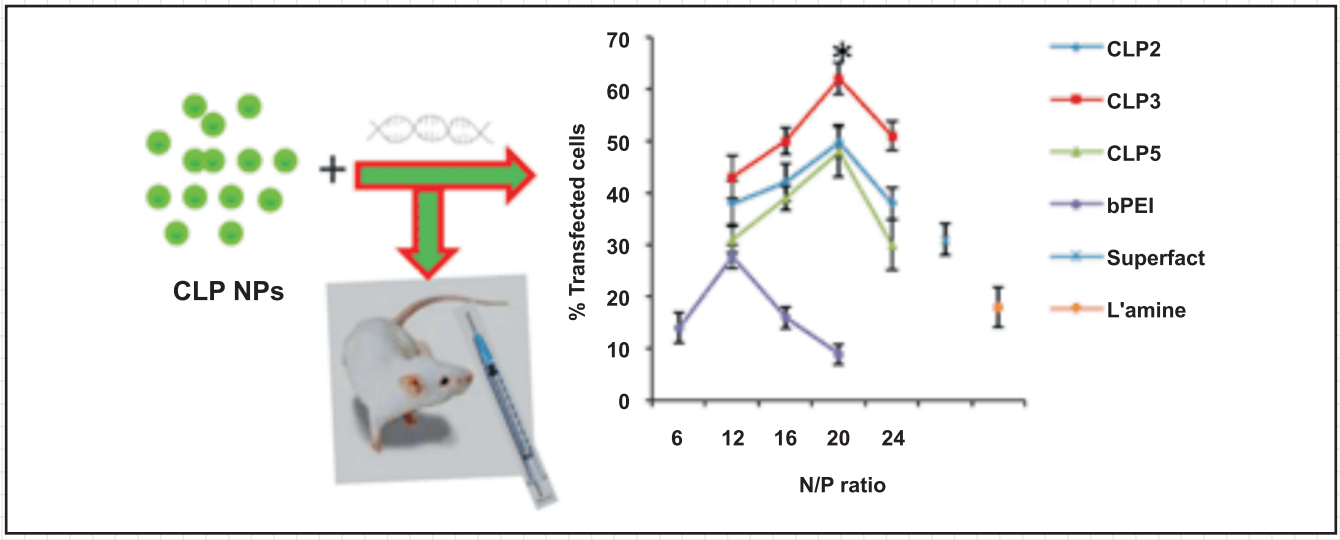
In spite of proficient results of several phytochemicals in preclinical settings, the conversion rate from bench to bedside is not very encouraging. Many reasons are attributed to this limited success, including inefficient systemic delivery and bioavailability under *in vivo* conditions. To achieve improved efficacy, polyphenolic constituents of black (theaflavin [TF]) and green (epigallocatechin-3-gallate [EGCG]) tea in poly(lactide-co-glycolide) nanoparticles (PLGA-NPs) were entrapped with entrapment efficacy of ~18% and 26%, respectively. Further, their preventive potential against 7,12-dimethylbenzanthracene (DMBA)-induced DNA damage in mouse skin using DNA alkaline unwinding assay was evaluated. Pre-treatment (topically) of mouse skin with either TF or EGCG (100 µg/mouse) doses exhibits protection of 45.34% and 28.32%, respectively, against DMBA-induced DNA damage. However, pretreatment with TF-loaded PLGA-

Cell receptor targeted antitumor activity of nanoparticles against Ehrlich's ascites tumors

NPs protects against DNA damage 64.41% by 1/20th dose of bulk, 71.79% by 1/10th dose of bulk, and 72.46% by 1/5th dose of bulk. Similarly, 51.28% (1/20th of bulk), 57.63% (1/10th of bulk), and 63.14% (1/5th of bulk) prevention was noted using EGCG-loaded PLGA-NP doses. These results showed that tea polyphenol-loaded PLGA-NPs have ~30-fold dose-advantage than bulk TF or EGCG doses. Additionally, TF- or EGCG-loaded PLGA-NPs showed significant potential for induction of DNA repair genes (XRCC1, XRCC3, and ERCC3) and suppression of DNA damage responsive genes (p53, p21, MDM2, GADD45α, and COX-2) as compared with respective bulk TF or EGCG doses. Taken together, TF- or EGCG-loaded PLGA-NPs showed a superior ability to prevent DMBA-induced DNA damage at much lower concentrations, thus opening a new dimension in chemoprevention research.
Srivastava et al.; International J Nanomedicine; 2013; 8; 1451-1462

Hydrophobic and membrane permeable polyethylenimine nanoparticles efficiently deliver nucleic acids *in vitro* and *in vivo*

Conjugation through primary amines is one of the most commonly used methods to modify cationic vectors for efficient gene delivery. Here, dimethyl suberimidate, a commercially available homobifunctional reagent bearing imidoesters at the termini has been used to crosslink branched polyethylenimine (bPEI) into its nanoparticles (crosslinked PEI nanoparticles, CLP NPs) specifically through primary amines without altering the total charge on the resulting NPs for interaction with biomolecules and cell membranes. By varying the degree of crosslinking, a small series of CLP NPs was prepared and evaluated for their capability to deliver nucleic acids *in vitro* and *in vivo*. Physico-chemical characterization revealed the size of the NPs in the range of 152 to 210 nm with zeta potential +35 to +38 mV. The plasmid DNA binding ability of these nanoparticles was examined by mobility shift assay, where the pDNA migration was found to be completely retarded by these NPs at an N/P ratio of 4 (cf. bPEI at N/P 3). In various mammalian cells, CLP/pDNA nanoplexes were not only found to be non-toxic but also exhibited significantly enhanced gene expression with one of the formulations, the CLP3/pDNA nanoplex, displaying the highest transfection efficiency, outperforming native bPEI and the selected commercial transfection reagents both in the presence and absence of serum. Further, the versatility of the vector, CLP3, was demonstrated by sequential delivery of GFP-specific siRNA to HEK293 cells, which resulted in 79% suppression of the target gene. Intracellular localization studies showed a significant population of the dual labeled nanoplex (CLP3/pDNA) in the nucleus in just 60 min of incubation. Luciferase reporter gene analysis in Balb/c mice post-intravenous administration of the CLP3/pDNA nanoplex showed the highest gene expression in their spleen. The study suggests that CLP NPs could be used as efficient gene delivery vectors for future gene therapy applications.
Tripathi et al.; Journal of Material Chemistry B; 2013; 1; 2515-2524



Efficient nucleic acids delivery by hydrophobic and membrane permeable polyethylenimine nanoparticles *in vitro* and *in vivo*

Functionalized graphene oxide mediated nucleic acid delivery

A simple preparation of linear polyethylenimine-grafted graphene oxide (LP-GO) conjugates and its efficacy to transfer nucleic acids into the mammalian cells was reported. Graphene oxide (GO), with epoxy functions on its surface, was treated with different amounts of linear polyethylenimine (lPEI), a non-toxic polymer, to obtain three different positively charged LP-GO conjugates (LP-GO-1 to LP-GO-3), capable of interacting with negatively charged nucleic acids (gel retardation assay) and transporting them efficiently into the cells. The results show that these conjugates not only exhibited considerably higher transfection efficiency but also possessed even better cell viability than lPEI. LP-GO-2, the best system in terms of transfection efficiency, showed improved buffering capacity compared to lPEI and provided sufficient stability to bound DNA against DNase I. Further, LP-GO-2 was used for the sequential delivery of GFP specific siRNA, which resulted in ~ 70% suppression of the target gene expression. Intracellular trafficking using fluorescence microscopy revealed that LP-GO-2 conjugate delivered pDNA in the nucleus within 1 h of exposure. The results indicate the prospect of using these conjugates as efficient carriers of nucleic acids for future gene therapy applications.
Tripathi et al.; Carbon; 2013; 51; 224-235.

Tea phenols in bulk and nanoparticle form modify DNA damage in human lymphocytes from colon cancer patients and healthy individuals treated *in vitro* with platinum based-chemotherapeutic drugs

Tea catechin epigallocatechin-3-gallate (EGCG) and other polyphenols, such as the a flavins (TFs), are increasingly proving useful as chemopreventives in a number of human cancers. They can also affect normal cells. The polyphenols in tea are known to have antioxidant properties that can quench free radical species, and pro-oxidant activities that appear to be responsible for the induction of apoptosis in tumor cells. The bioavailability of these natural compounds is an important factor that determines their efficacy. Nanoparticle (NP)-mediated delivery techniques of EGCG and TFs have been found to improve their bioavailability to a level that could enhanced their effectiveness as chemopreventives. The present study was conducted to compare the effects of TFs and EGCG, when used in the bulk form and in the polymer (poly[lactic-co-glycolic acid])-based NP form, in oxaliplatin- and satraplatin-treated lymphocytes as surrogate cells from colorectal cancer patients and healthy volunteers. NPs were examined for their size distribution, surface morphology, entrapment efficiency and release profile. Lymphocytes were treated in the Comet assay with oxaliplatin and satraplatin, washed and treated with bulk or NP forms of tea phenols, washed and then treated with hydrogen peroxide to determine single-strand breaks after crosslinking. The results of DNA damage measurements by the Comet assay revealed opposite trends in bulk and NP forms of TFs, as well as EGCG. Both the compounds in the bulk form produced statistically significant concentration-dependent reductions in DNA damage in oxaliplatin- or satraplatin-treated lymphocytes. In contrast, when used in the NP form both TFs and EGCG, although initially causing a reduction, produced a concentration-dependent statistically significant increase in DNA damage in the lymphocytes. These observations support the notion that TFs and EGCG act as both antioxidants and pro-oxidants, depending on the form in which they are administered under the conditions of investigation.
Alotaibi et al.; Nanomedicine (Lond); 2013; 8; 389-401

Novel polyethylenimine-derived nanoparticles for *in vivo* gene delivery

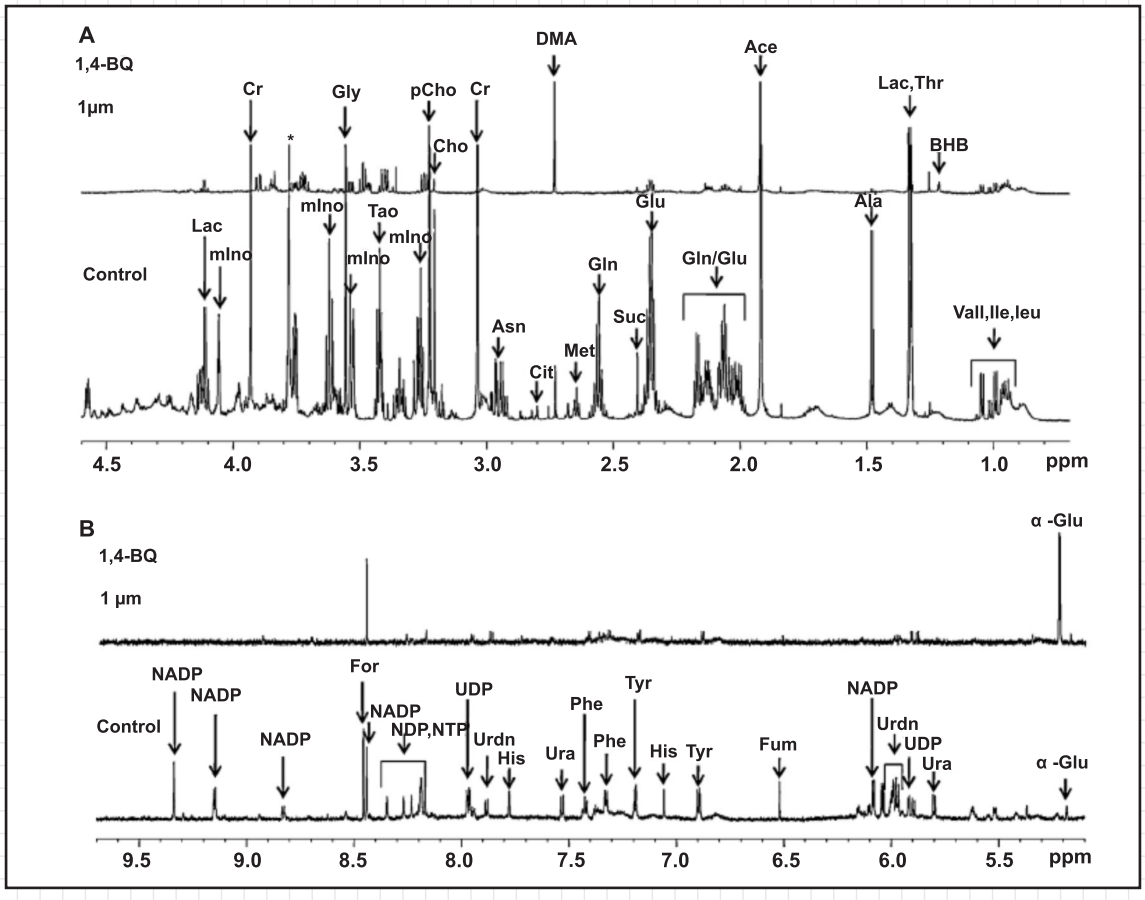
Branched and linear polyethylenimines (PEIs) are cationic polymers that have been used to deliver nucleic acids both *in vitro* and *in vivo*. Owing to the high cationic charge, the branched polymers exhibit high transfection efficiency, and particularly PEI of molecular weight 25 kDa is considered as a gold standard in gene delivery. These polymers have been extensively studied and modified with different ligands so as to achieve the targeted delivery. The application of PEI *in vivo* promises to take the polymer-based vector to the next level wherein it can undergo clinical trials and subsequently could be used for delivery of therapeutics in humans. This review focuses on the various recent developments that have been made in the field of PEI-based delivery vectors for delivery of therapeutics *in vivo*. The efficacy of PEI-based delivery vectors *in vivo* is significantly high and animal studies demonstrate that such systems have a potential in humans. However, we feel that though PEI is a promising vector, further studies involving PEI in animal models are needed so as to get a detailed toxicity profile of these vectors. Also, it is imperative that the vector reaches the specific organ causing little or no undesirable effects to other organs.
Patnaik et al.; Expert Opinion Drug Delivery; 2013; 10; 215-228

NMR-based metabonomics study of sub-acute hepatotoxicity induced by silica nanoparticles in rats after intranasal exposure

Silica nanoparticles (SiO_2 NPs) are widely used commercially; and their potential toxicity on human health has attracted particular attention. In the present study, the intranasal toxicological effect of 10nm and 80nm SiO_2 NPs (dosed at 150µg for 90 days) on rats was investigated using conventional approaches and metabonomics analysis of serum. Oxidative stress was measured by assessing Lipid peroxide (LPO) levels and enzymatic activities of Superoxide dismutase (SOD), Catalase (CAT), and Glutathione (GSH) levels in liver tissue homogenate. These biochemical observations were supplemented by histological examination of liver sections. SiO_2 NPs enhanced lipid peroxidation with concomitant reduction in SOD, CAT, and GSH content. In addition, SiO_2 NPs also produced alterations in hepatic histopathology. We also evaluated the effect of SiO_2 NPs on the activities of hepatic enzymes such as aminotransferases (ALT/AST) and alkaline phosphatase (ALP) which revealed significant increase in their activity when compared with control. Metabonomic profile of 90 days SiO_2 NPs treated rat sera exhibited significant increase in lactate, alanine, acetate, creatine and choline coupled with a considerable decrease in glucose level. These perturbations, on the whole, implicate impairment in tricarboxylic acid cycle and liver metabolism, which suggests that silica nanoparticles may have a potential to induce hepatotoxicity in rats.
Parveen et al., Cellular and Molecular Biology; 2012; 58; 196-203.

Polycyclic aromatic hydrocarbons and their quinones modulate the metabolic profile and induce DNA damage in human alveolar and bronchiolar cells

The release of particulate pollutants into the air through burning of coal, crude oil, diesel, coal tar, etc.



Polycyclic aromatic hydrocarbon and their quinones modulate the metabolic profile and induce DNA damage in human alveolar and bronchiolar cells

raises concerns of potential health hazards to the exposed human population. Polycyclic aromatic hydrocarbons (PAHs) are major toxic constituents of particulate matter (PM), which upon ingestion get metabolized to even more toxic metabolites such as quinones. The PAHs levels were assessed in both respirable particulate matter (RSPM, <10 µM size) and suspended particulate matter (SPM, >10 µM size) of urban ambient air (UAA) and that of major contributors viz. diesel exhaust particles (DEPs) and coal tar combustions emissions (CTCE). Seven US Environmental Protection Agency (USEPA) prioritized PAHs in RSPM and 10 in SPM were detected in UAA. Ten and 15 prioritized PAHs, respectively, were also detected in diesel exhaust particles (DEP) and coal tar combustion emission (CTCE) evidencing their release in the air. These PM associated PAHs for UAA, DEP and CTCE showed significant increase ($p < 0.05$) in mutagenicity and mammalian genotoxicity in the order CTCE > DEP > UAA. Human lung alveolar (A549) and bronchiolar (BEAS-2B) cells when treated with PAH-metabolites viz. 1,4-benzoquinone (1,4-BQ), hydroquinone (HQ), 1,2-naphthoquinone (1,2-NQ), 1,4-naphthoquinone (1,4-NQ) and 9,10-phenanthroquinone (9,10-PQ) showed metabolic modulation in these cell lines with significant depletion of principal cellular metabolites viz. NADP, uracil, asparagines, glutamine, and histidine and accumulation of di-methyl amine and beta-hydroxybutyrate, identified using ^{1}H NMR spectroscopy. These results suggest that PAH-quinones induce genotoxic effects by modulating the metabolic machinery inside the cells by a combined effect of oxidative stress and energy depletion. Our data for metabolic profiling of human lung cells could also help in understanding the mechanism of toxicity of other xenobiotics.

Gurbani et al.; International Journal of Hygiene and Environmental Health; 2013; 216; 553-565

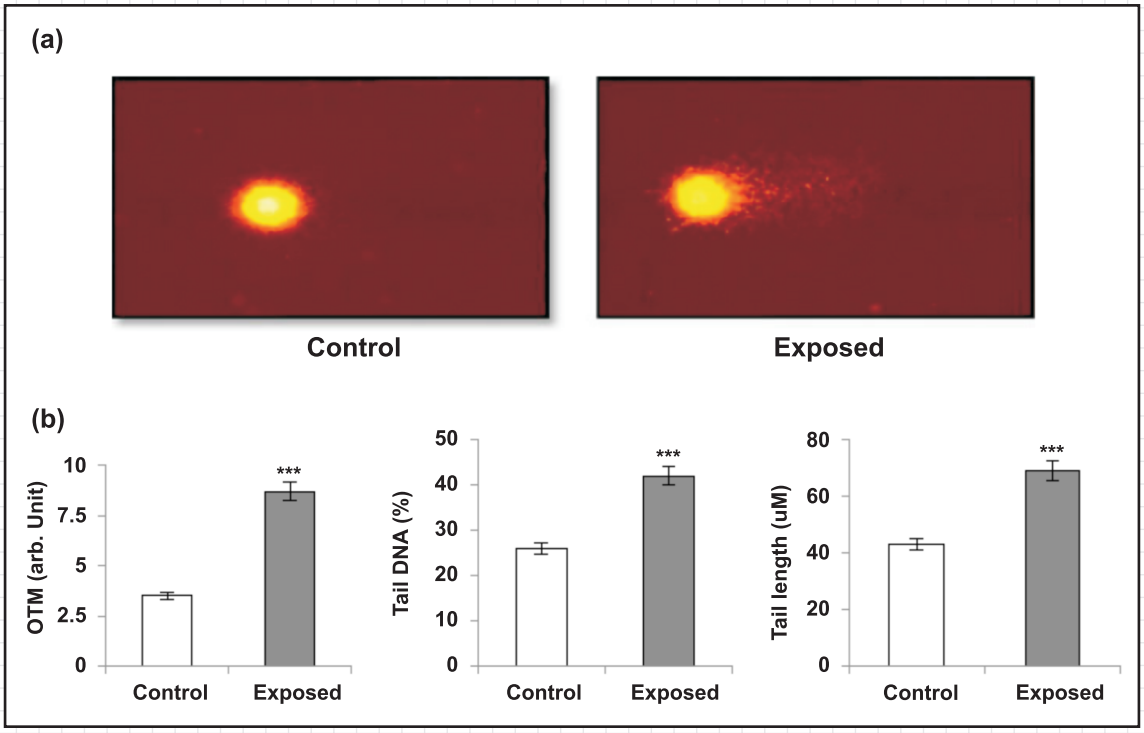
Comparative study on effects of two different types of titanium dioxide nanoparticles on human neuronal cells

Titanium dioxide (TiO_2) is among most frequently used nanoparticles (NPs). It is the present in a variety of consumer products, including food industry in which they are employed as an additive. The potential toxic effects of these NPs on mammal cells have been extensively studied. However, studies regarding neurotoxicity and specific effects on neuronal systems are very scarce and, to our knowledge, no studies on human neuronal cells have been reported so far. Therefore, the main objective of this work was to investigate the effects of two types of TiO_2 NPs, with different crystalline structure, on human SHSY5Y neuronal cells. After NPs characterization, a battery of assays was performed to evaluate the viability, cytotoxicity, genotoxicity and oxidative damage in TiO_2 NP-exposed SHSY5Y cells. Results obtained showed that the behaviour of both types of NPs were quite comparable. They did not reduce the viability of neuronal cells but were effectively internalized by the cells and induced dose-dependent cell cycle alterations, apoptosis by intrinsic pathway, and genotoxicity not related with double strand break production. Furthermore, all these effects were not associated with oxidative damage production and, consequently, further investigations on the specific mechanisms underlying the effects observed in this study are required.

Valdiglesias et al.; Food & Chemical Toxicology; 2013; 57; 352-361

2.45 GHz microwave irradiation-induced oxidative stress affects implantation or pregnancy in mice, *Mus musculus*

The present experiment was designed to study the 2.45 GHz low-level microwave (MW) irradiation-induced stress response and its effect on implantation or pregnancy in female mice. Twelve-week-old mice were exposed to MW radiation (continuous wave for 2 h/day for 45 days, frequency 2.45 GHz, power density = 0.033549 mW/cm2, and specific absorption rate = 0.023023 W/kg). At the end of a total of 45 days of exposure, mice were sacrificed, implantation sites were monitored, blood was processed to study stress parameters (hemoglobin, RBC and WBC count, and neutrophil/lymphocyte (N/L) ratio), the brain was processed for comet assay, and plasma was used for nitric oxide (NO), progesterone and estradiol estimation. Reactive oxygen species (ROS) and the activities of ROS-scavenging enzymes— superoxide dismutase, catalase, and glutathione peroxidase—were determined in the liver, kidney and ovary. We observed that implantation sites were affected significantly in MW-irradiated mice as compared to control. Further, in addition to a significant increase in ROS, hemoglobin ($p < 0.001$), RBC and WBC counts ($p < 0.001$), N/L ratio ($p < 0.01$), DNA damage ($p < 0.001$) in brain cells, and plasma estradiol concentration ($p < 0.05$), a significant decrease was observed in NO level ($p < 0.05$) and antioxidant enzyme activities of MW-exposed mice. Our findings led us to conclude that a low level of MW irradiation-induced oxidative stress not only suppresses implantation, but it may also lead to deformity of the embryo in case pregnancy continues. We also suggest



2.45 GHz Microwave Irradiation-Induced Oxidative Stress Affects Implantation or Pregnancy in Mice, *Mus musculus*

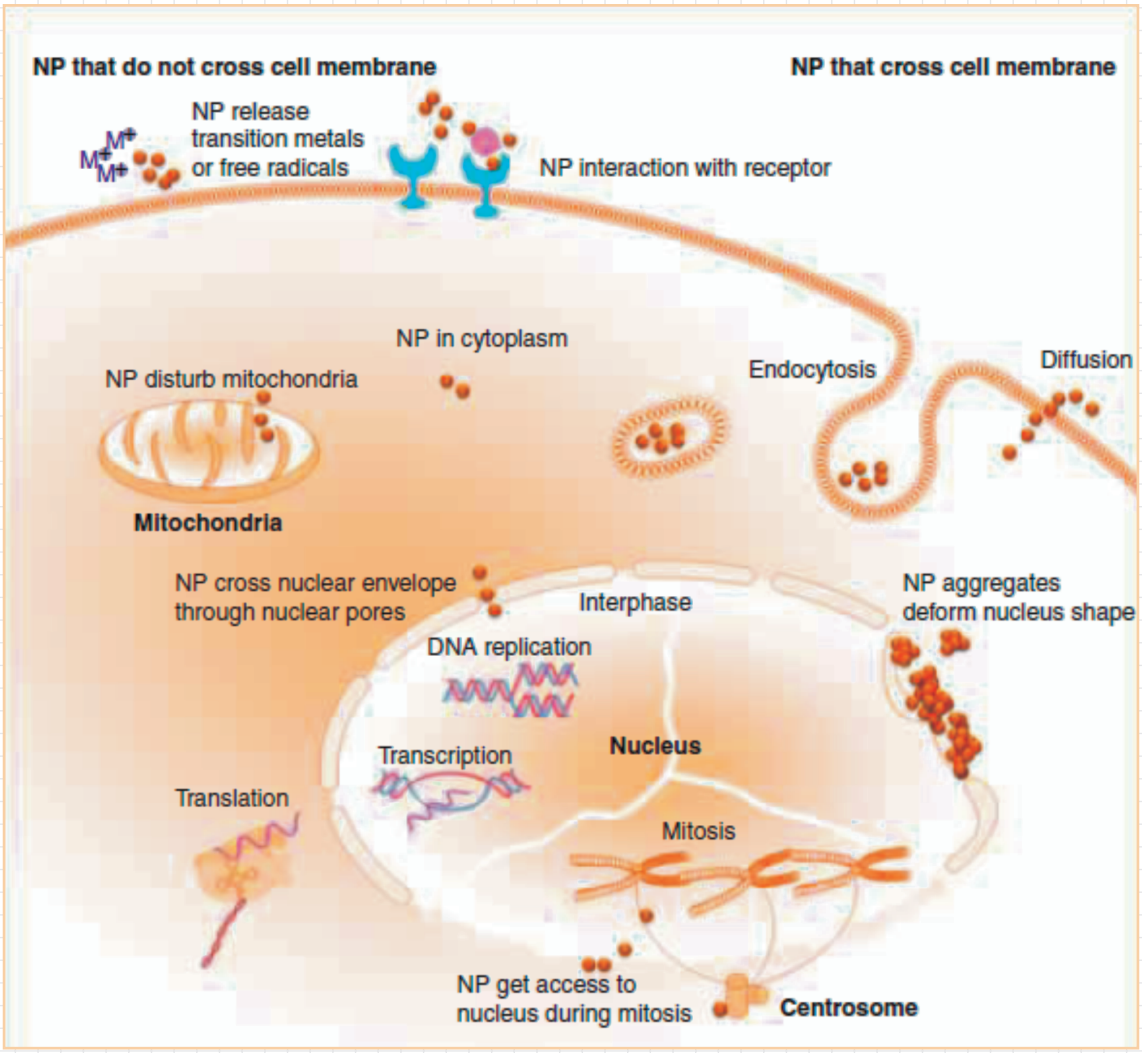
that MW radiation-induced oxidative stress by increasing ROS production in the body may lead to DNA strand breakage in the brain cells and implantation failure/resorption or abnormal pregnancy in mice.
Shahin et al.; Applied Biochemistry and Biotechnology; 2013; 169; 1727-1751

Methods for detection of oxidative stress and genotoxicity of engineered nanoparticles

The distinctive characteristics of engineered nanoparticles (ENPs) such as higher surface-to-volume ratio find immense applications in personal care products, food packaging, drug delivery systems, therapeutics & biosensors and others. The exponential increase in the ENP containing consumer products in the last 5 years has also increased their inadvertent release in the environment and a debate towards their adverse effects to the human and environment health. A variety of ENPs with different size, shape, and surface properties have been shown to induce genotoxicity, cytotoxicity, and oxidative stress in different cellular models. Here we describe the techniques and protocols used in the assessment of the genotoxicity (single-cell gel electrophoresis (comet) assay, cytokinesis block micronucleus assay) and oxidative stress parameters (reactive oxygen species, lipid peroxidation, and glutathione depletion) induced by the ENPs in the cells.
Kumar et al., Methods in Molecular Biology, 2013; 1028; 231-246

A review of *in vitro* and *in vivo* studies with engineered nanoparticles

Engineered nanoparticles (NPs) are widely used in different technologies but their unique properties might also cause adverse health effects. In reviewing recent *in vitro* and *in vivo* genotoxicity studies we discuss potential mechanisms of genotoxicity induced by NPs. Various factors that may influence genotoxic response, including physico-chemical properties and experimental conditions, are highlighted. From 4346 articles on NP toxicity, 112 describe genotoxicity studies (94 *in vitro*, 22 *in vivo*). The most used assays are the comet assay (58 *in vitro*, 9 *in vivo*), the micronucleus assay (31 *in vitro*, 14 *in vivo*), the chromosome aberrations test (10 *in vitro*, 1 *in vivo*) and the bacterial reverse mutation assay (13 studies). We describe advantages and potential problems with different methods and suggest the need for appropriate methodologies to be used for investigation of genotoxic effects of NPs, *in vitro* and *in vivo*.
Magdolenova et al.; Nanotoxicology. 2013 Mar 20. Epub ahead of print doi:10.3109/17435390.2013.773464



Mechanism of Genotoxicity. A review of *in vitro* and *in vivo* studies with engineered Nanoparticles

Presence of strong association of the major histocompatibility complex (MHC) class I allele HLA-A*26:01 with idiopathic hypoparathyroidism

The pathogenesis of isolated hypoparathyroidism, also referred to as idiopathic hypoparathyroidism (IH), is not clear. There is a paucity of information related to the immunogenetic basis of the disease due to its rarity. A recurrent theme of several autoimmune disorders is aberrant antigen presentation. We investigated the association of alleles of the human leukocyte antigen (HLA) class I and II loci with IH. A total of 134 patients with IH and 902 healthy controls from the same ethnic background participated in the study. There was a significant increase of HLA class I alleles HLA-A*26:01 [$P < 1.71 \times 10^{-34}$; odds ratio (OR) = 9.29; 95% confidence interval (CI) = 6.08–14.16] and HLA-B*08:01 ($P < 8.19 \times 10^{-6}$; OR = 2.59; 95% CI = 1.63–4.04) in patients with IH compared to healthy controls. However, the association of A*26:01 was primary because B*08:01 was in linkage disequilibrium with A*26:01. Although the major histocompatibility complex (MHC) is very polymorphic, several alleles of HLA loci share key residues at anchor positions in the peptide binding pockets such that similar peptides may be presented by different MHC molecules encoded by the same locus. These allelic forms with similar anchoring amino acids have been clustered in super types. An analysis of HLA-A locus supertypes A01, A02, A03, and A04 revealed that supertype A01 was significantly increased ($P < 9.18 \times 10^{-9}$; OR = 2.95) in IH compared to controls. However, this increase in the supertype A01 was contributed by

A*26:01 because 68.7% of the A01 samples had A*26:01. Other alleles of the supertype did not show any significant differences. The strong association of HLA-A*26:01 suggests an important role of MHC class I-mediated presentation of autoantigenic peptides to CD8+ cytotoxic T cells in the pathogenesis of IH. This data provided evidence for the autoimmune etiology of IH akin to other autoimmune disorders like type 1 diabetes and rheumatoid arthritis.

Goswami et al.; Journal of Clinical Endocrinology and Metabolism; 2012; 97; E1820-1824

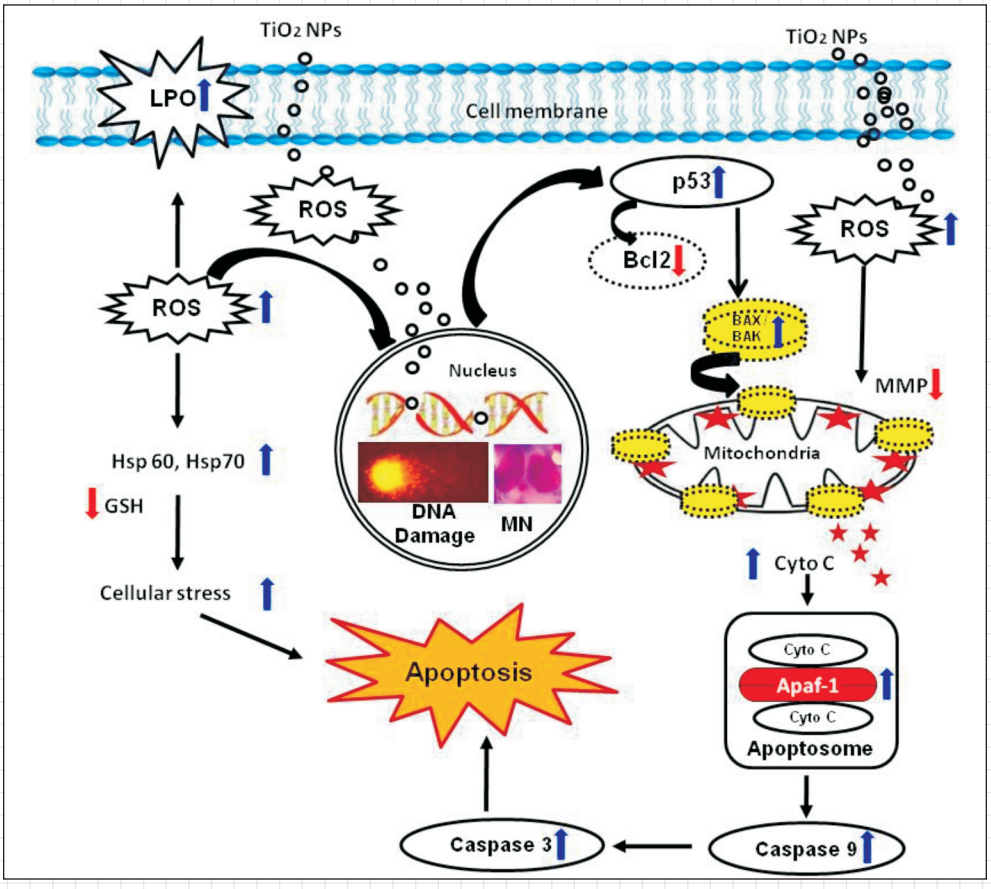
TiO_2 nanoparticles induce oxidative DNA damage and apoptosis in human liver cells

Titanium dioxide nanoparticles (TiO_2 NPs), widely used in consumer products, paints, pharmaceutical preparations and so on, have been shown to induce cytotoxicity, genotoxicity and carcinogenic responses *in vitro* and *in vivo*. The present study revealed that TiO_2 NPs induce significant ($p < 0.05$) oxidative DNA damage by the Fpg-Comet assay even at 1 µg/ml concentration. A corresponding increase in the micronucleus frequency was also observed. This could be attributed to the reduced glutathione levels with concomitant increase in lipid peroxidation and reactive oxygen species generation. Furthermore, immunoblot analysis revealed an increased expression of p53, Bax, Cyto-c, Apaf-1, caspase-9 and caspase-3 and decreased the level of Bcl-2 thereby indicating that apoptosis induced by TiO_2 NPs occurs via the caspase-dependent pathway. This study systematically shows that TiO_2 NPs induce DNA damage and cause apoptosis in HepG2 cells even at very low concentrations. Hence the use of such nanoparticles should be carefully monitored.

Shukla et al.; Nanotoxicology; 2013; 7; 48-60

Lipophilic and cationic triphenylphosphonium grafted linear polyethylenimine polymers for efficient gene delivery to mammalian cells

Synthetic chemical vectors have recently provided a versatile and robust platform for the safe and efficient delivery of exogenous genes. Here, for the first



TiO_2 Nanoparticles induce oxidative DNA Damage and Apoptosis in Human Liver Cells

time, a small series of N-butyltriphenylphosphonium bromide-grafted-linear polyethylenimine (BTP-g-lP) polymers (N–P hybrid polymers) have been evaluated for their ability to deliver genes into mammalian cell lines, viz., MCF-7 and A549 cells. Biophysical characterization revealed that the projected polymers efficiently interacted with plasmid DNA, and the resulting complexes displayed hydrodynamic diameters in the range of 249–307 nm with relatively higher zeta potential values of +31 to +34 mV (cf. lPEI, +26 mV). The tethering of lipophilic and cationic triphenylphosphonium moieties to linear PEI (lPEI) addressed several limitations associated with lPEI, such as solubility, the stability of the pDNA complexes and the timely release of pDNA for nuclear localization as assessed by protection and release assays. Also, the lipophilic interactions between cellular membranes and the pDNA complexes mediated the efficient cellular uptake and internalization of the pDNA complexes, resulting in significantly higher transfection efficiency in these cell lines, outperforming the GenePORTER 2™, Lipofectamine™ and Superfect™ used in the study for comparison purposes. Confocal studies using dual-labeled TMR-BTP-g-lP3/YOYO-1-pDNA complex in MCF-7 cells confirmed that the complex behaved more or less like native lPEI, as the substitution of the phosphonium moiety was too small to affect the intracellular trafficking. Furthermore, the versatility of the BTP-g-lP3 vector was established by GFP specific siRNA delivery, which resulted in [similar]79% suppression of targeted gene expression (cf. Lipofectamine™, [similar]55%). Altogether, the study demonstrates the potential of these hybrid polymers for the efficient delivery of nucleic acids for future gene therapy applications.

Bansal et al.; Journal of Materials Chemistry; 2012; 22; 25427-25436