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Table of content

List of Terms and Abbreviations

AMs, alveolar macrophages
BAL, bronchoalveolar lavage
CL, cardiolipin
CNF, carbon nano-fibers
CNM, carbonaceous nanomaterials
CNT, carbon nanotubes
Cyt c, cytochrome c
DOX, doxorubicin
EPO, eosinophil peroxidase
ESI-MS, electrospray ionization mass spectrometry
GNPs, gold nanoparticles
GO, graphene oxide
HOCl, hypochlorous acid
iNOS, inducible nitric oxide synthase
LC/ESI-MS, Mass Spectrometry Liquid Chromatography/Electrospray Ionization
LDH, lactate dehydrogenase
MALDI, Mass Spectrometry Matrix-assisted laser desorption/ionization mass spectrometry
MDSC, myeloid-derived suppressor cells
MPO, myeloperoxidase
MWCNT, multi-walled carbon nanotubes
PMNs, polymorphonuclear granulocytes
PL-OOH, phospholipid hydroperoxides
PLA, poly(lactic acid)
PLGA, poly(lactic-co-glycolic acid),
PS, phosphatidylserine
ROS, reactive oxygen species
RNS, reactive nitrogen species
SWCNT, single-walled carbon nanotubes

Abstract

Biopersistence of single- and multiwalled carbon nanotubes, graphene oxide (GO) and several other types of carbonaceous nanomaterials is an essential determinant of their health effects. In 2008, we have discovered (Nano Letters 8 (11), 3899-3903, 2008) that carbonaceous nanomaterials can be degraded by oxidative enzymes, such as peroxidases. Our extensive studies during the subsequent period – funded by this grant - have demonstrated that successful biodegradation is one of the major factors defining the life span and biological responses to nanoparticles. Our work in this area has been cited about 2,000 times and laid the foundation for a new field of nanotoxicology. Our detailed studies have revealed the role and contribution of different oxidative enzymes of inflammatory cells - myeloperoxidase, eosinophil peroxidase, lactoperoxidase, hemoglobin, and xanthine oxidase - to the reactions of nanoparticle biodegradation. We obtained essential results characterizing interactions of nanomaterials with hemoproteins dependent on the specific features of their physico-chemical and structural characteristics. Mechanistically, we deciphered the significance of immobilized peroxidase reactive intermediates vs diffusible small molecule oxidants (hypochlorous and hypobromous acids) for the overall oxidative biodegradation process in neutrophils and eosinophils. We also deciphered the peroxynitrite-driven pathways realized in macrophages via the engagement of NADPH oxidase- and NO synthase-triggered oxidative mechanisms. We established the importance of genetic factors and their manipulations for the enzymatic biodegradation in vivo. We documented possible involvement of oxidative machinery of other professional phagocytes such as microglial cells, myeloid-derived suppressor cells, in the context of biodegradation relevant to targeted drug delivery. Finally, we described a novel type of biodegradation realized via the activation of the "dormant" peroxidase activity of hemoproteins by nano-surfaces. Overall, our studies pave the way to developing new strategies for creating safe by design carbonaceous nanoparticles with optimized characteristics regulatable life-span in circulation.

Section 1 of the Final Progress report

Significant or Key Finding

We deciphered on the molecular level the machanisms and pathways through which the major oxidative enzymes of inflammatory cells interact and catalyze the process of biodegradation of carbonaceous nanomaterials. In addfition to detailed studies of myeloperoxidase in neutrophiles and eosinophil peroxidase in eosinophils, these discovery studies also included decoding of the mechanisms of iNOS/NADPH oxidase driven peroxynitrite biodegradation pathway realized in macrophages.

We discovered that the surface of nanomaterials may activate “dormant” peroxidase activities of hemoproteins normally not involved in the biodegradation process such as cytochrome c of intermembrane space of mitochondria.

In terms of the diversity of nanomaterials employed we explored in a comparative way and assessed pulmonary inflammation, fibrosis, oxidative stress markers and systemic immune responses to a braod range of materials, including respirable single-walled CNT (SWCNT), carbon nano-fibers (CNF), graphene/graphene oxide in comparison to and asbestos.

Our mechanistic studies in model biochemical systems and cells were extended to include in vivo studies on animals, including the animals with genetically manupilated propensities (eg, myeloperoxidase KO mice, NADPH oxidase KO mice) with regards to the major enzymatic mechanisms of oxidative biodegradation.

Given the extraordinary important role of oxidative stress-driven mechanisms as the environment in which biodegradation of nanopartciels takes place we pioneered the studies of redox phospholipidomics and presented the first description of the major phospholipidomics oxidized biomarkers generated by the inhalation exposure of animals to nanopartciles.

We established that nanoparticles realized their toxic effects and cause apoptotic cell death associated with selective oxidation of a mitochondria-specific phospholipid, cardiolipin.

Considering that bio-corona formed during the life cycle of nanoparticles in biological fluids and tissues modulates their biopersistence and interfacing with the organism we performed – for the first time, global lipidomics characterization of the bio-corona.

We established that coating nanopartciles with a specialized phospholipid, phosphatidylserine, acting as an “eat-me” signal for professional phagocytes navigates this modified nanopartciles to these cells thus providing for targeted interactions of nanopartciles with macrophages, dendritic cells and microglial cells.

Assuming that nanoparticles may affect immuno-modulatory effects on a number of disease conditions, including cancer, we innitiated studies of the effects of nanomaterials on tumor growth in the lung and establishe the fundamental mechanisms through which they can stimulate tumor growth.

Given the important role of new methods development in experimental research we developed several unique protocols to characterize interactions of nanoparticles with the major components of cells and tissues, particularly in the fields of lipidomics and redox lipidomics as well as imaging of individual lipids by mass-spectrometry and imaging nanopartcles and quantitative assessments of their contents and biodegradation/ persistence characteristics in the lung.

A detailed understanding of the molecular interactions between nanoparticles and biological nano-machinery - macromolecules, membranes, and intracellular organelles - is crucial for obtaining

adequate information on mechanisms of action of nanomaterials as well as a perspective on the long term effects of these materials and their possible toxicological outcomes. We developed, performed and analyzed the data on structure-based computational molecular modeling as a tool to understand and to predict the interactions between nanomaterials and nano-biosystems, particularly as they relate to oxidative enzymes involved in the biodegradation process .

Based on our analysis of the fundamental parameters underlying CNT degradation in vitro and in vivo, we developed new types of safe-by-design carbon-based nano-containers to target organs/cells, deliver their cargo, and biodegrade via peroxidase-driven mechanisms. These new types of carbonaceous nanomaterials represent an attractive therapeutic delivery option in nanomedicine.

Translation of Findings

Carbonaceous nanomaterials (CNM) are emerging as high-value nanoparticles that are abundant, renewable, and sustainable. In the recent years, CNMs have been studied for a wide variety of potential applications, including reinforcement phases in polymer composites, protective coatings, barrier/filter membrane systems, antimicrobial films, network structures for tissue engineering, and substrates for flexible electronics. Explosively growing applications of CNM in various industries imply increasing exposure levels in occupational and environmental settings. Considering that lung is the major portal of unintended nanomaterial entry into the human body, a detailed understanding of pulmonary toxicity of CNC materials is essential to address safety and health issues concerning CNC exposures. Moreover, studies of bio-persistence and biodegradation of CNM become particularly important. Such studies are also necessary to target the preventive measures. Due to limited or no data on biodegradation mechanisms in the contents of toxic effects of CNM, it is essential to explore underlying mechanisms of their biodegradation. Our completed studies explored and develop new methodology and characterized pathways and mechanisms of CNM biodegradation in the context of their toxicity, particularly in the lungs. We discovered major pathways through which oxidative anzymatic systems of inflammatory cells biodegrade CNMs thus determining their persistence in the body's tissues and fluids. This information and newly obtained knowledge are essential for risk characterization and assessment, development of toxicogenomic signatures of exposure to CN materials and development of control strategies for reducing exposures. Thus, our studies provide a roadmap for reducing potential hazards associated with exposure to CNMs. Such studies will also help us understand the benefits of emerging nanotechnologies while minimizing potential risks to human health during manufacturing/handling.

Research Outcomes/Impact

Prior to our studies, there has been no understanding of potential roles that oxidative enzymatic mechanisms, particularly those of inflammatory cells, may play in the processes of CNMS biodegradation thus determining the fate and biopersistence, hence toxicity of CNMs. Our work designated the appearance of a new field of nanotoxicology – toxicology of oxidative biodegradation of nanomaterials. This topic has been regularly discussed at essentially every scientific meeting of nanotoxicologists. The number of publications in this area of research has been growing in explosive way. The newly emerged understanding of the roles that inflammatory cells play in biodegradation of nanomaterials gives an entirely new perspective to exposure and risk assessments as they relate to occupational safety and health with regard to improved practices aimed at the reductions of work-related health issues and disease. Overall, our completed studies will have the major impact facilitating improvements in occupational safety and health and guiding the future directions of nanotoxicology research.

Section 2 of the Final Progress Report

Scientific Report

Background for the project

B.1. Nanotoxicology: an Emerging Discipline. Historically, the term nanotoxicology was coined in 2004-2005 although the question as to whether nanotechnology is dangerous was voiced at least some 5 years earlier. While substantial experience has been accumulated through the study of particles of different sizes and sources including ultra-fine particles prior to the introduction of the term *nanotoxicology*, the uniqueness of the physico-chemical properties of nanoparticles suggests that their interactions with cells and tissues may also be unpredictable. Furthermore, because cellular molecular machines and man-made nanoparticles have similar dimensions, the latter may directly interact and interfere with vital cellular processes. However, there may be unique features relevant for instance to electron transfer reactions and oxidative stress induction, again emphasizing the potentially poor predictive power of classical toxicology for biological effects of novel nanomaterials. *Nanotoxicology should be defined as a discipline studying the interference of engineered nanomaterials with the functions of cellular and extracellular nanomachineries.* This definition places emphasis on the specific responses which are directly related to the scaling and dimensions of nanomaterials. In addition to size, other physical and chemical properties of nanomaterials may also induce toxicological outcomes in unanticipated ways. The nanotechnologies are a moving target and possible adverse health effects of emerging materials are to be understood and prevented.

B.2. Cellular Recognition of Nanoparticles. A century ago, I. Mechnikov discovered the physiological process of phagocytosis by which blood cells take up and digest bacteria. In fact, most cells have some phagocytic function; however, higher organisms also have professional phagocytes (monocytes/macrophages and polymorphonuclear granulocytes or PMNs) equipped with dedicated receptors that are able to recognize foreign invaders. In addition to the recognition and clearance of microbes and particles/foreign debris, professional phagocytes (macrophages) also play an important role in the recognition and clearance of apoptotic cell corpses. The degree of recognition and internalization of nanomaterials by professional phagocytes (macrophages) is likely to influence their biodistribution and toxicity. How are nanoparticles taken up by cells? Geiser et al. reported that uptake of fluorescent polystyrene beads (78 nm) *in vitro* by porcine lung macrophages occurs through a passive (non-phagocytic) mechanism. In contrast, uptake of carboxylated polystyrene spheres (50 nm) *in vivo* was suggested to occur through particle opsonization with specific serum proteins resulting in recognition by scavenger receptors on hepatic macrophages. Other investigators have reported that opsonization of nanoparticles with serum proteins may inhibit cellular uptake. These differences may be related to the specific physico-chemical properties of the nanoparticles studied and to the cell-type specific cellular internalization pathways involved. Indeed, it may be relevant to consider the differences in phagocytic potential and repertoire of cell surface receptors between professional phagocytic cells (macrophages) and non-professional cell types including various cancer cells lines that abound in toxicological research laboratories. It is also important to study whether exposure to engineered nanomaterials impairs subsequent macrophage engulfment of other phagocytic prey, including microorganisms and apoptotic cells. Tissue homeostasis could be compromised if nanomaterials were to interfere with the normal clearance (waste-disposal) processes of macrophages.

B.3. Bio-Corona - Opsonization of Nanoparticles. The concept of particle opsonization by serum factors (proteins) was suggested in 1904 by Wright and Douglas. Moreover, Leo Vroman reported in a seminal publication almost 50 years ago that proteins adsorb to surfaces through a series of attachment/displacement steps (this phenomenon is now referred to as the Vroman effect). Recent studies have brought these fundamental considerations to the forefront of nanotoxicological and nanotechnological research. In particular, there is an emerging understanding that the “protein corona” i.e. the specific proteins coating the nanoparticle surface is one of the key factors determining the outcomes of nanomaterial interactions with biological systems. Several recent studies have thus shown

– perhaps not surprisingly – that the uptake as well the toxicity of engineered nanomaterials correlates with protein opsonization. Using a set of tailored copolymer nanoparticles that allowed the systematic investigation of the influence of size and composition (hydrophobicity) of the particles on their interaction with proteins, Cedervall et al. observed a clear dependence of the binding and dissociation parameters on protein identity and on the particle surface characteristics. Subsequent studies revealed a specific pattern of binding to the nanoparticle surface insofar as albumin, the most abundant serum protein, was found to be successively replaced by the higher-affinity and slower-exchanging apolipoproteins AI, AII, AIV, and E – the Vroman effect. In a more recent study, Ehrenberg et al. showed that nanoparticle surface chemistry, as it pertains to the protein adsorbing capacity of particles determines the cellular binding of nanoparticles, and furthermore, that cellular association is not dependent on the specific identity of adsorbed proteins, at least in studies of polystyrene nanoparticles and cultured human umbilical vein endothelial cells. Dobrovolskaia et al. identified 69 different plasma proteins bound to colloidal gold nanoparticles (30 and 50 nm) and studied the effect of protein binding on hydrodynamic size of the particles. Finally, our studies clearly identified lipids as essential components of bio-corona determining interactins of the nanoparticles with other constituents of tissue nd biofluids environments, Overall, one may argue that the boundaries between man-made nanomaterials and biological nanoorganisms (viruses) or even cellular debris (apoptotic bodies) begin to blur, in light of these observations, as the interaction with host cells is likely to depend, in all of these cases, on the nature of the protein and/or lipid or carbohydrate signals that are presented to cells.

B.4. Biodegradation of Nanoparticles. Degradability of the material is an important factor in the assessment of toxicity of nanomaterials. Non-degradable nanomaterials can accumulate in organs and also intracellularly where they can exert detrimental effects to the cell. For instance, long-term accumulation of medicinal gold salts (nanoparticles) in the body may result in adverse or toxic effects in patients. SWCNT are known to be biopersistent and may remain inside macrophages in spleen and liver for prolonged periods of time following parenteral administration; CNT have also been observed in the lungs of exposed mice up to 1 year after pharyngeal administration (Shvedova et al., 2014). On the other hand, biodegradable nanomaterials could also yield unpredictable toxic responses due to toxic degradation products. For instance, leaching of toxic core components such as cadmium from quantum dots with induction of oxidative stress has been suggested as a mechanism of *in vivo* toxicity of these nanomaterials. Controlled biodegradation of nanomaterials thus represents one of the important challenges not only in the field of nanotoxicology but also in nanomedicine, as the safe implementation of nanomaterials for biomedical purposes is contingent on the controlled degradation and/or clearance of the exogenous nanomaterials. Our studies revealed the mechanisms and pathways through which oxidiative enzymatic machinery of inflammatory cells can degrade different types of CNMs*. A novel "green' biochemical pathway for biodegradation of CNT in a physiologically relevant environment is based on the action of MPO – the reactive intermediates of the enzyme itself as well as potent oxidants – like HCl-OO- that are generated in PMNs and other inflammatory cells.*

B.5. Toxicity of nanomaterials. Nanotoxicological studies conducted to date have suggested correlations between different physico-chemical properties and the biological/toxicological outcomes of exposure of cells and tissues to nanomaterials. However, there is as yet no consensus as to which is the most important dose metric in deciding the toxicity of engineered nanomaterials: particle mass, particle number, surface area, surface chemistry, or all of these parameters together. Safety assessment of engineered nanomaterials is a key to the efficient development of a number of novel nano-applications essential not only for pharmaceuticals, biomimetics, tissue engineering but also to promote the well-being of humans during manufacturing and use. While numerous data are available on *in vitro* toxicity of nanomaterials there is an emerging understanding that *in vivo* assessments of nanomaterial toxicity are essential. In the absence of predictive and validated *in vitro* assays, *in vivo* testing of potentially toxic materials appears to be indispensable for human safety.

Evaluation of human exposure to engineered nanomaterials requires knowledge of the likelihood of exposure, changes in particle concentration over time, and identification and characterization of exposure directly prior to uptake. The respiratory system is a unique target encountering nanomaterials

via inhalation. In addition, dermal and gastrointestinal exposure, eye absorption and direct parenteral administration are also potential routes of entry, and may be associated with adverse effects. Significant interest in the respiratory system as a target for both beneficial and adverse effects of engineered nanomaterials is reflected by a growing number of relevant scientific publications during the past decade. Previous epidemiological studies have documented a strong association between so-called ultra-fine air pollution particles including particles in the nano-size range and respiratory and cardiovascular morbidity and mortality. Moreover, several epidemiological observations support the notion that ultrafine- and fine particles cause a higher rate of adverse respiratory outcomes as compared to coarse particles. Some, but not all of these effects, may be related to indirect actions of particles on components of the immune system, for instance through modulation of inflammatory cytokine secretion. Indeed, Dobrovolskaia and McNeil emphasized that engineered nanomaterials can either stimulate or suppress immune responses thus pointing to the fact that one of the fundamental questions in the field of nanotoxicology concerns the mechanisms through which nanoparticles are "sensed" by the immune system.

Due to a multitude of different nanomaterials with differences in physico-chemical properties, nanoparticles cannot be summarized in one homogenous group, but should be studied on a case-by-case basis. In particular, SWCNT and multi-walled carbon nanotubes (MWCNT) represent two important types of engineered nanomaterials with numerous applications and hence with a potential for human exposure. We have reported that exposure of C57BL/6 mice to SWCNT through pharyngeal aspiration causes dose-dependent granulomatous pneumonia, oxidative stress, acute inflammatory/cytokine responses, with fibrosis and decrease in pulmonary function. To avoid potential artifactual effects due to instillation/agglomeration associated with SWCNT, we also conducted inhalation exposures using stable and uniform SWCNT dispersions obtained by a newly developed aerosolization technique. SWCNT inhalation was more effective than aspiration in causing inflammatory responses, oxidative stress, collagen deposition, and fibrosis. BAL fluid cytology indicated a robust accumulation of PMNs and lymphocytes after inhalation exposure to SWCNT. Even 28 days post-inhalation, the numbers of PMNs and lymphocytes in BAL fluid of exposed animals remained significantly elevated compared to the control group. The principal histopathologic alterations in mice inhaling SWCNT were pulmonary inflammation, bronchiolar epithelial cell hypertrophy, and the presence of foreign material in the interstitium, intracellularly within individual macrophages or free of lung tissue and most frequently aggregated near bronchoalveolar junctions, often with juxtaposed alveolar macrophages. Throughout the post-exposure time course, macrophages were the principal inflammatory cells. In addition, anuclear macrophages were seen, suggesting the occurrence of abnormal mitoses or an apoptotic process involving dissolution of the cell nucleus (karyolysis). By 28 days post-exposure, the granulomatous lesions were often well-organized. Overall, our studies demonstrate that SWCNT cause unusual and robust pulmonary inflammation and fibrosis in mice, and suggest that the chain of pathological events is realized through interactions of inflammatory responses and oxidative stress culminating in the development of multifocal granulomatous pneumonia, interstitial fibrosis, and mutagenesis.

B.6. Nanomaterials and Oxidative Stress. Cell death has been universally associated with the initiation and propagation of free radical oxidation reactions and excessive accumulation of their products. However, the question as to whether these effects are causative to cellular destruction or rather stem from the injury triggered by other mechanisms remains, in most cases, unanswered. Excessive oxidative stress has been proposed as a common paradigm for the toxicities of engineered nanoparticles. While widely accepted, not all studies comply with this general notion, thus pointing to the need of careful scrutiny of this pervasive concept. Two major factors should be addressed in any claim of the potential involvement of oxidative reactions in pathogenic mechanisms: 1) catalysts and 2) sources of oxidizing equivalents. The importance of transition metals such as copper and iron in catalysis of redox oxidations remains undisputable. A point in case, manufacturing of CNT frequently involves the use of significant amounts of metals – Fe, Ni, Cr. One may assume that these metals could act as potent oxidation catalysts. Closely associated with this is the propensity of metals to participate in one-electron reduction of oxygen and production of so-called reactive oxygen species such as

superoxide radicals, hydrogen peroxide and hydroxyl radicals. The latter can act as an immediate and direct oxidizing entity causing oxidation of biomolecules in cells and tissues. While carbon nanotube-induced production of oxygen radicals relevant to the presence of adventitious metals has been well documented in simple model systems, the carbon nanotube-dependent formation of oxygen radicals in realistic physiological conditions is more equivocal because of the potent capacities of cells and biofluids to bind transition metals yielding redox-inactive complexes. Nonetheless, accumulation of oxidation products in proteins, DNA and lipids in cells and tissues of animals exposed to CNT has been reported by several independent laboratories. Notably, dietary manipulations of anti-/pro-oxidant status of animals achieved by maintaining them on vitamin E-deficient diet has been reported to exacerbate the inflammatory pulmonary response to aspired SWCNT. The major point, however, is the origin of these oxidation products: when and how have they been accumulated and what is their role in pathogenic events in exposed tissues?

The association between oxidation biomarkers with the direct induction of oxidative stress by CNT versus the indirect impact on oxidative pathways triggered by CNT should be carefully discriminated. For comparison, one may consider the initiation and execution of the apoptotic program, in which specific oxidation events are key to the execution process, while at the same time the disruption of electron transport during apoptosis massively generates superoxide radicals and hydrogen peroxide as a consequence of cell death. Simultaneous with the generation of reactive oxygen species, the transmembrane migration of a mitochondria-specific anionic phospholipid, cardiolipin (CL) from the inner to the outer mitochondrial membrane facilitates binding of this phospholipid with an intermembrane hemoprotein, cytochrome c (cyt c) resulting in the assembly of cyt c/CL complexes with peroxidase activity. In this way, both of the critical components needed for the induction of mitochondrial oxidative stress are in place: cyt c/CL catalytic complexes and a source of oxidizing equivalents emanating from the disrupted electron transport chain. Consequently, selective accumulation of phospholipid oxidation products takes place. Involvement of CL oxidation products in mitochondrial permeability transition and release of cyt c into the cytosol is also linked to the catalytic role of cyt c in oxidation of yet another anionic phospholipid, phosphatidylserine (PS), in extramitochondrial locations.

Oxidation and externalization of PS in the plasma membrane of apoptotic cells has been associated with the recognition/uptake of apoptotic cells by professional phagocytes. This PS-dependent pathway of cell clearance is thought to be essential for the resolution of acute inflammatory responses and to preclude chronic inflammation and fibrosis [19]. Notably, we have observed that early onset of fibrosis is characteristic of pulmonary inflammatory reactions induced by exposures of mice to SWCNT. The arrival of macrophages with their NADPH oxidase-driven production of superoxide radicals and hydrogen peroxide creates conditions for further progression of oxidative/nitrosative stress. Our recent studies have shown that NADPH oxidase-deficient mice respond to SWCNT exposure with a marked accumulation of PMNs and elevated levels of unengulfed apoptotic cells in the lungs, production of pro-inflammatory cytokines, decreased production of the pro-fibrotic cytokine, TGF-β, and significantly lower levels of collagen deposition, as compared to control mice. The latter studies thus demonstrate a role for NADPH oxidase-derived reactive oxygen species in determining the course of pulmonary responses to SWCNT. It is worthwhile noting that recent studies point to a role for a functional NADPH oxidase both for activation of the inflammasome, an intracellular molecular complex required for processing and secretion of the pro-inflammatory cytokine, IL-1β and for the regulation of antibacterial autophagy during phagocytosis.

In sum, oxidative stress commonly accompanies cytotoxic effects originating from exposure of cells and animals to engineered nanomaterials, including CNT. However, whether this remains a correlation or a causative effect remains to be elucidated. Specific mechanisms underlying the propensity of CNT to induce oxidative stress are not well understood. *In fact, enzymatic pathways acting as major generators of oxidative stress – such as MPO – may display quite a different function towards engineered nanomaterials – green chemical reactors for biodegradation of nanoparticles. The possibility and mechanisms of this novel pathway towards SWCNT – is the major tenet of this application.*

Significance: Engineered nanomaterials are novel materials that are manufactured in the same size-range as cellular nanomachines and biomolecules, suggesting that such artificial nano-machines can interfere with the highly orchestrated and structurally coordinated function of intracellular counterparts. There is an exponential growth in the number of publications on engineered nanomaterials. However, only approx. 1 in 50 (2%) of the total number of published articles on nanoparticles during the past 10 years were apparently devoted to the toxicity of nanoparticles. Engineered nanomaterials, as exemplified by CNT, can cause robust and unusual inflammatory responses in vivo with a rapid transition from the acute inflammatory phase to a chronic fibrotic phase; genotoxic effects have also been documented indicating that the realization of these events into a carcinogenic process may be possible. These toxic features of SWCNT limit their numerous industrial and biomedical applications. Studies of potential biodegradation pathways for SWCNT, although are clearly required, are lacking. The proposed projects will fill this gap by investigating the role and mechanisms through which MPO in PMNs can catalyze biodegradation of SWCNT. Discovery of enzymatic mechanisms for "green" biochemistry of CNT biodegradation may revolutionize the ways to regulate their distribution in the body and contribute to a roadmap to new effective approaches to decrease their potential toxicity.

Specific Aims:

Specific Aim 1 will determine molecular mechanisms, products and reaction pathways through which hMPO catalyzes biodegradation of SWCNT.

Specific Aim 2 will define the conditions maximizing biodegradation of SWCNT in human PMNs and determine possible contribution and role of PMN interacions with macrophages in the biodegradation process through the formation of redox phagocytic synapse.

Specific Aim 3 will elucidate hMPO-catalyzed biodegradation of single-walled carbon nanotubes by PMNs in vivo and quantitatively assess the contribution of the biodegradation process in mitigation of the SWCNT induced inflammatory responses in mouse lung.

Methodology

Particles. Adequate characterization of physical and chemical properties of nanomaterials is an absolute requirement for obtaining reproducible results. Carbon nanofibers were vapor grown (PR-24, LHT grade) and heat treated (up to 3,000°C) to graphitize chemically vapor-deposited carbon present on the surface of the pyrograf and to remove the iron catalyst. SWCNT (Unidym, Sunnyvale, CA) were manufactured using the high-pressure CO disproportionation process (HiPco) and purified with acid treatment to remove catalytic metal contaminants. CNF were obtained from Pyrograf Products. A UICC standard crocidolite asbestos was utilized as a positive fiber control. Total elemental carbon and trace metal analyses were performed by the Chemical Exposure and Monitoring Branch (DART/NIOSH, Cincinnati, OH). Elemental carbon was assessed according to the NIOSH Manual of Analytical Methods (NMAM) 5040, whereas trace metals were analyzed by nitric acid dissolution and inductively coupled plasma-atomic emission spectrometry (ICP-AES) following NMAM method 7300 for trace metals. Raman spectroscopy, near-infrared spectroscopy, and thermo-gravimetric analysis were used for purity assessment of HiPco SWCNT. Specific surface area was measured at −196°C by the nitrogen absorption-desorption technique (Brunauer Emmet Teller method, BET) using an SA3100 Surface Area and Pore Size Analyzer (Beckman Coulter, Fullerton, CA), and particle diameter was measured by transmission electron microscopy. Before pharyngeal aspiration, particles were ultrasonicated (30 s × 3 cycles) for improved dispersion of nanoparticles.

Generation of an aerosol of SWCNT for inhalation exposure. The generation system used to deliver respirable SWCNT structures (5 mg/m^3) at a flow rate of 10 l/min to two animal exposure chambers

each containing 12 mice was described previously. Briefly, aerosols containing SWCNT particles were generated using an aerosol dispersion system containing a powder feeder and a knife mill. The fluidized powder feeder, designed specifically for delivering the low-density material, allowed relatively constant feed rates over a period of 6 h. A knife mill was set up to provide high shear forces to tear apart agglomerates in the bulk material. Because the aerosolized material tends to form clumps, a static discharger containing ^{210}Po strips was used to reduce the electrical charges on the particles to prevent agglomerate formation due to contact charging. Before entering the inhalation chamber, the aerosol was passed through a settling chamber, followed by an air cyclone (GK 2.69; BGI, Waltham, MA) to remove the coarse portion of the particles from the aerosol by gravitational settling and centrifugal force. The cyclone had a 50% cutoff size of 4-μm aerodynamic diameter. The feed rate, mill speed, and air flow rate were adjusted to allow a target mass concentration of 5.0 mg/m^3 in the chamber, 5 h/day for 4 days. The mean flow rate through each animal chamber was 5 l/min. The resulting SWCNT aerosol was more dispersed, i.e., less agglomerated, than the SWCNT in suspension used for pharyngeal aspiration. This inhalation exposure resulted in a lung burden of 5 μg. Mice were killed 1 yr postinhalation. SWCNT concentration within the exposure chambers was monitored in real-time by a DataRAM (Thermo Fisher Scientific, Waltham, MA). Gravimetric samples were also taken every 30 min at two sites within each exposure chamber using 25-mm polyvinyl chloride filters at a flow rate of 1 l/min to calibrate the DataRAM.

Particulate aspiration. Mouse pharyngeal aspiration was used for bolus particulate administration. Briefly, after anesthetization with a mixture of ketamine and xylazine (62.5 and 2.5 mg/kg subcutaneous in the abdominal area), the mouse was placed on a board in a near vertical position and the animal's tongue extended with lined forceps. A suspension (~50 μl) of SWCNT (40 μg/mouse), CNF (40 and 120 μg/mouse), or asbestos (120 μg/mouse) prepared in PBS was placed posterior on the tongue, which was held until the suspension was aspirated into the lungs. Control mice were administered sterile Ca^{+2} + Mg^{+2}-free PBS vehicle. The mice revived unassisted after ~30–40 min. All mice in PBS, CNF, SWCNT, and asbestos groups survived this exposure procedure and exhibited no negative behavioral or health outcomes. This technique provided good distribution of particles widely disseminated in a peribronchiolar pattern within the alveolar region as was detected by histopathology. Mice were killed 1 yr following the exposure. To reveal long-term outcomes following SWCNT, CNF, and asbestos exposure, we used three different subsets of mice (n = 6/per each endpoint). Therefore, collection of BAL, lungs tissues for histopathology, and measurements of biochemical indices in lung homogenates were performed in separate samples obtained from different animals in experimental groups.

Obtaining BAL from mice. A subset of mice was weighed and killed with intraperitoneal injection of pentobarbital sodium (>100 mg/kg) and exsanguinated. The trachea was cannulated with a blunted 22-gauge needle, and BAL was performed using cold sterile PBS at a volume of 0.9 ml for first lavage (kept separate) and 1.0 ml for subsequent lavages. Approximately 5 ml of BAL fluid per mouse was collected in sterile centrifuge tubes. Pooled BAL cells for each individual mouse were washed in PBS by alternate centrifugation (800 g for 10 min at 4°C) and resuspension. Cell-free first-fraction BAL aliquots were stored at 4°C for LDH assays, whereas the remainder was frozen at −80°C until analyzed for protein and cytokine levels.

BAL cell counting and differentials. The degree of inflammatory response induced by pharyngeal aspiration of SWCNT, CNF, or asbestos was estimated by quantitating total cells, alveolar macrophages (AMs), and polymorphonuclear leukocytes (PMNs) recovered by BAL. Cell counts were performed using an electronic cell counter equipped with a cell-sizing attachment (Coulter model Multisizer II with a 256C channelizer; Coulter Electronics, Hialeah, FL). AMs and PMNs were identified by their characteristic cell shape in cytospin preparations stained with Diffquick (Fisher Scientific, Pittsburgh, PA), and differential counts of BAL cells were carried out. Three hundred cells per slide were counted.

Total protein concentration and LDH activity in the BAL fluid. Measurement of total protein in the BAL fluid was performed by a modified Bradford assay according to the manufacturer's instructions (Bio-Rad, Hercules, CA) with bovine serum albumin used as a standard. The activity of LDH was assayed spectrophotometrically by monitoring the reduction of nicotinamide adenine dinucleotide at 340 nm in the presence of lactate using a Lactate Dehydrogenase Reagent Set (Pointe Scientific, Lincoln Park, MI).

Lung lavage fluid cytokine analysis. Levels of cytokines were assayed in the acellular BAL fluid following SWCNT, CNF, or asbestos aspiration. The concentrations of TNF-α, monocyte chemoattractant protein (MCP)-1, IL-12, IL-6, IL-10, and IFN-γ (sensitivity of assay is 5–7.3 pg/ml) were determined using the BD Cytometric Bead Array Mouse Inflammation Kit (BD Biosciences, San Diego, CA).

Lung preparation for microscopic evaluation. Preservation of the lung was achieved by vascular perfusion of a glutaraldehyde (2%), formaldehyde (1%), and tannic acid (1%) fixative with sucrose as an osmotic agent. This method of fixation was chosen to prevent possible disturbances of the airspace distribution of deposited materials while maintaining physiological inflation levels comparable to that of the end expiratory volume. This was performed using protocols previously employed to study pulmonary effects of SWCNT. Briefly, animals were deeply anesthetized with an overdose of pentobarbital sodium by subcutaneous injection in the abdomen, the trachea was cannulated, and laparotomy was performed. Mice were then killed by exsanguination. The pulmonary artery was cannulated via the ventricle and an outflow cannula inserted into the left atrium. In quick succession, the tracheal cannula was connected to a 5 cmH_2O pressure source, and clearing solution (saline with 100 U/ml heparin, 350 mOsm sucrose) was perfused to clear blood from the lungs. The perfusate was then switched to the fixative. Coronal sections were cut from the lungs. The lungs were embedded in paraffin and sectioned at a thickness of 5 μm with an HM 320 rotary microtome (Carl Zeiss, Thornwood, NY).

Preparation of lung homogenates. The whole mouse lungs from a subset of mice were separated from other tissues and weighed before being homogenized with a tissue tearer (model 985-370; Biospec Products, Racine, WI) in PBS (pH 7.4) for 2 min. The homogenate suspensions were frozen at −80°C until processed.

Histopathology. Histopathological alterations were evaluated in hematoxylin and eosin-stained sections by a single board-certified veterinary pathologist familiar with the toxicological pathology of nanoparticles using guidelines for toxicological histopathology. Unmasked histopathology assessment was used because unmasked evaluation is generally accepted as the most accurate method for evaluating the toxicological histopathology of new agents and because deposition of SWCNT, CNF, and asbestos are visible in tissue section, making masked evaluation impossible because exposure status is apparent during the evaluation. SWCNT, CNF, and asbestos were visible in light microscopic sections, and their location in cells could be identified because of their ability to strongly absorb light. The identity of SWCNT, CNF, and asbestos in tissue section was confirmed by their presence only in the group exposed to the specific test article and by comparison with the distinctive microscopic appearance of each test article before exposure. The histopathology findings from the left and right lung and tracheobronchial lymph node of a sub-set of mice were evaluated to identify variations from normal histology. Following identification of exposure-related changes, numerical data on exposure-related inflammatory changes of the lung, pleura, and lung-associated lymph nodes were obtained using semiquantitative pathology scores reflecting the severity and distribution of morphological changes as previously described. Briefly, slides were scored for distribution (0, none; 1, focal; 2, locally extensive; 3, multifocal; 4, multifocal and coalescent; and 5, diffuse) and severity (0, none; 1, minimal; 2, mild; 3, moderate; 4, marked; and 5, severe). The pathology score was the sum of both distribution and the severity score. A separate score was determined for the inflammation of the lung (pneumonia) and inflammation of the pleura (pleuritis), but for each of these target tissues both the left and right lung were used to determine the pathology score. The presence or absence and the pathology score for

fibrosis were determined in the lungs of SWCNT and air-exposed mice in the SWCNT inhalation study in trichrome-stained sections. Lymph node inflammation (lymphadenitis) was evaluated and scored as described above in hematoxylin-stained sections of tracheobronchial lymph nodes. Additionally, the tracheobronchial lymph nodes were evaluated for the presence or absence of giant cells, and the pleura was evaluated for the presence or absence of mesothelial cell hyperplasia or cellular atypia. In mice aspirating SWCNT, CNF, and asbestos, the presence or absence of pulmonary fibrosis was determined after the histopathology assessment through the evaluation of trichrome-stained sections by a board certified pathologist at Colorado Histoprep (Fort Collins, CO). The obtained histopathology results also confirmed the seen presence of inflammation in the lungs of mice aspirating SWCNT (40 μg), CNF (40 μg), CNF (120 μg), and asbestos (120 μg).

Lung collagen measurements. Total lung collagen content was determined by quantifying total soluble collagen using the Sircol Collagen Assay kit (Accurate Chemical and Scientific, Westbury, NY). Briefly, whole lungs were homogenized in 0.7 ml of 0.5 M acetic acid containing pepsin (Accurate Chemical and Scientific, Westbury, NY) with 1:10 ratio of pepsin:tissue wet weight. Each sample was stirred vigorously for 24 h at 4°C and centrifuged, and 200 μl of supernatant was assayed according to the manufacturer's instructions.

*Raman spectroscopy***.** Assessments of SWCNTs retained in the lungs 1 yr postexposure were performed using Raman microscopy. This protocol has been successfully employed for the detection of SWCNTs in tissues and cells due to the presence of a characteristic tangential-mode G-band in their Raman spectra. The Raman spectra were measured using a Horiba Jobin-Yvon spectrometer connected to a CCD camera and a confocal microscope with an ×80 objective. Samples were excited using a 514.5-nm laser with ~5-μm spot size and a power of 0.3 mW at the sample, giving a power density of ~15 $\mu W/\mu m^2$. These conditions were kept constant across all samples. A calibration curve was constructed to estimate the amount of SWCNT in the lung slides using standard samples of SWCNT prepared as follows: 1 mg/ml of SWCNT was dispersed in 1% pluronic 127 to give a concentration of 50 μg/ml. Various volumes were dispersed on five microscope slide wells to obtain mass-per-surface-area concentrations in the range 1.875×10^{-9} $\mu g/\mu m^2$ to 1.5×10^{-7} $\mu g/\mu m^2$. The tissue sections containing both left and right lungs (n = 5; one slide per mouse) were first inspected visually with a ×10 objective for dark areas that contained SWCNT. Following this, the objective was changed to ×80 magnification, and laser beam was focused until the Raman signal of the G-band reached the maximum. A spectrum for that area was collected with an exposure time of 10 s. To obtain a good signal-to-noise ratio, a total of five spectra per chosen area were collected and averaged. Finally, the intensity of the G-band was translated into mass/area concentration using the calibration curve, and this number was multiplied by the estimated area of the region measured to obtain final mass per slide. Furthermore, the total amount of SWCNTs retained in the whole lung was estimated using the formula: (mass × average volume of mouse lung/volume of tissue section on each slide). An average volume of 10 × 5 × 0.005 mm^3 of lung tissue section on each slide and 520 mm^3 per mouse lung was considered for estimating the total mass in each case.

Detection of Apoptotic Cells in the Lung of Mice after SWCNT Inhalation. Cell apoptosis was determined by a fluorescence labeling apoptosis detection system (Promega, Madison,Wi). This system measures fragmented DNA in apoptotic cells by catalytically incorporating fluorescein 12 dUTP at the 3′OH ends using the TUNEL (TdT mediated dUTP Nick End Labeling) assay. Normal cells were fluorescently labeled red by counter-staining with propidium iodide. Positive control slides were prepared by treating sections with DNase prior to TUNEL. Negative control slides were prepared by omission of the TdT enzyme from the labeling solution. The number of TdT positive nuclei enclosed by a cell cytoplasm (apoptotic cells) per unit area were counted in 10 randomly selection fields (40×) from each animal. Cell counts were expressed as the number of apoptotic cells per unit area of section.

Myeloperoxidase Staining. Lung sections were hydrated with tap water and antigen retrieval was performed in citrate buffer solution, pH 6.0. The slides were pretreated with 3% hydrogen peroxide for

20 min to inhibit endogenous peroxidase and blocked using 2.5% normal horse serum (Vector Labs, Burlingame, CA) for 30 min to prevent nonspecific reactivity. Sections were incubated with anti-myeloperoxidase primary antibody (1:100, 4°C overnight incubation) (ab9535, AbCam, Cambridge, UK). Then, sections were treated with a peroxidase conjugated secondary antibody (ImmPRESS, MP-7401, Vector Labs) for 30 min at RT. After washing, color was developed by adding AEC Chromogen for 10 min (SK-4200, Vector Labs). Finally, slides were counterstained with hematoxylin and mounted using Faramount (DAKO, Glostrup, Denmark). Negative control sections were treated in the same way but primary antibodies were omitted. For the detection of macrophages, sections were incubated with anti-rabbit F4/80 antibody (1:50, 4° C overnight) (ab100790, AbCam, Cambridge, UK).

hMPO Contents in Cells and Its Release. Levels of hMPO in cells were determined by an ELISA kit (Alpco Diagnostics, NH) after 30 min incubation with samples. Neutrophils were centrifuged at 1000*g* for 10 min. The supernatant and pellet were obtained and used separately for hMPO measurements according to the manufacturer's manual. The amounts of hMPO were expressed as mg/mL.

Degradation of nanotubes in Vitro. SWCNTs were incubated in 50 mM phosphate buffer (pH 7.4) with MPO, EPO or different concentrations of peroxynitrite for 120 h at 37 C. Peroxynitrite was generated (a) by addition of peroxynitrite donor SIN-1, producing both nitric oxide (*NO) and superoxide (*O2) upon decomposition in aqueous solution, or (b) by simultaneous addition of a superoxide-generating system, containing xanthine oxidase/xanthine (XO/X) and a NO donor (PAPA NONOate or spermine NONOate). Aliquots of fresh H_2O_2, NaBr, PAPA NONOate, spermine NONOate, xanthine, and SIN-1 were added every 1.5 h (5 times a day); solutions contained MPO, EPO or xanthine oxidase were added in the morning and in the evening. Degradation of SWCNTs was assessed visually by a steady progression of fading color intensity and turbidity. In addition, aliquots were removed from the incubating bulk samples at different time points, and the biodegradation of SWCNTs was studied using transmission electron microscopy (TEM), ultraviolet-visiblenear-infrared absorption spectroscopy (UV-vis-NIR) and Raman spectroscopy.

Visible Near-Infrared Absorption Spectroscopy. The visNIR spectra were obtained from the samples using a PerkinElmer Lambda 750 UV/vis/NIR spectrophotometer (PerkinElmer, Waltham, MA, USA). Spectra were recorded using a 50 µL cuvette (Starna Cell Inc., Atascadero, CA, USA). Absorbance in the region of the S2 band was normalized by subtraction of scattering.

Lipid Extraction and 2D-High Performance Thin Layer Chromatography Analysis. Total lipids were extracted from lung homogenates by Folch procedure. Lipid extracts were separated and analyzed by 2D HPTLC. To prevent oxidative modification of phospholipids during separation plates were treated with methanol containing 1 mM EDTA, 100 µM DTPA prior to application and separation of phospholipids by 2D-HPTLC. The phospholipids were visualized by exposure to iodine vapors and identified by comparison with authentic phospholipid standards. For electrospray ionization mass spectrometry (ESI-MS) and analysis of phospholipid hydroperoxides (PL-OOH) by fluorescence high performance liquid chromatography (HPLC) using Amplex Red, the phospholipid spots on the silica plates were visualized by spraying the plates with deionized water. Subsequently, the spots were scraped from the silica plates and phospholipids were extracted in choloroform:methanol:water (10:5:1 v/v). Lipid phosphorus was determined by a micro-method.

Electrospray Ionization Mass Spectrometry. LC/ESI-MS was performed using a Dionex Ultimate™ 3000 HPLC coupled on-line to ESI and a linear ion trap mass spectrometer (LXQ Thermo-Fisher). The lipids were separated on a normal phase column (Luna 3 µm Silica 100A, 150×2 mm, (Phenomenex, Torrance CA)) with flow rate 0.2 mL/min using gradient solvents containing 5 mM CH_3COONH_4 (A – n-hexane: 2-propanol: water, 43:57:1 (v/v/v) and B - n hexane: 2-propanol: water, 43:57:10 (v/v/v). Analysis of phospholipid oxidized molecular species (hydroperoxy- and hydroxy-) was performed as described.To minimize isotopic interferences between isolated masses M+2, the spectra were acquired using an isolation width of 1.0 *m/z*. For identification of phopsholipids ESI-MS analysis was performed

by direct infusion into linear ion-trap mass spectrometer LXQ™ with the Xcalibur operating system (Thermo Fisher Scientific, San Jose, CA) as previously described.

2D-Liquid Chromatography Mass Spectrometry. CL separated by HPTLC was analyzed by LC/MS using a Prominence HPLC system (Shimadzu, Inc.) with a reverse phase C_8 column (Luna, 5 micron, 4.6mm × 15 cm, Phenomenex, Inc.). An isocratic solvent system (2-propanol:water: triethylamine:acetic acid (450:50:2.5:2.5, v/v/v/v) was used at a flow rate of 0.4 mL/min. Spectra were analyzed on Q-TOF Premier mass spectrometer (Waters, Inc.). Parameters were as follows: capillary voltage: 2.85 kV, negative mode; source temperature, 100 °C; desolvation gas, 400 L/hr; sampling cone, 60V; extraction cone, 4.5V; ion Guide, 3.0V. Tuning was optimized for oxidized and non-oxidized CL species across CL scan range.

MALDI-TOF Mass Spectrometry. CL extracts were analyzed in triplicate on a Voyager DE-STR MALDI-TOF-MS (Applied Biosystems/Life Technologies, Carlsbad, CA). High resolution mass measurements were done on an Ultraflex II MALDI-TOF-MS (Bruker Daltonics, Billerica, MA). Sample preparation was slightly modified from the method of Schiller et at. In brief, the CL extract was spotted onto the MALDI target, followed by 500mM 2,5-dihydroxybenzoic acid (DHB) in methanol containing 0.02% trifluoroacetic acid. Spectra were acquired in reflector-negative mode with external calibration. Masses of all detected molecular species were confirmed to within 0.02 Da.

Liquid Chromatography/Electrospray Ionization Mass Spectrometry of Oxygenated Fatty Acids. Oxygenated fatty acids were analyzed by LC/ESI-MS after hydrolysis of major classes of phospholipids with porcine pancreatic PLA_2 (1 U/μL) in 25 mM phosphate buffer containing 1.0 mM Ca, 0.5 mM EGTA and 0.5 mM SDS (pH 8.0 at RT for 30 min). Aliquots of extracted lipids (5 μL) were injected into a C_{18} reverse phase column (Luna, 3 μm, 150 × 2 mm) and eluted using gradient solvents (A and B) containing 5 mM ammonium acetate at a flow rate of 0.2 mL/min. Solvent A was tetrahydrofuran/methanol/water/CH_3COOH, 25:30:50:0.1 (v/v/v/v). Solvent B was methanol/water 90:10 (v/v). The column was eluted during first 3 min isocratically at 50% B, from 3 to 23 min with a linear gradient from 50% solvent B to 98% solvent B, then 23-40 min isocratically using 98% solvent B, 40-42 min with a linear gradient from 98% solvent B to 50% solvent B, 42-28 min isocratically using 50% solvent B for equilibration of the column. Hydroperoxy-fatty acids: 13S-OOH-9Z,11E-octadecadienoic acid, 9-OH-10E,12Z-octadeacadienoic acid, 15S-OOH-5Z,8Z,11Z13E

Statistics. Treatment-related differences were evaluated using one-way ANOVA, using the Dunnett's Multiple Comparisons to control and Student's unpaired *t*-test with Welch's correction for unequal variances, as appropriate. Statistical significance was considered at $P < 0.05$. Data are presented as means ± SE.

Results and Discussion

Long-term effects of carbon containing engineered nanomaterials. As stated above, enzymatic oxidative biodegradation of carbonaceous materials by the machinery of inflammatory cells is one of the importanr factors determining biopersistence and their fate in tissues and biofluids. Therefore, we were interested in assessing of the “longevity” and long-term effects of SWCNT in the lung and their association with the activities of the inflammatory cells. Single-walled carbon nanotubes (SWCNT), composed of a rolled-up cylindrical sheet of graphene, and carbon nanofibers (CNF), formed from stacked graphene nanocones, are carbonaceous nanoparticles sharing fibrous morphology with a well-known, naturally occurring, toxic fiber, asbestos. Both short- and long-term outcomes of pulmonary exposure to asbestos, ranging from inflammation and fibrosis to mesothelioma and lung cancer, are well described. The hallmark geometric feature of individual SWCNT and CNF particles, high aspect ratio, makes them similar to asbestos fibers. However, chemical composition, physical dimensions, and mechanical and surface properties of SWCNT and CNF are very different from asbestos. Thus it remains uncertain whether SWCNT and CNF would follow the asbestos toxicity paradigm.

Acute and subchronic (up to 28 or 90 days) consequences of pulmonary exposure to SWCNT and CNF have been previously reported, where SWCNT, CNF, and asbestos have been shown to induce inflammation and fibrosis. Notably, morphological features of acute and subchronic inflammatory response to SWCNT were different from those seen after asbestos or CNF exposure. Foci of granulomatous lesions and collagen deposition were associated with dense particle-like SWCNT agglomerates, whereas no granuloma formation was found following exposure to nonagglomerated fiber-like CNF or asbestos.

Malignant consequences of asbestos exposure are lung cancer and mesothelioma formation. High biopersistence, pulmonary penetration, fibrous morphology, the ability to generate reactive oxygen species, as well as inflammatory and genotoxic effects associated with these phenomena or occurring independently have been implicated in asbestos carcinogenicity. Several recent reports suggested that SWCNT or multi-walled carbon nanotubes (MWCNT) induced DNA damage, micronuclei formation, disruption of the mitotic spindle, and polyploidy. We have previously shown that CNF and asbestos were capable of inducing aneugenic (chromosomal malsegregation) or clastogenic (chromosome breakage) events in human small airway epithelial cells. Furthermore, exposure to SWCNT has been implicated in an increased incidence of K-ras oncogene mutations in vivo in an acute inhalation study. Considering that genetic instability combined with chronic inflammation are important factors contributing to carcinogenicity, evaluation of genotoxic effects of carbonaceous nanoparticles in vivo represents a relevant topic of investigation.

Our data provide evidence that, up to 1 yr after exposure, SWCNT, CNF, and asbestos persist in the lung and regional lymphatics and elicit genotoxic effects and pulmonary fibrosis. To address the concerns raised about the relevance of the high dose rate after bolus instillation animal exposure, we also compared the data obtained from inhalation and pharyngeal aspiration protocols for SWCNT at 1 yr postexposure.

Oxidative stress induced by carbonaceous nanomaterials. It is commonly accepted that the cytotoxicity of nanoparticles such as CNTs is largely derived from oxidative stress resulting from their small size and large specific surface area. Moreover, the oxidative stress also arises from increased levels of reactive oxygen species (ROS) that include superoxide radical anions and hydroxyl radicals. As a natural byproduct of cellular respiration process in mitochondria, the ROS are normally balanced by a series of anti-oxidant enzymes. However, the cellular homeostasis can be disrupted when the intracellular ROS levels are significantly levitated by environmental stress (*e.g*. exposure to nanoparticles), thereby leading to oxidative stress. It is believed that nanoparticles can trigger the formation of ROS through two different pathways. Some transition metal-based nanoparticles can catalyze decomposition of peroxides and form ROS directly such as the Fenton reaction triggered by iron catalytic nanoparticles from unpurified SWCNTs; while in more general cases, the ROS are generated by phagocytic cells of the immune system (*i.e*. neutrophils, eosinophils, and macrophages) in response to the extrinsic nanoparticles. When phagocytes actively internalize nanoparticles, the massive ROS generation process is considered to be NADPH oxidase-dependent, where upon activation, NADPH oxidase ensembles at the phagolysosomal membrane and transfers electrons to oxygen to form superoxide in a process known as “oxidative burst”. In turn, superoxide anions will dismutate to H_2O_2, which is an initiator of the aforementioned peroxidase cycle of MPO in neutrophils and EPO in eosinophils. CNTs, in particular, because of their large surface activity, are highly likely to induce oxidative burst.

Role of oxidative stress in the in vitro *degradation of CNTs.* It is commonly thought that oxidative stress triggers cell damage and death, and this phenomenon may be the cause of many diseases and cancers. However, the beneficial effects of oxidative stress are receiving more and more attention in recent years as studies revealed the important immune functions of ROS in “oxidative signaling,” which activates the inflammatory response in phagocytic cells, as well as in the attack and degradation of pathogens. The massive generation of strong ROS in response to pathogen engulfment has a large enough oxidative potential to break C-C and C-H bonds in bacteria and viruses thereby leading to their

degradation. This oxidative clearance of pathogens also applies to degradation of carbonaceous nanomaterials including CNTs as they share similar sizes and elemental compositions with bacteria or viruses.

The *in vitro* degradation of CNTs is more likely to occur in the activated phagocytic cells with high oxidative stress. As demonstrated by in test tube results, innate peroxidases, such as MPO in neutrophils and EPO in eosinophils, play a pivotal role in oxidative degradation of CNTs. Under natural inflammatory response, neutrophils are attracted and activated by chemoattractants at the inflammatory sites, and MPO is released thus being able to act both intra-and extra-cellularly. H_2O_2, which originated from NADPH oxidase, forms inside the phagosome, activates MPO, and leads to the production of reactive enzymatic intermediates and oxidants (*e.g*. hydroxyl radicals, HClO, *etc*.), both of which are responsible for CNT degradation. As a result, the phagosomes of the phagocytes provide the exact environment facilitating the degradation of CNTs as simulated under test tube conditions. Both H_2O_2-generating enzyme (*e.g*. NADPH oxidase) and oxidative peroxidase are essential factors for the degradation of CNTs inside phagocytes under oxidative stress. The presence of halide ions also facilitates the degradation process by forming strong oxidative hypohalous acids. Finally, in the *in vitro* experiment, chemoattractants and degranulation promoting agent must be added to promote degradation. While chemoattractants activate the phagocytic cells, and degranulation promoting agent (*e.g*. cytochalasin B) trigger the release of peroxidase.

Cellular internalization of CNTs. The *in vitro* degradation of CNTs may occur either inside or outside of the cells depending on the distribution of the peroxidase upon cellular activation. If the peroxidase is localized mainly inside of the cells, then CNTs must be pre-opsonized for efficient internalization. Opsonization can be achieved by functionalization of CNTs with immunoglobulins, which specifically target receptors on phagocytes. For example, in our previous study of CNT degradation in neutrophils, most of the MPO was translocated into the intracellular phagolysosomal space, where degradation ensued. Therefore, the nanotubes were targeted with IgG for enhanced cellular uptake. A significant increase in uptake and degradation of IgG-nanotubes by neutrophils occurred relative to non-functionalized nanotubes. Additionally, coating with specialized “eat-me” signals such as phosphatidylserine also makes CNTs recognizable by different professional phagocytes. In contrast, in the study of CNT degradation by eosinophils, opsonization of CNTs was not required as the biodegradative peroxidase EPO is exocytosed upon cellular activation.

Enzymatic oxidative degradation of carbonaceous nanoparticles. The chemical oxidative degradation of pristine carbonaceous materials using strong acids and oxidants (such as mixtures of sulfuric acid and hydrogen peroxide, different chemical generators of hydroxyl radicals) has been known for quite some time. However, the biological relevance of these oxidative processes remained elusive in spite of the fact that the catabolic pathways for oxidative degradation of different organic molecules in the body (e.g., by different P450 isoforms) have been well characterized. One of the first indications that biologically relevant peroxidase reactions may be responsible for degradation of nanomaterials came from experiments with single-walled carbon nanotubes (SWCNTs) by a plant enzyme, horseradish peroxidase (HRP). Subsequent detailed studies of the mechanisms and the reaction products demonstrated that other bio-peroxidases, particularly those present in inflammatory cells, can also effectively oxidatively “metabolize” carbonaceous nanomaterials. Indeed, a number of different oxidative enzymes have been tested and found effective as a mechanism of nanoparticle biodegradation. The list of enzymes includes myeloperoxidase (MPO), eosinophil peroxidase (EPO), lactoperoxidase, hemoglobin and xanthine oxidase. Contrary to HRP, another plant metallo-enzyme Mn peroxidase, was shown to degrade pristine but not carboxylated SWCNTs. These studies also established that two types of reactive intermediates – those formed within the protein (particularly oxo-ferryl iron (Fe4 += O) of heme-peroxidases (Compound I)) as well as freely diffusable low molecular weight oxidants such as hypochlorous and hypobromous acids (HOCl and HOBr) – can be responsible for the oxidative modification of carbonaceous nanomaterials. The relative contribution of these two types of oxidants to the overall degradation process may vary dependently on the type of enzyme,

conditions (particularly pH), pro-/anti-inflammatory status, etc. In all cases, however, the presence of catalytic metals is necessary for triggering the degradation process.

Numerous studies emphasized the effects of diversified organic compounds, particularly hydrophobic molecules, with the expression of different isoforms of CYP450 and their activity. Moreover, particular carbonaceous materials can also cause robust changes in the functions of CYP450 system. This catalytic responsiveness of CYP450 has led to the development and applications of carbonaceous nanoparticles complexes with these hemoproteins as CNT-conjugated P450-biosensors.

Surprisingly, to the best of our knowledge there have been no studies demonstrating the propensity of CYP450 to biodegrade nanoparticles. This may represent an interesting direction of research because highly reactive Compound I is generated during P450 catalyzed metabolism of xenobiotics or direct interaction of these hemoproteins with H_2O_2 or organic hydroperoxides (peroxidase shunt).

In contrast to highly likely and straightforward capacities of CYP450 to be involved in the degradation of carbonaceous nanoparticles, the potential role of catalase is less obvious. In contrast to widely opened and solvent exposed active sites, characteristic of most hemoprotein-based peroxidases, the heme of catalases is deeply buried into the protein structure and connected with the surface via a very long and narrow access channel. Catalase compound I is accessible only for very small molecules to effectively fulfill the function of oxoferryl iron to either oxidize H_2O_2 or one of the aromatic residues of the protein thus leading to the formation of tyrosyl of tryptophanyl radicals. The peroxidase activity of native catalase is relatively low. It is possible, however, that catalase monomers formed upon dissociation of multi-meric protein and/or their structural re-arrangements on the surface of nano-materials that will lead to the heme exposure and appearance of peroxidase-like activity with a typical catalytic competence to oxidize phenolic compounds. Interestingly, this type of peroxidase activity of catalase has been explored in reactions of controllable degradation of C_3N_4 to obtain biocompartible fluorescent N-C dots.

Oxidative degradation of nanoparticles by inflammatory cells. Among many encounters with the gateway cells of the body, interactions of nanoparticles with immune/inflammatory cells are of particular interest for at least two reasons. Inflammatory cells are “armed” with different types of generators of reactive oxygen and nitrogen species (ROS and RNS, respectively) as well as hypohalous acids. These chemical agents have strong oxidizing redox potentials enabling their reactivity towards the carbonaceous surface of nanoparticles. Notably, oxygenation (along with nitration, chlorination) of the lipid-protein “corona” of nanoparticles generates clusters of hydrophilic and/or negatively charged functionalities recognizable by inflammatory cell receptors, thus triggering a vicious cycle of interactions leading to a severe inflammatory response. In professional phagocytes, the major events include engulfment, uptake, possible intracellular metabolism, and digestion. Two major factors – enzymatic machinery generating reactive intermediates and a source of oxidizing equivalents (e.g., H_2O_2, lipid hydroperoxides) – are required for the effective degradation of carbonaceous nanomaterials. While oxidative enzymes are always constitutively expressed in certain types of immune cells, the levels of their expression may be increased many-fold by pro-inflammatory conditions, including those triggered by the nanoparticles.

Neutrophils are the first line of responders to pro-inflammatory stimulation and their `response reaction includes the activation of MPO-driven pathways. In the context of biodegradation, two mechanisms – immobilized reactive intermediates of the protein itself and highly diffusible small molecule oxidants such as hypochlorous acid (HOCl) – have been identified as components of the oxidative process. The role and contribution of these two factors into the overall degradation may depend on the specific conditions (e.g., the presence of sufficient amounts of Cl^- ions) in the microenvironment. *In vitro*, activation of neutrophils (by fMLP and cytochalasin B or by serum opsonized zymosan) is required to achieve significant levels of oxidative biodegradation. Normally occurring opsonization of particles in the body – stimulating their uptake by phagocytes – can be mimicked by their functionalization with immunoglobulins to facilitate the particles' uptake by neutrophils. Under these conditions, neutrophils

respond by oxidative burst detectable by the generation of superoxide anion radicals which dismutate to yield H_2O_2. The latter is required to feed the peroxidase reaction of MPO, thus causing oxidative biodegradation of nanoparticles. The efficiency of this pathway has been documented for SWCNTs (including their PEGylated forms) and GO. Not only active MPO but also sufficiently high activity of NADPH oxidase – generating superoxide radicals – is necessary to maintain the degradation process. The essentiality of this function of NADPH oxidase has been demonstrated both pharmacologically (using its inhibitors) as well as genetically (using NADPH oxidase deficient animals).

Eosinophils – another class of immune cells – combat multicellular parasitic organisms engaging a specialized peroxidase, EPO, capable of generating hypobromous acid (HOBr) and low levels of hypochlorous acid at acidic pH. In murine eosinophils activated by cytochalasin B plus platelet-activating factor (PAF), the oxidizing enzyme, EPO, is released to cause extracellular degradation of SWCNTs.

Macrophages - employ the complex of reactions leading to the production of peroxynitrite, another potent oxidant capable of oxidative degradation of carbonaceous nanomaterials. Two enzymatic systems – NADPH oxidase and NO synthase – produce superoxide radicals and $NO_{\bullet}$, respectively. Both of these molecules are not reactive enough to oxidatively biodegrade nanoparticles. However, $O_2^{-}{}_{\bullet}$ and $NO_{\bullet}$ can effectively react to yield peroxynitrite, whose oxidizing potency is sufficient to cause biodegradation. *In vitro*, effective biodegradation capacity has been demonstrated for several types of macrophages such as RAW 264.7, THP-1, and human monocyte-derived macrophages. Because peroxynitrite-dependent oxidation reactions are independent of the direct binding of the reactive protein intermediates with nanoparticles, the degradation process driven by macrophages is independent on the specific positioning of the oxidative machinery on the surface of nanoparticles. As a result, different types of nanomaterials – oxidatively pre-modified as well as pristine – may undergo degradation by macrophages. It is also possible that macrophages "prime" the nano-objects to generate "oxidized" sites where released enzymes of neutrophils and eosinophils can selectively "land" to propagate the process initiated by macrophages.

It is possible that other types of immune cells – specific to particular organs and/or disease conditions – may be involved in biodegradation of carbonaceous nanomaterials. For example, microglial cells in the brain can act similar to macrophages and catalyze the reactions leading to peroxynitrite and ROS formation, hence effectively biodegrading nanoparticles. The evidence for the occurrence and effectiveness of this pathway in the brain is accumulating. Another example is myeloid-derived suppressor cells (MDSC) — a heterogenous population of immature cells from the myeloid lineage. As a result of an altered hematopoiesis, amounts of MDSCs can be highly increased in severe disease conditions, particularly chronic infections and cancer. These pathological conditions lead to over-expression of NADPH oxidase, iNOS and MPO in MDSC creating a highly pro-oxidant intracellular environment. These specific features of MDSC may be exploited for the targeted degradation of nanomaterials for optimized delivery of drugs. MDSC-derived oxidants can open carbon nano-cups (NCNCs) loaded with antitumor drug paclitaxel and corked with gold nanoparticles (GNPs) and release paclitaxel.

Biodegradation of nanomaterials by inflammatory cells in vivo*: role in pulmonary inflammation and fibrosis.* There is a common opinion about biopersistence of nanoparticles in the body. While extended circulation of drug nano-carriers with payloads may be desirable, the ineffective elimination of nanoparticles from the organs after unintentional exposure or as a result of therapeutic attempts seems to represent a serious problem. Poorly degradable nanomaterials can accumulate in organs and inside cells where they can cause detrimental effects. Even with regards to carbonaceous nanomaterials that are readily susceptible to biodegradation, carbon nanotubes may remain inside macrophages in the spleen and liver for prolonged periods of time following parenteral administration. Moreover, SWCNTs have been observed in the lungs of exposed mice up to one year after pharyngeal administration. Overall, however, high-aspect, bulky carbonaceous nanomaterials tend to have longer retention times within the tissues and are less effectively cleared than short functionalized particles that are readily

taken up and degraded by phagocytes. Notoriously, the appearance of nanoparticles in tissues triggers robust inflammatory responses. Given that immune cells can spend some of their pro-oxidant potential on biodegradation of the nanoparticles, and thus display a weakened immune response, studies of nanoparticle biodegradation *in vivo* and possible regulation of biodegradation in the context of inflammation *in vivo* became necessary. These issues have also stimulated the concepts of creating safe-by-design nanoparticles as well as employment of inflammatory cells for targeted drug delivery. In line with the ability to take up and biodegrade carbonaceous nanomaterials, professional phagocytes are believed to be mostly accountable for the clearance of the engulfed nanoparticles *in vivo*. Several studies established the association between the clearance of carbonaceous nanoparticles in the lungs and the amounts of neutrophils and macrophages in the respective tissues. These correlational relationships imply that, indeed, biodegradation reactions taking place in inflammatory cells are substantial contributors to the overall elimination of nanoparticles from the tissues. In support of this conclusion, the data on the time course of inflammatory responses and SWCNTs elimination in mice with k/o MPO clearly demonstrated the dependence of these biomarkers on the genetic manipulations with the major biodegrading oxidative enzyme of neutrophils, MPO. Quantitative imaging clearly demonstrated the link between MPO-catalyzed degradation of nanoparticles and one of the hallmarks of the inflammation – pulmonary fibrosis – in wild type (WT) versus MPO k/o animals. The role of NADPH oxidase as a supplier of superoxide for the subsequent reactions of dismutation (to generate H_2O_2 as a fuel for MPO) or with $NO\cdot$ (to produce peroxynitrite in macrophages) has been revealed in experiments with genetically manipulated animals. Clearance of SWCNTs was 10-fold less effective in NADPH oxidase-deficient mice ($gp91^{phox(-/-)}$mice) *vs* WT animals. Photoacoustic imaging also documented significantly reduced rate of SWCNTs clearance in the lung of NADPH-deficient mice compared to WT control animals. There are clear experimental indications that microglial cells – with their highly developed oxidative enzymatic machinery similar to that in macrophages – are primarily responsible for the biodegradative elimination of MWCNTs from the brain.

Advances in the development, production and applications of nanomaterials inevitably lead to numerous chemical and biochemical interactions at the interfaces of biological systems with nanoparticles that result in a variety of responses by the former as well as modifications of the latter. One can imagine that during the repetitive cycles of these interactions both the materials and the organisms are affected resulting in alterations that may change the consequences and the meaning of the interfacing partners. One of the first important modifications of the nanoparticles in biological systems is the formation of protein-lipid “corona”. The composition and properties of “corona” are dependent on the local microenvironments in biological fluids, tissues and cells, thus determining the specificity of nanoparticles-evoked reactions. The physicochemical characteristics of the corona can also undergo marked changes due to metabolic conversions in the body and also via chemical reactions catalyzed by active ingredients of nanoparticles. This, in turn, triggers strongly modified biological responses. Among the constituents of the protein corona, there may be enzymes contributing to the biodegradation process such as peroxidases, isoforms of CYP450, lysosomal hydrolases, etc.
Overall, nanoparticles tend to either be readily degradable or resistant to degradation in terms of their sensitivity to biodegradation. The first group includes nano-liposomes and polymeric nano-arrangements (such as dendrimers, micelles). Effective degradation of these nanomaterials is particularly important in the context of drug delivery aimed at the achievement of the prolonged circulation of the nano-vehicle with the payload. The second group comprises carbon-based nanoparticles with sp^2 hybridization of carbon atoms (i.e. carbon nanotubes, nanohorns and graphene family materials). These nanomaterials are more persistent and can either display prolonged life-time at the sites of their entry or migrate to distant locations. The mechanisms underlying the resistance of the latter type of nanoparticles to biodegradation are important not only for their biomedical applications but also in regards to unintended exposures in occupational and environmental settings.
While studies of nanoparticles degradation have been conducted essentially from the time of their discovery and initial applications (mostly in the field of anticancer therapy), the discovery of *in vivo* biodegradation of carbonaceous nanomaterials by enzymatic machinery of inflammatory cells and enhancement of the enzymatic degradation of carbon nanotubes by surface modification caused a new wave of interest to this issue. This was mostly driven by exploration of new approaches to regulate the

life-time of nanoparticles in desirable ways: increasing the circulation time of drug nano-carriers and enhancing the biodegradation process of nanomaterials causing inflammatory responses and toxicity after inadvertent exposures. Notably, a variety of microbial biodegradation enzymatic mechanisms have been described with the emphasis on their potential role in biodegradation of environmental nanoparticles.

The in vivo biodegradation of CNTs in the brain. Several studies investigated the fate of MWCNTs in mouse neuronal tissue. Amine-functionalized MWCNTs were stereotactically injected into the motor cortex of a mouse brain, where microglia functioned as the primary professional phagocytes for the brain's immune system. TEM imaging was performed on parenchyma samples near the injection site 2 days post-injection. While widespread microglia internalization of MWCNTs was considered to be the predominant mechanism of early tissue response, the micrographs revealed that this nanomaterial was also internalized into different types of brain cells including neurons. Aggregates of MWCNTs were localized in the possible phagolysosomal vesicles through active endocytosis or phagocytosis, and individualized MWCNTs were mainly internalized in the cytoplasm through a direct membrane translocation pathway. Besides the intact nanotubes, many internalized MWCNTs were observed to undergo severe structural deformation yielding amorphous debris, which indicated the widespread initiation of the degradation process in microglia on day 2 after injection. This structural deformation was confirmed by Raman spectra that were taken of the injected brain tissue on days 2 and 14 after injection, which demonstrated an overall reduction in the intensity of the D and G peaks coupled with an increase in background noise thereby indicating diminishing CNT content. A decrease in the D to G band ratio was observed, however, which might indicate the incomplete degradation of MWCNTs in microglia as was observed in another degradation experiment conducted on MWCNTs. While the authors attributed the possible *in vivo* degradation of MWCNTs to the highly efficient phagocytosis capacity of microglia cells that possess both an oxidative lysosomal environment with low pH and an abundance of hydrolytic enzymes, the actual mechanism of degradation remained hypothetical.

Can oxidative mechanisms contribute to biodegradation of non-carbonaceous nanomatrials? Unlike peroxidase-catalyzed degradation of CNTs and graphene oxide, the biodegradation of other nanoparticles may undergo completely different pathways depending on their chemical properties. The most inert gold nanoparticles (NPs) are rarely reported to undergo biodegradation in cells with their eventual *in vivo* fate likely to include retention inside organs such as the liver and spleen. In contrast, iron oxide NPs can be gradually degraded in the acidic environment of endosomes or lysosomes after cellular uptake thereby releasing ferric ions. Quantum dots (QDs) such as CdSe and CdTe were also reported to undergo a pH-dependent degradation, and the presence of intracellular hypochlorous acid and hydrogen peroxide may facilitate the degradation process. The degradation of QDs is not desired because the released Cd^{2+} ions are highly cytotoxic, which, in turn, greatly limits the biomedical applications of QDs. Different types of inorganic NPs, which include semiconductor, metal, metal oxide, and lanthanide-doped QDs are commonly encapsulated in polymers or coated with lipids or surfactants to preserve the as-synthesized properties of the nanomaterial. The surface properties of these carbonaceous coatings, which are highly vulnerable to oxidative biodegradation, will impact the interactions of NPs with cells and components of biofluids thus defining their fate and effectiveness in the body. Therefore, peroxidase-catalyzed biodegradation reactions can affect not only carbonaceous NPs but also many other types of NPs that utilize carbon-based coatings as a part of their formulation.
A large group of biocompatible polymeric NPs has been developed as novel drug delivery vehicles such as poly(lactic acid) (PLA), poly(lactic-*co*-glycolic acid) (PLGA), *etc.*, which can be degraded through either enzymatic or hydrolytic pathways. Along these lines, de Gracia Lux *et al.* developed polymeric capsules that contained aryl boronic ester protecting groups integrated into their polymer design [95]. Due to the cleavage of the boronic ester by biological levels of H_2O_2 (*i.e.* 50–100 μM), which resulted in the degradation of the polymer backbone, these nanoparticle demonstrated the ability to release cargo when incubated in physiological environments such as with activated neutrophils.
Ideally, delivery vehicles (*e.g*. CNTs or graphene derivatives) that transport theranostic payloads should be disposed of after arriving at their target destination sites. To this end, while many chemical methodologies have been developed to “oxidatively cut” and degrade CNT, the aggressive nature of

these oxidants precludes their possible employment in physiologically relevant environments (*i.e.* tissues and body biofluids). Consequently, this prompted an active search for possible enzymatic mechanisms and pathways whereby mild and controlled oxidation reactions would effectively biodegrade CNTs. The discovery of peroxidase-driven biodegradation processes is an important milestone on the path to regulated and targeted spatiotemporal degradation of CNT. Through either noncovalent functionalization or covalent attachment through stimuli-cleavable groups, specialized signaling molecules of lipid and/or protein nature will facilitate the internalization of CNTs. Also, one can envision the attachment of chemical/biological species that will activate the professional phagocytes. Acute and resolution phases of the body's inflammatory response are characterized by the predominant accumulation of myeloperoxidase (MPO)-rich PMNs and macrophages, respectively. One can envision that MPO-driven oxidative reaction will represent the dominant pathways for the CNTs biodegradation during the acute phase. In contrast, macrophages, which are relatively poor in MPO, may utilize a different oxidizing system such as peroxynitrite generators to be involved in the CNT biodegradation process. In this regard, NADPH-oxidase and NO synthases (*e.g.* iNOS) may be particularly important as sources of superoxide radicals and $NO^{\bullet}$, respectively. By producing these reactive oxygen and nitrogen species (*i.e.* ROS and RNS), macrophages are involved in the production of peroxynitrite, whose high oxidizing potential is sufficient to trigger CNT oxidative degradation. Therefore, by tailoring functionalization, one can envision a possible tool for regulating the biodegradation of CNTs coated with important payloads. Alternatively, nano-containers with encapsulated freight can be utilized in ways where oxidative biodegradation of the vehicle will facilitate the release of the inner contents as they reach the desired targets. While different types of nano-containers have been designed and fabricated, approaches towards the utilization of peroxidase-catalyzed oxidative biodegradation on these types of delivery nano-devices in target organs/cells have not yet been developed and represent an exciting future area of research.

*Pseudo-peroxidase degradation by adventitious transition metals present in nanomaterials***.** The production of carbonaceous nanomaterials is often associated with the employment of significant amounts of transition metal catalysts, including iron, copper, manganese, etc. The presence of these metals should be inevitably associated with the pseudo-peroxidase function inherent to nano-materials. Moreover, electron donor–acceptor specificity of nano-environments may be conducive to the unusual peroxidase-like activities of metals not traditionally associated with redox catalysis such as gold, silver, etc. Indeed, many studies have documented the peroxidase-like activities of a variety of metal-containing nanoparticles inherent to their structure or present as adventitious metals. Intrinsic catalytic activity of graphene oxide (GO) may be associated with its paramagnetic properties. As an illustration, we present EPR spectra of GO samples demonstrating the presence of Mn(II) inclusions with paramagnetic propensities and narrow paramagnetic signal of GO structure. Notably, the Mn-containing GO samples displayed peroxidase-like activity as revealed by their ability to oxidize typical peroxidase substrates Amplex Red and dichlorofluorescein (DCFH). This type of peroxidase activities associated with integrated or adventitious metals in nanoparticles can act as an important biodegradation factor. It may act as a self-propelled biodegradation mechanism, including one that may be intentionally built-in as a self-biodegradation factor.

Awakening of dormant peroxidase activity during interactions of hemoproteins with nano-surfaces. Specific interactions of nanomaterials with hemoproteins that can trigger the conversion of hexa- to penta-coordinated states of the heme iron in “dormant” peroxidases are of particular interest in the context of biodegradation. As a typical example, one can consider interactions of cytochrome *c* (cyt *c*), the hexa-coordinated hemoprotein in the intermembrane space of mitochondria, with GO. The negative charges on GO's surface favor binding to basic proteins such as cyt *c* that has eight positive charges on its surface. Extensive previous work established a very peculiar behavior of cyt *c* upon its interactions with negatively charged phospholipids, particularly, cardiolipin, a unique doubly-charged phospholipid of mitochondria. During this interaction, the protein undergoes structural rearrangements leading to its conversion from the hexa-coordinated to the penta-coordinated electron configuration resulting in “unmasking” of its peroxidase activity. This phenomenon has been extensively studied in mitochondria, cells, and tissues and its role in the execution of apoptotic cell death program and

consequences for tissue damage have been well established. Notably, these specific interactions of hemoproteins with nano-surfaces are meaningful in the context of degradation of nanomaterials. The schema of these interactions and several sets of experimental data illustrate the pathways and significance of GO binding of cyt *c* resulting in unfolding of the protein, weakening of the heme iron/Met80 sulfur bond and low to high spin transition leading to peroxidase activation. The respective transitions of cyt *c* can be characterized by spectral changes. Accordingly, cyt *c* displays significant peroxidase activity upon its binding to the GO surface. Most importantly, the peroxidase reactive intermediates of cyt *c* can directly oxidize GO causing its degradation detectable by visible-NIR spectroscopy, TEM and XPS**.** Thus, by unfolding and activating cyt *c* into a peroxidase, GO inflicts self-degradation. It is likely that cyt *c*/GO interactions represent a prototypical example of a very interesting new type of biodegradation reactions triggered by interactions of different hemoproteins with charged nano-surfaces. This new type of biodegradation reaction may lead to the design and development of new generations of nano-platforms for drug delivery as well as for modulating the physicochemical characteristics of nanomaterials. Indeed, oxidative modifications of GO have been associated with the changes of its conducting-semiconducting characteristics. In this regard, the entire family of globins, particularly a recently discovered cytoglobin can represent a promising instrument for the controlled and targeted modification/degrade action of carbonaceous nanomaterials.

Alternatives (non-peroxidase) to enzymatic oxidative degradation of nanomaterials. In addition to peroxidase-based mechanisms, other oxidative metabolic reactions may contribute to the biodegradation process. Among the physiologically relevant mechanisms, peroxynitrite generating reactions have been identified as potent mechanisms of nanoparticle biodegradation. Oxidative biodegradation of SWCNTs via superoxide/$NO^{\bullet}$ → peroxynitrite-driven pathways of activated macrophages facilitate clearance of nanoparticles from the lung. This particular pathway includes two enzymatic components producing $NO^{\bullet}$ and superoxide radicals, respectively — iNOS and NADPH oxidase. Interestingly, another generator of superoxide radicals, xanthine oxidase, can also contribute to degradation of oxidized multi-walled carbon nanotubes (ox-

Employment of enzymatic biodegradation of nano-containers for targeting inflammatory cells in cancer. Peroxidase degradation of payloads vs nano-containers — significance for drug delivery. Design and development of nano-platform based carriers for drug delivery represents one of the active fields for biomedical applications. In this context, effective timely degradation of drug carriers becomes particularly important but must be optimized with regards to nano-carrier vs drug payload degradation. It has been well documented that oxidative enzymes of inflammatory cells, particularly MPO, can catalytically destroy different types of small organic molecules, including drugs, in circulation. This wasteful drug metabolism may be exceptionally strong in pro-inflammatory conditions associated with increased amounts and activation of inflammatory cells. This raises the question of possible “protective” role of nano-carriers in preventing unnecessary degradation of payloads and preservation of their therapeutic potential. Notably, experimental assessments of nano-carrier vs drug degradation have not been adequately addressed. A recent study compared degradation of an antitumor drug, doxorubicin (DOX), in free form vs its conjugate with SWCNTs in the presence of MPO or ONOO-generating systems. The evaluations in simple biochemical enzymatic systems clearly demonstrated that the SWCNTs-associated drug molecules (DOX-SWCNTs) degraded more slowly than free DOX. Notably, cytostatic and cytotoxic effects of free DOX, but not nanotube-carried drug, on melanoma and lung carcinoma cell lines were abolished in the presence of tumor-activated MDSC known to express high levels of MPO, NADPH oxidase and iNOS thus providing enhanced myeloperoxidase- and peroxynitrite-induced conditions for biodegradation of organic molecules. Optimizing the balance between the degradation and resistance of the drug carrier and the payload towards the oxidants generated by inflammatory cells is critical to meet the needs for safety and prolonged circulation while orchestrating the stability and therapeutic effect of the drug. This strategy opens opportunities for exploring new parameters in biodegradation and developing controllable degradation properties by chemical modification of the surface of nanotubes.

Global phospholipidomics analysis reveals selective pulmonary peroxidation profiles upon inhalation of single-walled carbon nanotubes. Remarkable progress in nanotechnology has paved the way for a very broad utilization of different engineered nanomaterials, including single walled carbon nanotubes (SWCNT), for various technological and biomedical applications. In spite of such impressive benefits, the unique physico-chemical properties of SWCNT have raised concerns about potential risks for human health associated with their use. In fact, animal studies as well as new epidemiologic data indicate that exposure to SWCNT without sufficient protective measures may cause pulmonary toxicity, granuloma formation, chronic pulmonary inflammation leading to fibrosis and also mutagenic effects in the lungs. The generation of reactive oxygen species (ROS) culminating in severe oxidative stress is commonly viewed as a mechanism of nanomaterial-induced toxicity. Indeed, ROS driven peroxidation of lipids - particularly polyunsaturated phospholipids in cellular membranes - is considered to be one of the major mechanisms of lung injury triggered by SWCNT. In support of this notion, previous studies have shown that mice that are maintained on a diet deficient for vitamin E, the major lipid-soluble antioxidant, display an increased sensitivity to SWCNT-induced pulmonary inflammation and enhanced pro-fibrotic responses.

In addition to the non-specific deleterious effects of lipid peroxidation, oxidized phospholipids have also been recognized as important regulators of cell signaling, with essential physiological functions in signal transduction. In particular, lipid peroxidation has been implicated in the execution of apoptosis (programmed cell death) and in the subsequent removal of apoptotic cell corpses by phagocytic cells of the immune system (programmed cell clearance), a process that is crucial for the resolution of inflammatory responses. Moreover, enzymatic oxidation is involved in the biodegradation of SWCNT by the neutrophil myeloperoxidase. Therefore, peroxidation reactions may be viewed not only as a by-product of cellular oxidative stress but also as an important signaling event in physiological as well as pathophysiological processes including responses to nanomaterial exposure. We have pioneered the field of oxidative lipidomics and developed quantitative approaches to identification and characterization of individual molecular species of peroxidized phospholipids in different tissues, including the lung, after exposure to a variety of insults. We performed global mass spectrometry (MS)-based oxidative lipidomics analysis of all major classes of pulmonary phospholipids to determine whether random or selective profiles are characteristic of the lung responses after the inhalation exposure of mice to SWCNT. We demonstrated, for the first time, the specific peroxidation profiles of cellular phospholipids in the lungs of mice exposed to non-purified (iron-containing) SWCNT. We found that the exposure to SWCNT results in a selective lipid peroxidation rather than in non-specific free radical oxidation. Furthermore, the fact that we could detect specific peroxidation of CL and PS, and a concurrent elevation in the number of apoptotic cells, suggests the involvement of mitochondria-dependent apoptosis as well as macrophage disposal of apoptotic cells in the regulation of the inflammatory response to SWCNT. Further studies are warranted to uncover the source(s) of selective, likely enzymatic, lipid peroxidation triggered by exposure to SWCNT. In addition, oxidative lipidomics protocols may be applied to the study of other engineered nanomaterials, in the lung or in other tissues.

Conclusions

The currently accelerating progress in nanotechnologies has already accepted the “safe-by-design” principle as a necessary requisite in the development of new nanomaterials. This principle has to include the “safe-by-biodegradation” component, providing for the optimized life-time and clearance of nanoparticles from the body. Biopersistence of CNMs has been long viewed as the major factor contributing to the toxic effects of these nanomaterials in the body. Our discovery of the enzymatic CNT degradation processes opened new opportunities for the regulation of CNT distribution and fate *in vivo* by controlling inflammatory response and/or employing SWCNT-metabolizing enzymes. Essentially our studies have laid the fundation for the new field of nantoxicology. Indeed, the number of studies relating to the field of CNMs and biodegradation has grown vigorously. For example, PubMed revealed >15 entries using the search terms “oxidative biodegradation” and “carbon nanotubes” before 2008; an additional 280 publications have been added since then. Although research in the field has greatly expanded, significant amount of work is still warranted. To date, most research on enzyme-catalyzed

biodegradation has focused on different peroxidases. It is likely that other hemo-proteins with peroxidase activity merit investigation, including different forms of cytochrome P450, cytochrome c, *etc*. The specific roles and significance of these enzymes in biodegradation and regulation of inflammatory responses should be explored. Additionally, the assessment of the intermediates of biodegradation represents another area of research that merits investigation because the likely products—aliphatic and oxidized polyaromatic hydrocarbons—will provide details about the mechanism of CNM biodegradation and may exert their own specific, possibly toxic, effects on the body, which may also have to be taken into account.

As a result of our paradigm shifting research on enzyme-catalyzed biodegradation, one can envision futuristic applications in areas like drug delivery or imaging applications. For example, enzymes that result in biodegradation could be encapsulated inside nitrogen-doped nanocapsules, a CNM that could be employed to deliver drug or imaging cargo along with all the components for its self-elimination from the body. More practically, relatively non-toxic and inexpensive enzymes may be instrumental in environmental/occupational biodegradation of CNMs contamination introduced *via* spills, waste products in landfills, *etc*. Finally, an interesting avenue of research will be applying enzymatic biodegradation to other non-carbonaceous nanomaterials to mitigate toxicity.

Publications:
List of publiations:

Zou C, Synan MJ, Li J, Xiong S, Manni ML, Liu Y, Chen BB, Zhao Y, Shiva S, Tyurina YY, Jiang J, Lee JS, Das S, Ray A, Ray P, Kagan VE, Mallampalli RK: [2016] LPS impairs oxygen utilization in epithelia by triggering degradation of the mitochondrial enzyme Alcat1. J Cell Sci. 129(1):51-64. PMCID: PMC4732295.

Mao G, Qu F, St Croix CM, Tyurina YY, Planas-Iglesias J, Jiang J, Huang Z, Amoscato AA, Tyurin VA, Kapralov AA, Cheikhi A, Maguire J, Klein-Seetharaman J, Bayır H, Kagan VE: [2016] Mitochondrial Redox Opto-Lipidomics Reveals Mono-Oxygenated Cardiolipins as Pro-Apoptotic Death Signals. ACS Chem Biol. 11(2):530-40. PMID: 26697918**.**

Vlasova II, Kapralov AA, Michael ZP, Burkert SC, Shurin MR, Star A, Shvedova AA, Kagan VE: [2016] Enzymatic oxidative biodegradation of nanoparticles: Mechanisms, significance and applications. Toxicol Appl Pharmacol. 15;299:58-69. PMCID: PMC4811710.

Shvedova AA, Kisin ER, Yanamala N, Farcas MT, Menas AL, Williams A, Fournier PM, Reynolds JS, Gutkin DW, Star A, Reiner RS, Halappanavar S, Kagan VE: [2016] Gender differences in murine pulmonary responses elicited by cellulose nanocrystals. Part Fibre Toxicol. 13(1):28. PMCID: PMC4898310.

Kagan VE, Jiang J, Huang Z, Tyurina YY, Desbourdes C, Cottet-Rousselle C, Dar HH, Verma M, Tyurin VA, Kapralov AA, Cheikhi A, Mao G, Stolz D, St Croix CM, Watkins S, Shen Z, Li Y, Greenberg ML, Tokarska-Schlattner M, Boissan M, Lacombe ML, Epand RM, Chu CT, Mallampalli RK, Bayır H, Schlattner U: [2016] NDPK-D (NM23-H4)-mediated externalization of cardiolipin enables elimination of depolarized mitochondria by mitophagy. Cell Death Differ. 23(7):1140-51. PMCID: PMC4946882**.**

Maguire JJ, Tyurina YY, Mohammadyani D, Kapralov AA, Anthonymuthu TS, Qu F, Amoscato AA, Sparvero LJ, Tyurin VA, Planas-Iglesias J, He RR, Klein-Seetharaman J, Bayır H, Kagan VE: [2016] Known unknowns of cardiolipin signaling: The best is yet to come. Biochim Biophys Acta. pii: S1388-1981(16)30221-9. PMID: 27498292.

Sparvero LJ, Amoscato AA, Fink AB, Anthonymuthu T, New LA, Kochanek PM, Watkins S, Kagan VE, Bayır H: [2016] Imaging mass spectrometry reveals loss of polyunsaturated cardiolipins in the cortical

contusion, hippocampus, and thalamus after traumatic brain injury. J Neurochem. [Epub ahead of print]. PMID: 27591733.

Zhao Y, Burkert SC, Tang Y, Sorescu DC, Kapralov AA, Shurin GV, Shurin MR, Kagan VE, Star A: [2015] Nano-gold corking and enzymatic uncorking of carbon nanotube cups. J Am Chem Soc. 137(2):675-84. PMCID: PMC4308760.

Ji J, Baart S, Vikulina AS, Clark RS, Anthonymuthu TS, Tyurin VA, Du L, St Croix CM, Tyurina YY, Lewis J, Skoda EM, Kline AE, Kochanek PM, Wipf P, Kagan VE, Bayır H: [2015] Deciphering of mitochondrial cardiolipin oxidative signaling in cerebral ischemia-reperfusion. J Cereb Blood Flow Metab. 35(2):319-28. PMCID: PMC4426750.

Kisin ER, Yanamala N, Farcas MT, Gutkin DW, Shurin MR, Kagan VE, Bugarski AD, Shvedova AA: [2015] Abnormalities in the male reproductive system after exposure to diesel and biodiesel blend. Environ Mol Mutagen. 56(2):265-76. PMCID: PMC4946425.

Shvedova AA, Kisin ER, Yanamala N, Tkach AV, Gutkin DW, Star A, Shurin GV, Kagan VE, Shurin MR: [2015] MDSC and TGFβ Are Required for Facilitation of Tumor Growth in the Lungs of Mice Exposed to Carbon Nanotubes. Cancer Res. 75(8):1615-23. PMCID: PMC4401633.

Tyurina YY, Polimova AM, Maciel E, Tyurin VA, Kapralova VI, Winnica DE, Vikulina AS, Domingues MR, McCoy J, Sanders LH, Bayır H, Greenamyre JT, Kagan VE: [2015] LC/MS analysis of cardiolipins in substantia nigra and plasma of rotenone-treated rats: Implication for mitochondrial dysfunction in Parkinson's disease. Free Radic Res. 49(5):681-91. PMCID: PMC4430340.

Seo W, Kapralov AA, Shurin GV, Shurin MR, Kagan VE, Star A: [2015] Payload drug vs. nanocarrier biodegradation by myeloperoxidase- and peroxynitrite-mediated oxidations: pharmacokinetic implications. Nanoscale. 7(19):8689-94. PMCID: PMC4582775.

Kagan VE, Tyurina YY, Tyurin VA, Mohammadyani D, Angeli JP, Baranov SV, Klein-Seetharaman J, Friedlander RM, Mallampalli RK, Conrad M, Bayir H:[2015] Cardiolipin signaling mechanisms: collapse of asymmetry and oxidation. Antioxid Redox Signal. 22(18):1667-80. PMCID: PMC4486147.

Balasubramanian K, Maeda A, Lee JS, Mohammadyani D, Dar HH, Jiang JF, St Croix CM, Watkins S, Tyurin VA, Tyurina YY, Klöditz K, Polimova A, Kapralova VI, Xiong Z, Ray P, Klein-Seetharaman J, Mallampalli RK, Bayir H, Fadeel B, Kagan VE: [2015] Dichotomous roles for externalized cardiolipin in extracellular signaling: Promotion of phagocytosis and attenuation of innate immunity. Sci Signal. 8(395):ra95. PMCID: PMC4760701.

Mandal A, Hoop CL, DeLucia M, Kodali R, Kagan VE, Ahn J, van der Wel PC: [2015] Structural Changes and Proapoptotic Peroxidase Activity of Cardiolipin-Bound Mitochondrial Cytochrome c. Biophys J. 109(9):1873-84. PMCID: PMC4643272.

Shvedova AA, Yanamala N, Kisin ER, Tkach AV, Murray AR, Hubbs A, Chirila MM, Keohavong P, Sycheva LP, Kagan VE, Castranova V: [2014] Long-term effects of carbon containing engineered nanomaterials and asbestos in the lung: one year postexposure comparisons. Am J Physiol Lung Cell Mol Physiol. 306(2):L170-82, PMC3920208.

Tyurin VA, Balasubramanian K, Winnica D, Tyurina YY, Vikulina AS, He RR, Kapralov AA, Macphee CH, Kagan VE: [2014] Oxidatively modified phosphatidylserines on the surface of apoptotic cells are essential phagocytic 'eat-me' signals: cleavage and inhibition of phagocytosis by Lp-PLA2. Cell Death Differ. doi: 10.1038/cdd.2014.1. [Epub ahead of print] PMID: 24497278.

Chu CT, Bayır H, Kagan VE: [2014] LC3 binds externalized cardiolipin on injured mitochondria to signal mitophagy in neurons: Implications for Parkinson disease. Autophagy. 10(2):376-378, PMID: 24351649.

Stoyanovsky DA, Sparvero LJ, Amoscato AA, He RR, Watkins S, Pitt BR, Bayir H, Kagan VE: [2014] Improved spatial resolution of matrix-assisted laser desorption/ionization imaging of lipids in the brain by alkylated derivatives of 2,5-dihydroxybenzoic acid. Rapid Commun Mass Spectrom. 28(5):403-12, PMID: 24497278.

Jiang J, Bakan A, Kapralov AA, Silva KI, Huang Z, Amoscato AA, Peterson J, Garapati VK, Saxena S, Bayir H, Atkinson J, Bahar I, Kagan VE: [2014] Designing inhibitors of cytochrome c/cardiolipin peroxidase complexes: mitochondria-targeted imidazole-substituted fatty acids. Free Radic Biol Med. 71:221-30. PMCID: PMC4216591.

Tyurina YY, Poloyac SM, Tyurin VA, Kapralov AA, Jiang J, Anthonymuthu TS, Kapralova VI, Vikulina AS, Jung MY, Epperly MW, Mohammadyani D, Klein-Seetharaman J, Jackson TC, Kochanek PM, Pitt BR, Greenberger JS, Vladimirov YA, Bayır H, Kagan VE: [2014] A mitochondrial pathway for biosynthesis of lipid mediators. Nat Chem. 6(6):542-52. PMCID: PMC4201180.

Kagan VE, Kapralov AA, St Croix CM, Watkins SC, Kisin ER, Kotchey GP, Balasubramanian K, Vlasova II, Yu J, Kim K, Seo W, Mallampalli RK, Star A, Shvedova AA: [2014] Lung macrophages "digest" carbon nanotubes using a superoxide/peroxynitrite oxidative pathway. ACS Nano. 8(6):5610-21. PMCID: PMC4072413.

Shurin MR, Yanamala N, Kisin ER, Tkach AV, Shurin GV, Murray AR, Leonard HD, Reynolds JS, Gutkin DW, Star A, Fadeel B, Savolainen K, Kagan VE, Shvedova AA: [2014] Graphene oxide attenuates Th2-type immune responses, but augments airway remodeling and hyperresponsiveness in a murine model of asthma. ACS Nano. 8(6):5585-99. PMCID: PMC4072415.

Amoscato AA, Sparvero LJ, He RR, Watkins S, Bayir H, Kagan VE:[2014] Imaging mass spectrometry of diversified cardiolipin molecular species in the brain. Anal Chem. 86(13):6587-95. PMCID: PMC4082390.

Mohammadyani D, Tyurin VA, O'Brien M, Sadovsky Y, Gabrilovich DI, Klein-Seetharaman J, Kagan VE: [2014] Molecular speciation and dynamics of oxidized triacylglycerols in lipid droplets: Mass spectrometry and coarse-grained simulations. Free Radic Biol Med. 76:53-60. PMCID: PMC4276254.

Yanamala N, Kapralov AA, Djukic M, Peterson J, Mao G, Klein-Seetharaman J, Stoyanovsky DA, Stursa J, Neuzil J, Kagan VE: [2014] Structural re-arrangement and peroxidase activation of cytochrome c by anionic analogues of vitamin E, tocopherol succinate and tocopherol phosphate. J Biol Chem. 289(47):32488-98. PMCID: PMC4239604.

Schlattner U, Tokarska-Schlattner M, Ramirez S, Tyurina YY, Amoscato AA, Mohammadyani D, Huang Z, Jiang J, Yanamala N, Seffouh A, Boissan M, Epand RF, Epand RM, Klein-Seetharaman J, Lacombe ML, Kagan VE: [2013] Dual function of mitochondrial Nm23-H4 protein in phosphotransfer and intermembrane lipid transfer: a cardiolipin-dependent switch. J Biol Chem. 288(1):111-21, PMC3537004.

Andón FT, Kapralov AA, Yanamala N, Feng W, Baygan A, Chambers BJ, Hultenby K, Ye F, Toprak MS, Brandner BD, Fornara A, Klein-Seetharaman J, Kotchey GP, Star A, Shvedova AA, Fadeel B, Kagan VE: [2013] Biodegradation of Single-Walled Carbon Nanotubes by Eosinophil Peroxidase. Small. doi: 10.1002/smll.201202508. [Epub ahead of print]. PMID: 23447468.

Kotchey GP, Zhao Y, Kagan VE, Star A: [2013] Peroxidase-mediated biodegradation of carbon nanotubes in vitro and in vivo. Adv Drug Deliv Rev. 65(15):1921-32, PMC3855904.

Tyurina YY, Winnica DE, Kapralova VI, Kapralov AA, Tyurin VA, Kagan VE: [2013] LC/MS characterization of rotenone induced cardiolipin oxidation in human lymphocytes: implications for mitochondrial dysfunction associated with Parkinson's disease. Mol Nutr Food Res. 57(8):1410-22, PMC3810210.

Chu CT, Ji J, Dagda RK, Jiang JF, Tyurina YY, Kapralov AA, Tyurin VA, Yanamala N, Shrivastava IH, Mohammadyani D, Qiang Wang KZ, Zhu J, Klein-Seetharaman J, Balasubramanian K, Amoscato AA, Borisenko G, Huang Z, Gusdon AM, Cheikhi A, Steer EK, Wang R, Baty C, Watkins S, Bahar I, Bayır H, Kagan VE: [2013] Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. Nat Cell Biol. 15(10):1197-205, PMC3806088.

Yanamala N, Hatfield MK, Farcas MT, Schwegler-Berry D, Hummer JA, Shurin MR, Birch ME, Gutkin DW, Kisin E, Kagan VE, Bugarski AD, Shvedova AA: [2013] Biodiesel versus diesel exposure: enhanced pulmonary inflammation, oxidative stress, and differential morphological changes in the mouse lung. Toxicol Appl Pharmacol. 272(2):373-83, PMID:23886933.

Kotchey GP, Gaugler JA, Kapralov AA, Kagan VE, Star A: [2013] Effect of antioxidants on enzyme-catalysed biodegradation of carbon nanotubes. J Mater Chem B Mater Biol Med. 1(3):302-309, PMC3634595.

Yanamala N, Kagan VE, Shvedova AA: [2013] Molecular modeling in structural nano-toxicology: interactions of nano-particles with nano-machinery of cells. Adv Drug Deliv Rev. 65(15):2070-7, PMC3819408.

Tyurina YY, Tungekar MA, Jung MY, Tyurin VA, Greenberger JS, Stoyanovsky DA, Kagan VE: [2012] Mitochondria targeting of non-peroxidizable triphenylphosphonium conjugated oleic acid protects mouse embryonic cells against apoptosis: role of cardiolipin remodeling. FEBS Lett. 586(3):235-41. PMCID: PMC3273856

Shvedova AA, Pietroiusti A, Fadeel B, Kagan VE: [2012] Mechanisms of carbon nanotube-induced toxicity: focus on oxidative stress. Toxicol Appl Pharmacol. 1;261(2):121-33, PMID: 22513272.

Samhan-Arias AK, Ji J, Demidova OM, Sparvero LJ, Feng W, Tyurin V, Tyurina YY, Epperly MW, Shvedova AA, Greenberger JS, Bayır H, Kagan VE, Amoscato AA: [2012] Oxidized phospholipids as biomarkers of tissue and cell damage with a focus on cardiolipin. Biochim Biophys Acta. 2012 Mar 23. [Epub ahead of print] PMID: 22464971

Shvedova AA, Kapralov AA, Feng WH, Kisin ER, Murray AR, Mercer RR, St Croix CM, Lang MA, Watkins SC, Konduru NV, Allen BL, Conroy J, Kotchey GP, Mohamed BM, Meade AD, Volkov Y, Star A, Fadeel B, Kagan VE: [2012] Impaired clearance and enhanced pulmonary inflammatory/fibrotic response to carbon nanotubes in myeloperoxidase-deficient mice. PLoS One. 7(3):e30923. Epub 2012 Mar 30. ID:PMC3316527.

Shi J, Karlsson HL, Johansson K, Gogvadze V, Xiao L, Li J, Burks T, Garcia-Bennett A, Uheida A, Muhammed M, Mathur S, Morgenstern R, Kagan VE, Fadeel B: [2012] Microsomal glutathione transferase 1 protects against toxicity induced by silica nanoparticles but not by zinc oxide nanoparticles. ACS Nano. 6(3):1925-38. PMCID:PMC3314313

Kapralov AA, Feng WH, Amoscato AA, Yanamala N, Balasubramanian K, Winnica DE, Kisin ER, Kotchey GP, Gou P, Sparvero LJ, Ray P, Mallampalli RK, Klein-Seetharaman J, Fadeel B, Star A, Shvedova AA, Kagan VE: [2012] Adsorption of Surfactant Lipids by Single-Walled Carbon Nanotubes in Mouse Lung upon Pharyngeal Aspiration. ACS Nano. 2012 Apr 6. [Epub ahead of print] PMID:22463369.

Murray AR, Kisin ER, Tkach AV, Yanamala N, Mercer RR, Young SH, Fadeel B, Kagan VE, Shvedova AA: [2012] Factoring-in agglomeration of carbon nanotubes and nanofibers for better prediction of their toxicity versus asbestos. Part Fibre Toxicol. 2012 Apr 10;9(1):10. [Epub ahead of print] PMID:22490147

Sparvero LJ, Amoscato AA, Dixon CE, Long JB, Kochanek PM, Pitt BR, Bayir H, Kagan VE: [2012] Mapping of phospholipids by MALDI imaging (MALDI-MSI): realities and expectations. Chem Phys Lipids. 165(5):545-62, PMID: 22692104.

Murray AR, Kisin E, Inman A, Young SH, Muhammed M, Burks T, Uheida A, Tkach A, Waltz M, Castranova V, Fadeel B, Kagan VE, Riviere JE, Monteiro-Riviere N, Shvedova AA: [2012] Oxidative Stress and Dermal Toxicity of Iron Oxide Nanoparticles In Vitro. Cell Biochem Biophys. Jun 5, [Epub ahead of print], PMID: 22669739.

Tkach AV, Yanamala N, Stanley S, Shurin MR, Shurin GV, Kisin ER, Murray AR, Pareso S, Khaliullin T, Kotchey GP, Castranova V, Mathur S, Fadeel B, Star A, Kagan VE, Shvedova AA: [2012] Graphene Oxide, But Not Fullerenes, Targets Immunoproteasomes and Suppresses Antigen Presentation by Dendritic Cells. Small. Aug 13, doi: 10.1002/smll.201201546. [Epub ahead of print] PMID: 22887961

Shvedova AA, Tkach AV, Kisin ER, Khaliullin T, Stanley S, Gutkin DW, Star A, Chen Y, Shurin GV, Kagan VE, Shurin MR: [2012] Carbon Nanotubes Enhance Metastatic Growth of Lung Carcinoma via Up-Regulation of Myeloid-Derived Suppressor Cells. Small. Sep 20, doi: 10.1002/smll.201201470. [Epub ahead of print]. PMID: 22996965.

Ji J, Kline AE, Amoscato A, Samhan-Arias AK, Sparvero LJ, Tyurin VA, Tyurina YY, Fink B, Manole MD, Puccio AM, Okonkwo DO, Cheng JP, Alexander H, Clark RS, Kochanek PM, Wipf P, Kagan VE, Bayır H: [2012] Lipidomics identifies cardiolipin oxidation as a mitochondrial target for redox therapy of brain injury. Nat Neurosci. 15(10):1407-13, PMID: 22922784.

Kotchey GP, Hasan SA, Kapralov AA, Ha SH, Kim K, Shvedova AA, Kagan VE, Star A.: [2012] A natural vanishing act: the enzyme-catalyzed degradation of carbon nanomaterials. Acc Chem Res. 45(10):1770-81, PMC3473158.

Tyurin VA, Yanamala N, Tyurina YY, Klein-Seetharaman J, Macphee CH, Kagan VE.: [2012] Specificity of Lipoprotein-Associated Phospholipase A(2) toward Oxidized Phosphatidylserines: Liquid Chromatography-Electrospray Ionization Mass Spectrometry Characterization of Products and Computer Modeling of Interactions. Biochemistry. 51(48):9736-50, PMC3567262.

Samhan-Arias AK, Tyurina YY, Kagan VE: [2011] Lipid antioxidants: free radical scavenging versus regulation of enzymatic lipid peroxidation. J Clin Biochem Nutr. 48(1):91-5. PMCID: PMC3022072.

Kisin ER, Murray AR, Sargent L, Lowry D, Chirila M, Siegrist KJ, Schwegler-Berry D, Leonard S, Castranova V, Fadeel B, Kagan VE, Shvedova AA.: [2011] Toxicol Genotoxicity of carbon nanofibers: Are they potentially more or less dangerous than carbon nanotubes or asbestos? Appl Pharmacol. 252(1):1-10. PMID:21310169.

Kotchey GP, Allen BL, Vedala H, Yanamala N, Kapralov AA, Tyurina YY, Klein-Seetharaman J, Kagan VE, Star A.: [2011] The enzymatic oxidation of graphene oxide. ACS Nano. 5(3):2098-108. PMC3062704.

Belikova NA, Glumac AL, Kapralova V, Cheikhi A, Tyurina YY, Vagni VA, Kochanek PM, Kagan VE, Bayir H.: [2011] A high-throughput screening assay of ascorbate in brain samples. J Neurosci Methods. 201(1):185-90. PMCID: PMC3276367.

Jiang J, Maeda A, Ji J, Baty CJ, Watkins SC, Greenberger JS, Kagan VE.: [2011] Are mitochondrial reactive oxygen species required for autophagy? Biochem Biophys Res Commun. 412(1):55-60 PMCID: PMC3171134.

Tkach AV, Shurin GV, Shurin MR, Kisin ER, Murray AR, Young SH, Star A, Fadeel B, Kagan VE, Shvedova AA.: [2011] Direct effects of carbon nanotubes on dendritic cells induce immune suppression upon pulmonary exposure. ACS Nano. 5(7):5755-62. PMCID: PMC3170729.

Tyurin VA, Cao W, Tyurina YY, Gabrilovich DI, Kagan VE.:[2011] Mass-spectrometric characterization of peroxidized and hydrolyzed lipids in plasma and dendritic cells of tumor-bearing animals. Biochem Biophys Res Commun. 413(1):149-53. PMID: 21872574.

Tyurina YY, Kisin ER, Murray A, Tyurin VA, Kapralova VI, Sparvero LJ, Amoscato AA, Samhan-Arias AK, Swedin L, Lahesmaa R, Fadeel B, Shvedova AA, Kagan VE.: [2011] Global phospholipidomics analysis reveals selective pulmonary peroxidation profiles upon inhalation of single-walled carbon nanotubes. ACS Nano. 5(9):7342-53. PMCID: PMC3321726.

Kapralov AA, Yanamala N, Tyurina YY, Castro L, Samhan-Arias A, Vladimirov YA, Maeda A, Weitz AA, Peterson J, Mylnikov D, Demicheli V, Tortora V, Klein-Seetharaman J, Radi R, Kagan VE.: [2011] Topography of tyrosine residues and their involvement in peroxidation of polyunsaturated cardiolipin in cytochrome c/cardiolipin peroxidase complexes. Biochim Biophys Acta. 1808(9):2147-55. PMCID: PMC3321730.

Atkinson J, Kapralov AA, Yanamala N, Tyurina YY, Amoscato AA, Pearce L, Peterson J, Huang Z, Jiang J, Samhan-Arias AK, Maeda A, Feng W, Wasserloos K, Belikova NA, Tyurin VA, Wang H, Fletcher J, Wang Y, Vlasova II, Klein-Seetharaman J, Stoyanovsky DA, Bayîr H, Pitt BR, Epperly MW, Greenberger JS, Kagan VE.: [2011] A mitochondria-targeted inhibitor of cytochrome c peroxidase mitigates radiation-induced death. Nat Commun. 11;2:497. doi: 10.1038/ncomms1499. PMID:21988913

Ray NB, Durairaj L, Chen BB, McVerry BJ, Ryan AJ, Donahoe M, Waltenbaugh AK, O'Donnell CP, Henderson FC, Etscheidt CA, McCoy DM, Agassandian M, Hayes-Rowan EC, Coon TA, Butler PL, Gakhar L, Mathur SN, Sieren JC, Tyurina YY, Kagan VE, McLennan G, Mallampalli RK.: [2010] Dynamic regulation of cardiolipin by the lipid pump Atp8b1 determines the severity of lung injury in experimental pneumonia. Nat Med. 16(10):1120-7. PMID: 20852622.

Sparvero LJ, Amoscato AA, Kochanek PM, Pitt BR, Kagan VE, Bayir H.: [2010] Mass-spectrometry based oxidative lipidomics and lipid imaging: applications in traumatic brain injury. J Neurochem. 115(6):1322-36. PMID:20950335.

Herber DL, Cao W, Nefedova Y, Novitskiy SV, Nagaraj S, Tyurin VA, Corzo A, Cho HI, Celis E, Lennox B, Knight SC, Padhya T, McCaffrey TV, McCaffrey JC, Antonia S, Fishman M, Ferris RL, Kagan VE, Gabrilovich DI.: [2010] Lipid accumulation and dendritic cell dysfunction in cancer. Nat Med. 16(8):880-6. PMCID: PMC2917488.

Huang Z, Jiang J, Belikova NA, Stoyanovsky DA, Kagan VE, Mintz AH.: [2010] Protection of normal brain cells from γ-irradiation-induced apoptosis by a mitochondria-targeted triphenyl-phosphonium-nitroxide: a possible utility in glioblastoma therapy. J Neurooncol. 100(1):1-8. PMID:20835910

All other information and media dissemination

Kagan, VE: [2016] Plenary Lecture at the Congress of Occupational Medicine, Rome, Italy, September 14-26.

Tyurin VA, Veglia F, Amoscato A, Angelini R, Murphy M, Kapralov A, Mohammadyani1 D, Gabrilovich DI and Kagan VE: [2016] LC-ESI-MS/MS analysis of oxygenated lipid droplets in dendritic cells treated with tumor explant supernatants. 64th American Society for Mass Spectrometry Conference on Mass Spectrometry and Allied Topics June 5 - 9, San Antonio, TX.

Tyurina Y, Amoscato A, Tyurin Vl, Qu F, Mao G, Bayir H, Kagan V: [2016] Identification and quantification of oxygenated arachidonoyl- and adrenoyl-phosphatidylethanolamines as ferroptotic death signals using oxidative phospholipidomic. 64th American Society for Mass Spectrometry Conference on Mass Spectrometry and Allied Topics June 5 - 9, San Antonio, TX**.**

Schlattner U, Boissan M, Montagnac G, Tokarska-Schlattner M, Cottet-Rousselle C, Desbourdes C, Lacombe M-L, Griparic L, Huang Z, Tyurina YY, Jiang J, van der Bliek AM, Roux A, Chavrier P, Kagan VE: [2015] Mitochondrial NM23-H4/NDPk-D is Multifunctional: Fueling Mitochondrial GTPase OPA1 and Triggering Mitophagy [abstract]. In: Biophysical Journal, January; 108(2):369a. DOI:10.1016/j.bpj.2014.11.2020.

Kagan VE, Jiang AND Kagan VE, Huang Z, Tyurin VA, Tyurina Y, Stolz D, Watkins S, Mallampalli R: [2014] Signaling Role of Cardiolipin Externalization in Elimination of Damaged Mitochondria in Lung Epithelial Cells. Poster presented at: 53rd Annual Meeting for Society of Toxicology; [abstract]. In: The Toxicologist, v.138, Issue 1, Mar 23-27; Phoenix, Arizona.

Kagan VE: [2014] ROS and Oxidative Lipidomics of Apoptotic Signaling. Gordon Research Conference: Oxygen Radicals from Detection to Disease, February 9-14, Ventura Beach Marriott, Ventura, CA.

A. A. Kapralov, C. M. St. Croix, S. C. Watkins, E. R. Kisin, G. P.Kotchey , K. Balasubramanian, I. I. Vlasova, H. Bayir, A. Star, A.A. Shvedova, V. E. Kagan A: [2014] New Role of Lung Macrophages in Carbon Nanotubes-Induced Injury. Oxidative Biodegradation via Superoxide/Peroxynitrite Pathway. "NANOTOX 2014, 7th International Nanotoxicology Congress", April 23--26, Antalya, Turkey.

M. Shurin, A. V. Tkach, E. Kisin, T. Khaliullin, S. Stanley, D. Gutkin, A. Star, Y. Chen, G. Shurin, V. Kagan and A. A. Shvedova: [2013] Carbon Nanotubes Enhance Metastatic Growth of Lung Carcinoma via Up-Regulation of Myeloid-Derived Suppressor Cells. 52nd Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. 1, V. 132, Abstract No. 461, p. 98, March 10-14, San Antonio, TX.

Y. Tyurina, D. Winnica, V. Kapralova, V. Tyurin and V. Kagan: [2013] Cardiolipin As a Biomarker of Mitochondrial Dysfunction Associated with Parkinson's Disease. 52nd Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. 1, V. 132, Abstract No. 1888, p. 401, March 10-14, San Antonio, TX.

V. Tyurin, W. Cao, J. Loomen, V. Kagan and D. Gabrilovich: [2013] Lipid Droplets with Oxygenated Fatty Acids and Triglycerides in Dendritic Cells: Possible Role in Antigen Presentation in Cancer. 52nd Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. 1, V. 132, Abstract No. 2008, p. 428, March 10-14, San Antonio, TX.

V. Kagan, V. Tyurin, S. Poloyac, M. Epperly, J. Greenberger, H. Bayir and Y. Tyurina: [2013] Mitochondrial Cardiolipin As a Substrate for Cytochrome C Catalyzed Production of Oxygenated Lipid Mediators. 52nd Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. 1, V. 132, Abstract No. 2009, p. 428, March 10-14, San Antonio, TX.

Star, A. Kapralov, A. Amoscato, V. Tyurin, W. Seo, M. Epperly, J. Greenberger, Y. Tyurina and V. Kagan: [2013] Development of a Mitochondria-Targeted Nano-Complex of Imidazole-Substituted Oleic Acid As a Radiomitigator. 52nd Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. 1, V. 132, Abstract No. 2010, p. 428, March 10-14, San Antonio, TX.

V. Kagan: [2012] RedOx Endeavors of Nanoparticles: from Oxidative Lipidomics to Oxidative Biodegradation, Emerging Trends In Free Radicals, Antioxidants and Nutraceuticals in Health, Disease and Radiation Biology, Satellite Meeting of the Society for Free Radical Resaerch-India (SFRR-India), Department of Biochemistry, College of Medicine & JNM Hospital, WBUHS, Kalyani January 10-11.

S. C. Stanley, A. V. Tkach, M. R. Shurin, G. V. Shurin, E. Kisin, A. R. Murray, S. Pareso, S. Leonard1, S. H. Young1, B. Fadeel3, S. Mathur4, A. Star, G. P. Kotchey, V. Castranova, V. E. Kagan and A. A. Shvedova: [2012] Pulmonary exposure of graphene oxide and fullerenes causes inflammation and modifies the immune response. 51th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.126, Suppl. 1, Abstract No. 669, p.145, March 11-15, San Francisco CA.

J. Frostegård, V. Tyurin, Y. Tyurina, A. Amoscato, J. Su, K. Balasubramanian, A. Maeda, B. Fadeel, J. Greenberger, R. Mallampali, B. Pitt and V. Kagan: [2012] Interrelationship of peroxidation and immunogenicity of cardiolipin: an oxidative lipidomics approach. 51th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.126, Suppl. 1, Abstract No. 760, p.165, March 11-15, San Francisco CA.

A. Samhan-Arias, V. Tyurin, Y. Tyurina, H. Bayir, V. Kagan and A. Amoscato: [2012] 2D-LC-MS Analysis of oxidized lipid species: application to the study of cardiolipin in apoptosis and damages tissue. 51th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.126, Suppl. 1, Abstract No. 1180, p.254, March 11-15, San Francisco CA.

L. J. Sparvero, A. Amoscato, B. Pitt and V. Kagan: [2012] MALDI mass spectrometric imaginf of cardiolipin and its oxidation products direct from tissue sections in a lung oxidative damage model. 51th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.126, Suppl. 1, Abstract No. 1182, p.254, March 11-15, San Francisco CA.

A. R. Murray, E. Kisin, A. Tkach, S. H. Young, V. Castranova, B. Fadeel, V. E. Kagan and A. A. Shvedova: [2012] Comparative toxic effects of nickel oxide nanoparticels. 51th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.126, Suppl. 1, Abstract No. 1388, p.299, March 11-15, San Francisco CA.

A. Tkach, G. V. Shurin, M. R. Shurin, E. R. Kisin, A. R. Murray, S. H. Young, A. Star, B. Fadeel, V. E. Kagan and A. A. Shvedova: [2012] Carbon nanotubes induce immune suppression via direct effect on dendritic cells. 51th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.126, Suppl. 1, Abstract No. 1279, p.276, March 11-15, San Francisco CA.

V. A. Tyurin, H. Zhao, Y. Tyurina, M. W. Epperly, J. S. Greenberger and V. E. Kagan: [2012] Mono-lyso-cardiolipin – biomarker of mitochondrial dysfunction unduced by total body irradiation. LC-ESI-MS study. 51th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.126, Suppl. 1, Abstract No. 2034, p.438, March 11-15, San Francisco CA.

Y. Tyurina, M. A. Tungekar, M. Jung, V. A. Tyurin, D. A. Stoyanovsky and V.E. Kagan: [2012] Mitochondria targeting of nonperoxidizable triphenylphosphonium conjugated oleic acid protects mouse embryonic cells against apoptosis: role of cardiolipin remodeling. 51th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.126, Suppl. 1, Abstract No. 2035, p.438, March 11-15, San Francisco CA.

J. Atkinson, A. Kapralov, N. Yanamala, Y. Tyurina, A. Amoscato, L. Pearce, J. Peterson, Z. Huang, J. Jiang, A. Samhan-Arias, A. Maeda, W. Feng, N. Belikova, V. Tyurin, H. Wang, J. Fletcher, Y. Wang, I. Vlasova, J. Klein-Seetharaman, D. Stoyanovsky, H. Bayir, B. Pitt, M. Epperly, J. Greenberger and V. Kagan: [2012] A mitochondria-targeted imidazole substituted fatty acid inhibits cytochrome c peroxidase and mitigates radiation induced death. 51th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.126, Suppl. 1, Abstract No. 2036, p.439, March 11-15, San Francisco CA.

Z. Huang, J. Greenberger, V. Kagan and H. Bayir: [2012] A combination of anti-apoptotic /anti-necrotic inhibitors protects NCCIT cells from gamma-irradiation induced cell death. 51th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.126, Suppl. 1, Abstract No. 2046, p.441, March 11-15, San Francisco CA.

V. E. Kagan, V. A. Tyurin, K. Balasubramanian, D. E. Winnica, C. H. Macphee and Y. Tyurina: [2012] Role of oxygenated phosphatidylserine and its metabolites – lyso-phosphatidylserine and its metabolites – lyso-phosphatidylserines produces after oxidation and hydrolysis by plasma lipoprotein-associated phospholipase A2 in clearance of apoptotic cells by macrophages: LC-ESI-MS study. 51th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.126, Suppl. 1, Abstract No. 2056, p.443, March 11-15, San Francisco CA.

A. Amoscato, W. Feng, V. E. Kagan and Y. Tyurina: [2012] Rotenone-induced oxidized molecular species of cardiolipin in human lymphocytes: can they be used as biomarkers of mitochondrial dysfunction associated with Parkinson's disease? 51th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.126, Suppl. 1, Abstract No. 2450, p.528, March 11-15, San Francisco CA.

V. Kagan: [2012] Oxidative Biodegradation of Nanomaterials. NanoSafety Congress - Turkey, Antalya, April 26-29.

V. Kagan: [2012] Oxidative Stress Biomarkers of Exposure to Carbon Nanotubes - Mass-Spectrometry Based Oxidative Lipidomics, NanoSafety Congress - Turkey, Antalya, April 26-29.

V. Kagan: [2011] Redox Interactions of Carbon Nanotubes: Pathways and Consequences, 4th NANOMUNE Consortium Meeting, Stockholm, Sweden, June 17.

V. Kagan: [2011] Oxidative Stress and Nanoparticles: Badness is Only Spoiled Goodness, NT11-Satellite Symposium: Carbon Nanomaterial Biology, Medicine & Toxicology University of Cambridge, Cambridge, July 15-16.

V. Kagan: [2011] Redox Adventures of Carbon Nanotubes: from Oxidative Lipidomics to Biodegradation, THE 2nd International School "Nanomaterials and nanotechnologies in living systems safety and nanomedicine" Moscow, Russia, September 21.

V. Kagan: [2011] Redox Adventures of Carbon Nanotubes: from Oxidative Lipidomics to Biodegradation, Third Annual Conference of the American Society for Nanomedicine, November 9-11, Universities at Shady Grove, Rockville, Maryland.

V. Kagan: [2011] Biodegradation of carbon-based nanomaterials, oxidative lipidomics. Carbon Nanotubes: From Safety Assessment to Biomedical Applications, One – day Workshop, 29th November, Brussels.

Kagan V.E: [2011] Use of oxidative lipidomics and signaling by oxidized lipids in safety screening of nanoparticles. 50th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.120, Suppl. 2, Abstract No. 25, p.5, March 6-10, Washington DC.

Tyurina YY, Tyurin VA, Sparvero LJ, Amoscato AA, Kapralova V, Kisin E, Murray A, Shi B, Fadeel A, Shvedova AA and Kagan VE: [2011] Oxidative lipidomics reveals selective, but not random, pulmonary phopsholipid peroxidation after inhalation of carbon nanotubes. 50th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.120, Suppl. 2, Abstract No. 252, p.1179, March 6-10, Washington DC.

Kaparalov AA, Yanamala N, feng WH, Fadeel B, Star AA, Shvedova AA, Kagan VE: [2011] Biodegradation of carbon nanotubes by eosinophil peroxidase. 50th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.120, Suppl. 2, Abstract No. 54, p.10, March 6-10, Washington DC.

Kisin E, Murray AR, Sargent L, Lowry D, Siegrist K, Chirila M, Schwegler-Berry D, Leonard S, Castranova V, Fadeel B, Kagan VE, Shvedova AA: [2011] Comparative genotoxicity of fibrous particles: carbon nanofibers, single-walled carbone nanotubes, and asbestos. 50th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.120, Suppl. 2, Abstract No. 1180, p.252, March 6-10, Washington DC.

Tkach A, Kisin E, Murray AR, Shurin GV, Shurin MR, Young SH, Star A, Fadeel B, Kagan VE, Shvedova AA: [2011] Pulmonary exposure to carbonaceous nanoparticles affects local and systemic immunity. 50th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.120, Suppl. 2, Abstract No. 1189, p.254, March 6-10, Washington DC.

Murray AR, Kisin E, Inman AO, Young SH, Muhammed M, Burks T, Uheida A, Tkach A, Waltz AM, Castranova V, Fadeel B, Riviere JE, Kagan VE, Monteiro-Riviere NA, Shvedova AA: [2011] Iron oxide nanoparticels cause oxidative stress and dermal toxicity. 50th Annual Meeting for Society of Toxicology; The Toxicologist: Suppl. V.120, Suppl. 2, Abstract No. 2072, p.444, March 6-10, Washington DC.

Kagan VE: [2010] Oxidative Stress and Biodegradation of Single-Walled Carbon Nanotubes: Toxicological and Biomedical Implications. 2nd Nobel Forum Mini-Symposium on Nanotoxicology: Understanding the Interactions of Engineered Nanomaterials with Biological Systems Nobel Forum, Karolinska Institutet, Stockholm, Sweeden, October 23.
2010;100(1):1-8. PMID:
20835910

1. Cumulative Inclusion Enrollment Table: N/A

2. Inclusion of gender and minority study subject: N/A

3. Inclusion of Children: N/A

4. Materials available for the other investigators.

The project generated several useful resources. The results obtained during the fulfillment of the funded project include:

a) data and conditions on bio-degradation of carbonaceous nanomaterials by different oxidizing enzymes in vitro;
b) data on biodegradation of carbonaceous nanomaterials by different oxidizing enzymes of inflammatory cells;
c) data on bio-degradation of carbonaceous nanomaterials in the lung of animals after inhalation or aspiration in vivo;
d) materials on pulmonary toxicity of carbonaceous nanomaterials;
e) information on mass-spectrometric characterization of phospholipid oxidation products accumulating in the lung of mice exposed to SWCNT.

All this information is shared with the research community in the form of presentations and posters at national and international meetings as well as through systematic publications of results in peer-reviewed journals as shown above.