HELLENIC REPUBLIC
National and Kapodistrian
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EST. 1837

Toxicity of Metallic Nanoparticles in the CNS

A Thesis Submitted to the Department of Pharmacy, School of Health Sciences,
School of Medicine
National and Kapodistrian University of Athens,

Interdisciplinary Postgraduates Studies Program "Nanomedicine"

Master of Sciences

By

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Department of Pharmacy, School of Health Sciences, School of Medicine

National and Kapodistrian University of Athens
Greece
September , 2021

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Dedication

This Dissertation is dedicated to my parents

who have given me invaluable educational opportunities and have been a source of motivation and strength during moments of discouragement and despair.

Thanks for your great support and continuous care.
You are the reason of what I become today.

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ABSTRACT

Due to their tiny size and their unique physicochemical properties, metal nanoparticles have discovered a variety of uses in various fields related to industry and medicine. Over the years, scientists have shown particular interest in the extent to which the understanding of the human race has evolved in terms of the influence of nanoparticles on living organisms, especially humans. All this interest in the sensitivity of the structure of the central nervous system as it is considered one of the most sensitive and exposed organs of the human body that are affected by the metal nanoparticles. Because of this, it is necessary to determine the ways in which they are exposed and how understandable the mechanisms by which the nanoparticles affect the nervous tissue are considered. The information is based on the literature review, where studies have shown important information from official forums and articles related to the neurotoxicity of metal nanoparticles. The route of entry of the nanoparticles is described, as well as their classification, penetration through the cell membrane and the blood-brain barrier. There are also a number of studies that report on neurotoxicity in vitro and in vivo, as well as some of the functions that may be to blame for the negative effects of metallic nanoparticles on the human central nervous system.

KEYWORDS

Nanoparticles, Neurotoxicity, Brain, Environmental exposure, Oxidative Stress.

ΠΕΡΙΛΗΨΗ

Τα μεταλλικά νανοσωματίδια εξαιτίας του μικροσκοπικού τους μεγέθους και των αποκλειστικών φυσικοχημικών ιδιοτήτων τους έχουν ανακαλύψει διαλεκτές χρήσεις σε διάφορους τομείς που αφορούν την βιομηχανία και την ιατρική. Επομένως, επί συναρτόμενα έτη διακρίνεται μια αύξηση του ενδιαφέροντος ανάμεσα στους επιστήμονες για τη εξέλιξη της κατανόησής μας για την επιρροή των νανοσωματιδίων στους ζώντες οργανισμούς, ιδιαιτέρως στους ανθρώπους. Λαμβάνοντας υπόψη την ευαισθησία της δόμησης του κεντρικού νευρικού συστήματος το οποίο θεωρείται ένα από τα όργανα που είναι πιο επισφαλή στις δυσμενείς επιδράσεις των μεταλλικών νανοσωματιδίων. Για το λόγο αυτό, είναι απαραίτητο να προσδιορίσουν οι τρόποι έκθεσης και να γίνουν αντιληπτοί οι μηχανισμοί της επήρειας των νανοσωματιδίων στον νευρωνικό ιστό. Σε αυτήν την βιβλιογραφική ανασκόπηση, επιχειρείται η προβολή των σημερινών πληροφορίων που σχετίζονται με τη νευροτοξικότητα των μεταλλικών νανοσωματιδίων βασιζόμενοι πάντα σε επιλεγμένα επιστημονικά δημοσιεύματα. Γίνεται περιγραφή της οδού της εισόδου των νανοσωματιδίων, καθώς και η κατάταξη τους, η εισχώρηση μέσω της κυτταρικής μεμβράνης και του φραγμού αίματος-εγκεφάλου. Επίσης, παρουσιάζεται μια έρευνα που σχετίζεται με τη νευροτοξικότητα in vitro και in vivo, καθώς και κάποιοι από τις λειτουργίες που ενδέχεται να φταίνε για τις απορριπτικές επιδράσεις των μεταλλικών νανοσωματιδίων στο κεντρικό νευρικό σύστημα.

ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ

Νανοσωματίδια, Νευροτοξικότητα, Εγκέφαλος, Έκθεση στο περιβάλλον, Οξειδωτικό Στρες.

INTRODUCTION

With the lightning spread of nanotechnology, nanomaterials (NPs) seem to have great potential for use due to their special properties in recent years (Khan, 2017). Of all the types of NPs, metal nanoparticles (MNPs) have been of considerable interest due to the special properties of NPs as mentioned above such as their small size which contrasts with the higher surface to volume ratio as well as distinct electronic, magnetic, optical and mechanical properties as well as the shape of the particles. MNPs mainly include metal nanoparticles and metal oxide nanoparticles and MNPs have been widely included in a wide variety of products and industries, such as electronics, cosmetics, paints, food additives and biological and medical systems (Andra, 2018, Parada, 2018). With the widespread use of MNPs, it is inevitable that MNPs will explore the environment or come into direct contact with humans. Therefore, potential threats to human health and the environment have emerged due to their value for attention (Made, 2017). MNPs have the ability to enter the body in a variety of ways, such as through the breath, gastrointestinal tract, or skin, and through the blood or lymphatic system, to enter and accumulate in various organs of the body (Yah, 2012). Previous studies on metal nanoparticles and metal oxide nanoparticles, including nano-Cu, nano-Ag, nano-Ni, nano-TiO2 and nano-ZnO, have shown that MNPs result in lung and gastrointestinal tract and in the course they entered the systemic circulation while in the course they were concentrated in possible target organs such as the liver and the mononuclear phagocytic system (Gustafson, 2015). The liver occupies the second position of exposure, as it has been shown to accumulate MNP in much larger quantities compared to the rest of the body (Almeida, 2011). Meanwhile, MNPs that are concentrated in the liver result in the interaction of liver cells and the ability to alter the structure and motility of liver cells. The liver is a complex network of interconnected cells, containing about 60-80% of hepatocytes, and the extra cells include Kupffer cells, hepatic sinusoidal endothelial cells, hepatic astral cells, and so on. The influences of MNPs associated with liver cells determine the fate of MNPs administered in vivo and the outcome of hepatotoxicity. In vivo studies aim to concentrate MNPs in the body organs, while most in vitro studies target liver cells and do not summarize changes in subcellular levels and their association with hepatotoxicity. It is important, therefore, to summarize the research on the hepatotoxicity of MNPs in animals, at the cellular and

subcellular level, but also in relation to the molecular mechanism and its effects. Also, the physicochemical features of MNPs, such as size, surface characteristics and chemical composition would change and affect their potential toxicity. From these events, the aim of this literature review is to collect and conference the effects of hepatotoxicity of MNPs both in vitro and in vivo, especially those involved at subcellular levels as well as to highlight the molecular function of these MNPs.

The advent of nanoparticle systems has had a strong impact on a wide range of disciplines, opening up new possibilities and energies in a wide range of uses. The characteristics of nanomaterials may differ from those presented in their bulk form and, in some cases, may provide completely unexpected physical and chemical characteristics. This is one of the reasons why today many industries and manufacturers use nanomaterials and nanotechnologies in the products they produce as they believe that they can thus benefit from the modern possibilities that provide them.

Many of the nanomaterial inputs are in the fields of medicine and technology, and many studies explore the possibilities and uses of biosensors, biomaterials, tissue engineering, DNA modification, and drug delivery systems (Chen et al. 2006; Lu et al. 2008 • Kim et al. 2009; Sun et al. 2009; Kirkpatrick & Bonfield 2010). Another branch that has benefited from these developments is microbiology, where the inhibitory effect of nanoparticles on microbes may be seen as an aid in the eradication and control of focal diseases. However, the influence on microbes also needs to be thoroughly investigated, as it reveals the potential influence of nanoparticles on living organisms. Inaccurate information related to the toxicity of nanoparticles in various areas of the human body reveals that the issue of protection remains incomplete. Several studies have further promoted this issue. For example, synthetic nanomaterials have been shown to provide dialect-activated regions that have the potential to induce carcinogenic, mutagenic, or cytotoxic effects (Seemayer et al. 1990; Seaton et al. 2010).

The nanoparticles are 100 times smaller in size than normal red blood cells, which makes them have more potential for interaction, and it is suggested that the nanoparticles interact with proteins, DNA (Seeman 2006) and lung cells. However, knowledge about the interaction of nanoparticles with poppy cells is alive and other biological systems are vital, especially as the dynamics and use of such technologies

has increased, urging health professionals and the general public to use such materials. . As we will give in the example, metal nanoparticle systems are easy to apply and a number of antimicrobial properties are useful in the production of products that we use every day, the range of which ranges from a simple deodorizer to specialized Ren computer machines (Allaker &).

Exactly the same goes for nuclear technology, the sudden appearance of personal computers and genetically modified crops / animals, more than 68% of people now estimate that nanotechnology "will do the things that will provide a profitable future" (Royal Academy of Engineering 2004).

CHAPTER 1 CENTRAL NERVOUS SYSTEM

1.1 Anatomy of Central Nervous System

The central nervous system is connected by the brain to the spinal cord.

The central nervous system is considered to be one of the most complex and concurrent organ systems in the body. It provides data through the intervention of the spinal cord and processes it through the brain. The nervous system acts as a guide that directs the actions of the human body in everyday life while at the same time it has the control of the function of the internal organs, such as the movement of the muscles, the dilation of the blood vessels until the learning of the anatomy and physiology data. . One wonders how the commanders all this? The answer is simple through signals, electrical and chemical, that lie between the cells (Johnson, 2013).

In combination, the central nervous system (CNS) and the peripheral nervous system (PNS) transmit and examine sensory information while being responsible for the functions of the human body. The control center is dependent on the brain and spinal cord (the CNS). They receive information while at the same time commanding feedback from the sensory organs and nerves throughout the system, while processing the data they receive and transmitting the commands back again. The PNS carries pathways through the neurons that transmit signals which they receive and send back. The cranial nerves are made up of twelve pairs that intertwine the brain with the eyes, ears and other sensory organs as well as the muscles of the head and neck. Thirty-one pairs of spinal nerves break down from the spinal cord into the tissues of the chest, abdomen, and limbs. Each nerve is responsible for transmitting information to the sensory organs, motion command, or both (Zimmerman-Viehoff 2016).

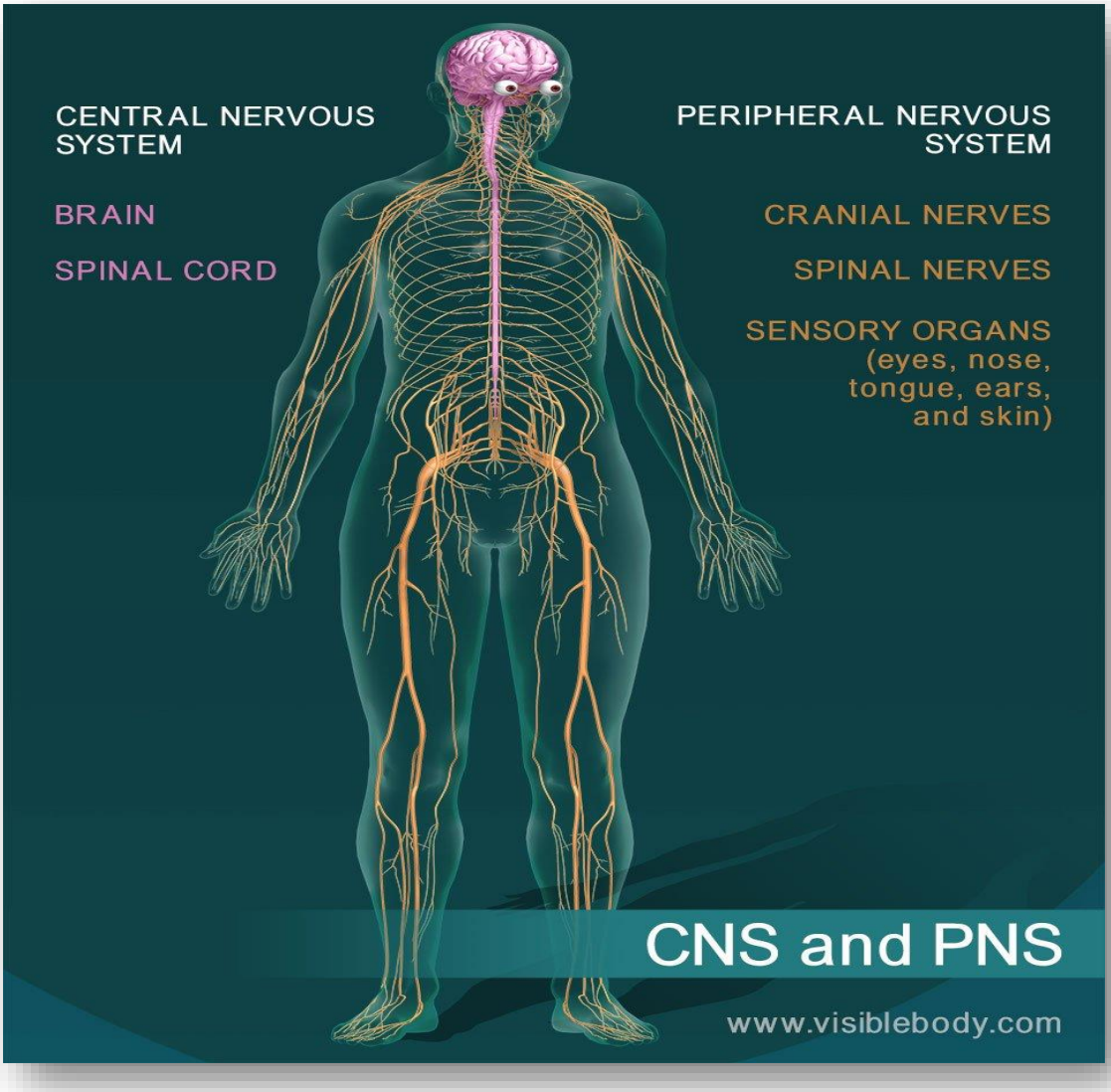


Image 1.1.1 The Brain and Spinal Cord Are the Central Nervous System. Nerves and Sensory Organs Make Up the Peripheral Nervous System (Zimmerman-Viehoff 2016).

1.1.1 Neurons

Neurons are cells that contain nerve tissue that travels from the brain to the spinal cord to the farthest nerve branch. Neurons are cells that are charged and transmit electrical messages to transmit information through the body. A typical neuron consists of the cell body, dendrites, and a terminal axis (Arthur 2010). Dendrites receive information from body tissues or other neurons and in turn transmit it to the cell body. If an outgoing message occurs, it zippers the axis to its axon terminal and

passes to the next neuron or target cell. This conductive dexterity transmits data up and down nerve pathways through the central nervous system with astonishing speed. Nearly 100 billion neurons provide the brain with its tremendous processing power (Bunsawat 2015).

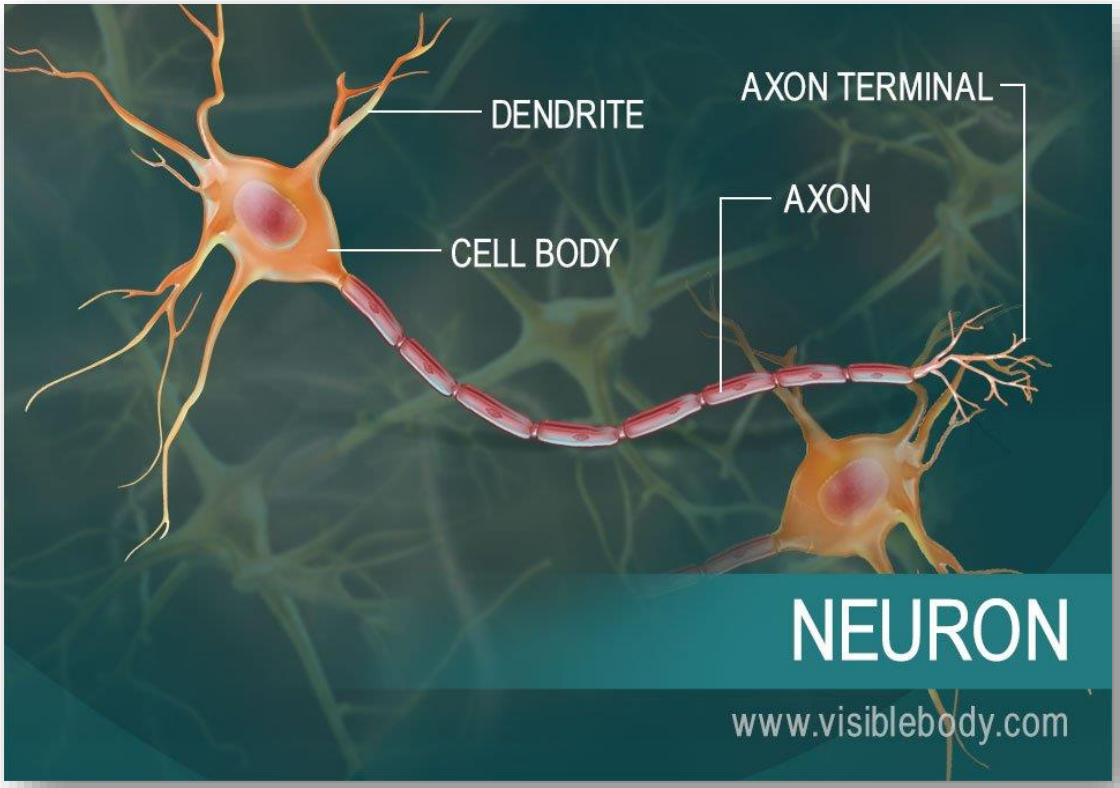


Image 1.1.1.1 Neurons in Nervous Tissue Relay Rapid-Fire Signals (Bunsawat 2015).

Neurons have the ability to transmit signals sent by the central nervous system as electrical signals. These alerts reach every neuron and respond to chemicals called neurotransmitters. Neurotransmitters travel in groups, in spaces between neurons and other tissues and cells in the body (Miller 2020). Neurotransmitters are classified into two types: stimulants and inhibitors. Stimulant neurotransmitters say this and their name stimulates electrical signals in other neurons and encourages responses from the body's cells. Inhibitory neurotransmitters work in the opposite way, they discourage signals and cellular responses (Zimmerman-Viehoff 2016). The nervous system regulates through the chemicals released the action of the muscles, glands and its own nerve pathways.

1.1.2 Spinal Cord

The spinal cord is an elongated cylinder of bodies of neuronal cells, bundles of axons and other cells, which is protected by connective tissue and bone. It is related to the brain in the medulla oblongata and passes under the spine, the concave tunnel enclosed in the vertebrae of the spine. The spinal cord is part of the central nervous system which functions as a kind of superhighway. The information of the sensory organs as well as the commands that are received are browsed up and down, while then they move towards the part of the brain. These signals travel rapidly in and out of the spinal cord through the spinal nerves — the "ramps inside and outside the ramp" that break down to power the limbs, trunk, and pelvis. Some of the signals received need a simple but immediate response. The spinal cord may release a reflex command without the intervention of the brain (Johnson, 2013).

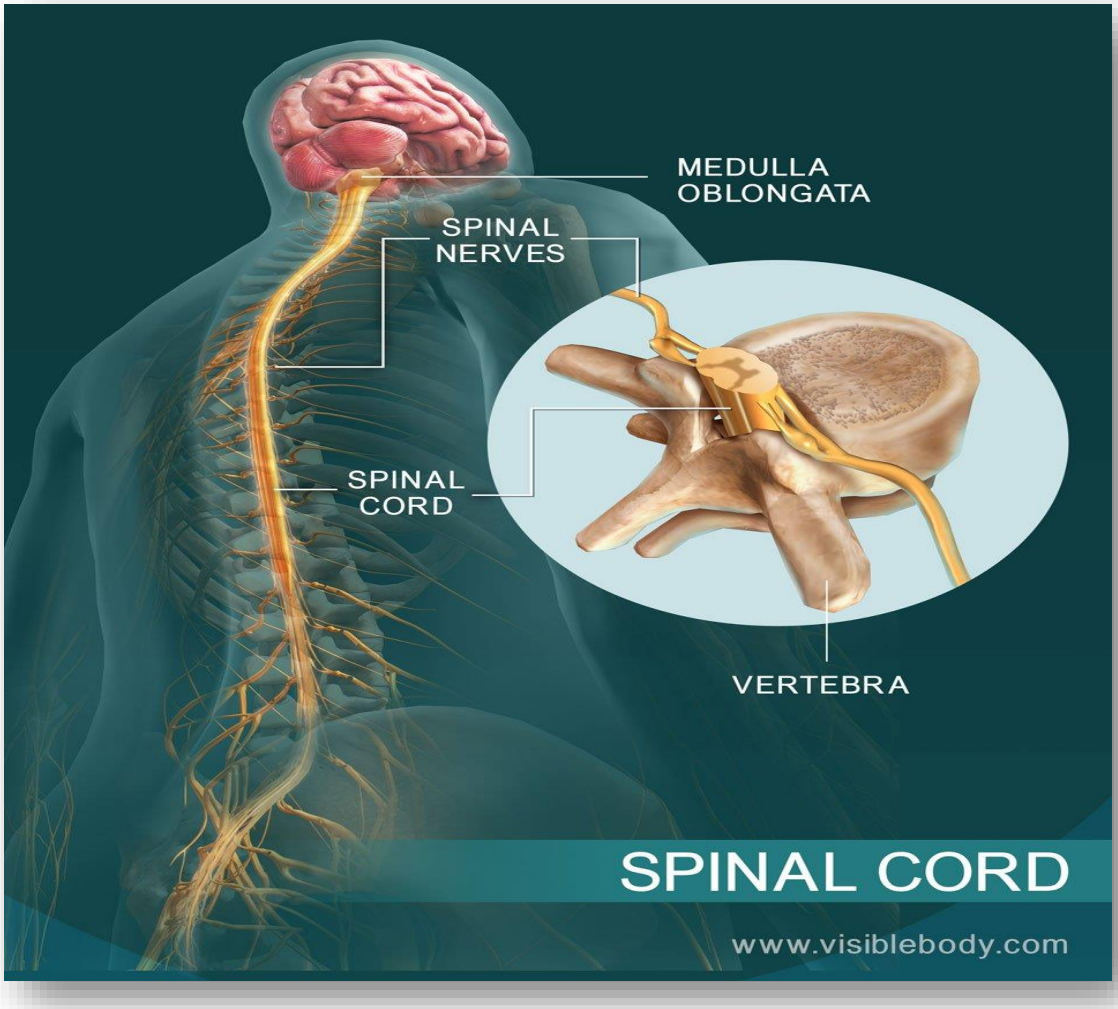


Image 1.1.2.1 The Spinal Cord Transmits Signals to and from the Brain and Commands Reflexes (Bunsawat 2015).

1.1.3 The Brain

The nervous system not only likes route commands and processing it provides much more. Sometimes specific smells take us back to older memories. The border system is responsible for all this. The parietal system creates two paired rings within the brain, consisting of the hippocampus and the amygdala, in conjunction with other structures and pathways (Miller 2020). The parietal system as well as other parts of the brain do not provide a single function but multiple functions at different levels of action of the nervous system. It strengthens the field of memory and smell and manages a series of emotions. The smell of food from a pot may have taken you back to a memory of grandma's food. It is also possible to travel to a dinner party from another era and flood you with feelings of nostalgia, joy and even remorse (Hadhazy, 2010).

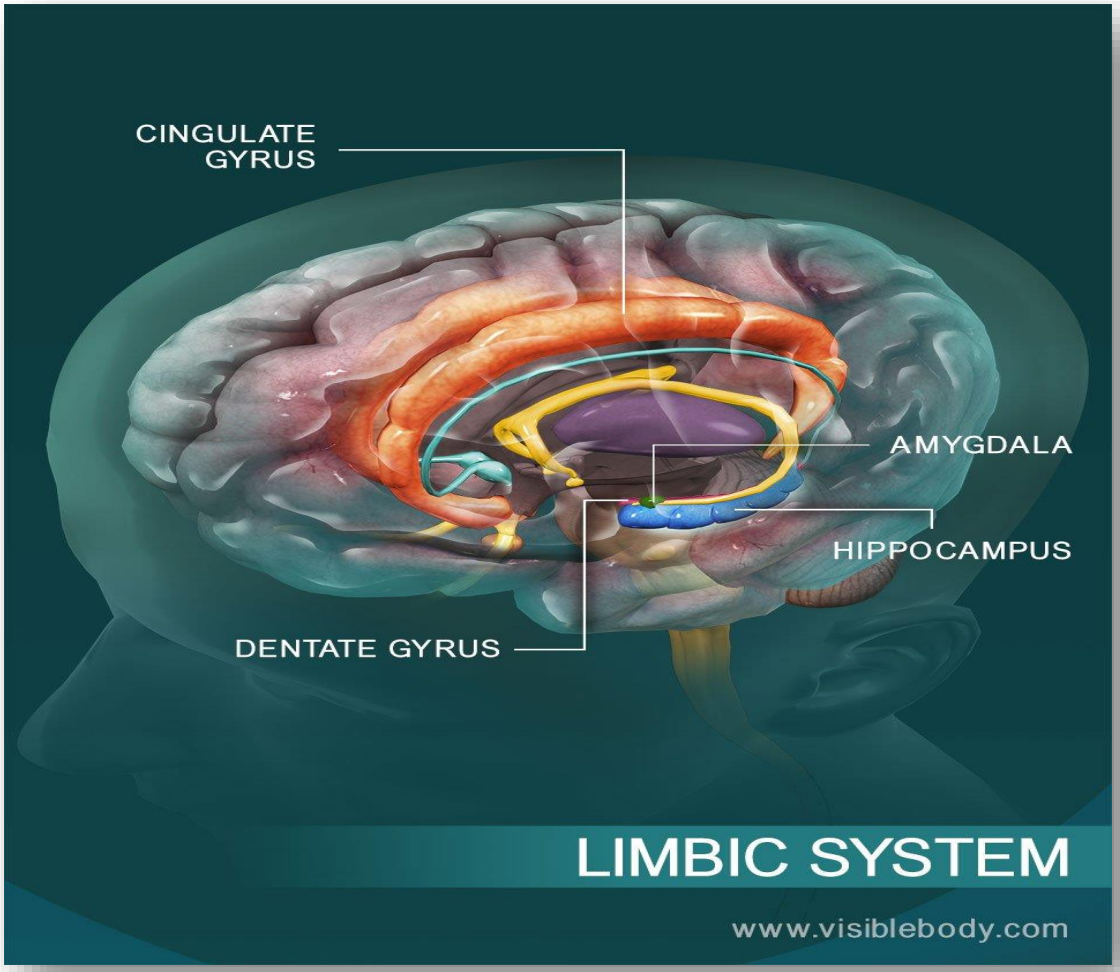


Image 1.1.3.1 The Brain Connects Perceptions to Complex Thought, Memory, and Emotion (Hadhazy, 2010).

1.2 Parts of the Nervous System

1.2.1 Peripheral Nervous System (PNS)

The peripheral nervous system (PNS) is one of the two components that make up the nervous system of bipolar animals, with the other part being the central nervous system (CNS). The PNS consists of nerves and ganglia which are located outside the brain and spinal cord (Pranav 2013). The most important action of the PNS is to connect the CNS to the extremities and other organs, making use of a relay between the brain and the spinal cord and the other organs (Goldstein 2016). While the CNS is observed to be protected by the spine and skull or by the blood-brain barrier, this is true in the PNS as it is exposed to toxins and mechanical injuries (Goldstein 2016).

The peripheral nervous system is divided into the bodily nervous system and the autonomic nervous system. In the somatic nervous system, the cranial nerves are part of the PNS with the exception of the optic nerve (cranial nerve II), along with the retina. The second cranial nerve is not a true peripheral nerve but a tract of the diencephalon (Johnson 2013). Cranial nerve ganglia originated in the CNS. However, the remaining ten cranial nerve axons extend beyond the brain and are therefore considered part of the PNS (Bunsawat 2015). The autonomic nervous system exerts involuntary control over smooth muscle and glands (Bunsawat 2015). The connection between CNS and organs allows the system to be in two different functional states: sympathetic and parasympathetic.

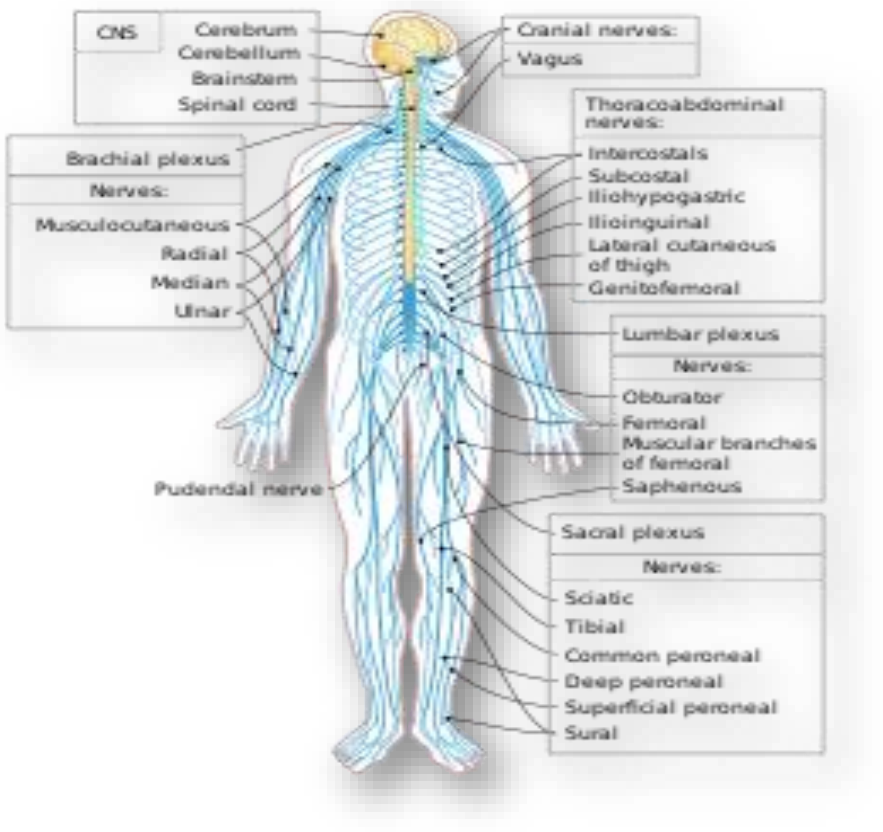


Image 1.2.1.1 Peripheral nervous system (Bunsawat 2015).

1.2.2 Central Nervous System (CNS)

The central nervous system (CNS) is the part of the nervous system consisting primarily of the brain and spinal cord (Thau 2020). The CNS is so named because the brain integrates the received information and coordinates and influences the activity of all parts of the bodies of bilaterally symmetric animals—i.e., all multicellular animals except sponges and jellyfish. It consists of a large nerve running from the anterior to the posterior, with the anterior end is enlarged into the brain. Not all animals with a central nervous system have a brain, although the large majority do (Gilbert 2014).

The rest of this article exclusively discusses the vertebrate central nervous system, which is radically distinct from all other animals.

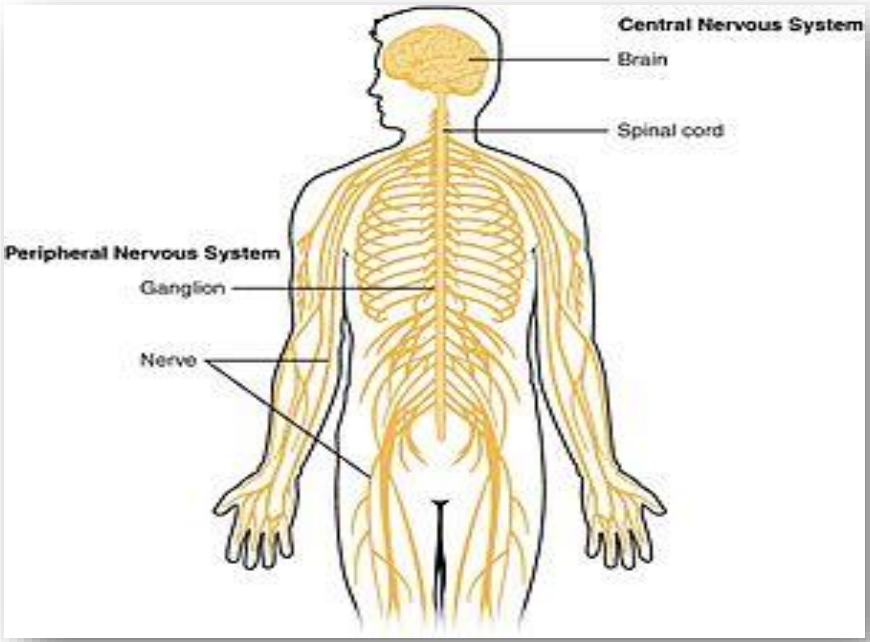


Image 1.2.2.1 Central nervous system (Gilbert 2014).

1.2.3 Autonomic Nervous System (ANS)

The autonomic nervous system (ANS), formerly the vegetative nervous system, is a division of the peripheral nervous system that supplies smooth muscle and glands, and thus influences the function of internal organs (Matic 2014). The autonomic nervous system is a control system that acts largely unconsciously and regulates bodily functions, such as the heart rate, digestion, respiratory rate, pupillary response,

urination, and sexual arousal (Laight 2013). This system is the primary mechanism in control of the fight-or-flight response.

The autonomic nervous system through the brain carries reflexes that are channeled into the spinal cord and organs. It works autonomously while its actions include the control of breathing, the regulation of the heart (the center of heart control), the vasomotor activity (vasomotor center) and some direct actions such as coughing, sneezing, swallowing and vomiting. These then pass to other sections and are also connected to autonomic subsystems and the peripheral nervous system. The hypothalamus, located in the upper part of the brain stem, acts as the one that incorporates the energies that are autonomous, receiving autonomous regulatory penetration from the parietal system (Alberts 2012).

The autonomic nervous system has three branches: the sympathetic nervous system, the parasympathetic nervous system and the enteric nervous system. (Matic 2014, Laight 2013). Some textbooks do not include the enteric nervous system as part of this system (Pranav 2013).The sympathetic nervous system is often considered the "fight or flight" system, while the parasympathetic nervous system is often considered the "rest and digest" or "feed and breed" system. In many cases, both of these systems have "opposite" actions where one system activates a physiological response and the other inhibits it. An older simplification of the sympathetic and parasympathetic nervous systems as "excitatory" and "inhibitory" was overturned due to the many exceptions found. A more modern characterization is that the sympathetic nervous system is a "quick response mobilizing system" and the parasympathetic is a "more slowly activated dampening system", but even this has exceptions, such as in sexual arousal and orgasm, wherein both play a role (Alberts 2012).

There are inhibitory and excitatory synapses between neurons. Relatively recently, a third subsystem of neurons that have been named non-noradrenergic, non-cholinergic transmitters (because they use nitric oxide as a neurotransmitter) have been described and found to be integral in autonomic function, in particular in the gut and the lungs (Monda 2009).

Although the ANS is also known as the visceral nervous system, the ANS is only connected with the motor side (Monda 2009). Most autonomous functions are

involuntary but they can often work in conjunction with the somatic nervous system which provides voluntary control.

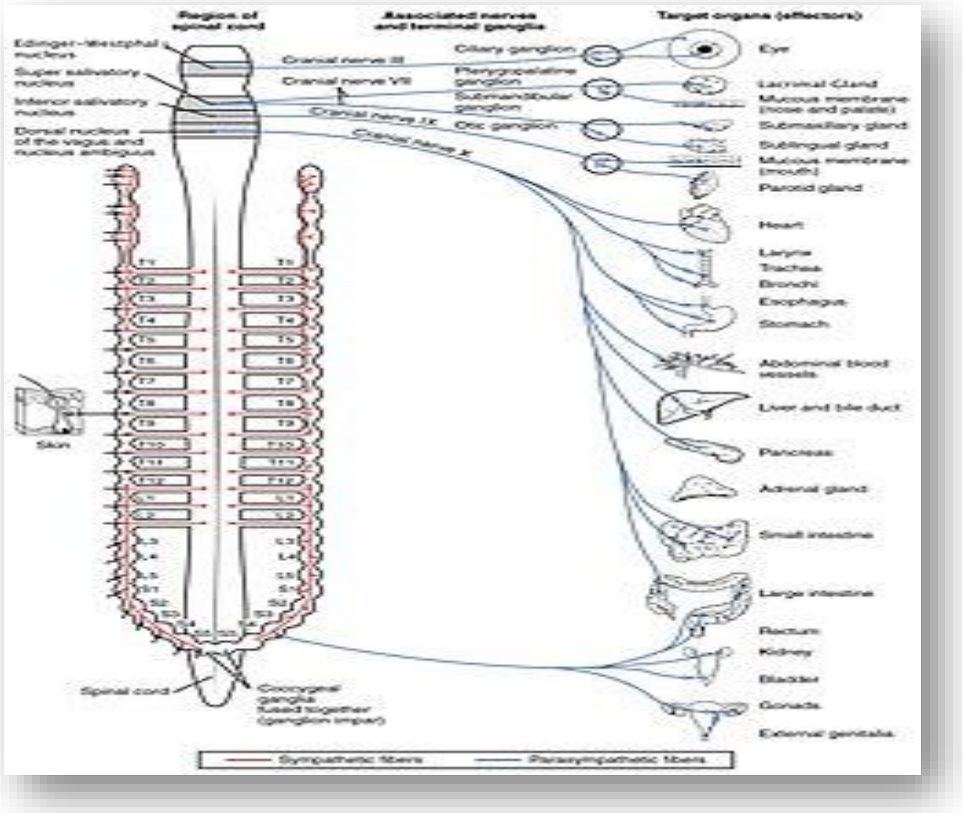


Image 1.2.3.1 Autonomic nervous system innervation (Monda 2009).

CHAPTER 2 METALLIC NANOPARTICLES

2.1 Definition of Metallic Nanoparticles

Metallic nanoparticles are, as the name suggests, nanosized metals with at least one of the dimensions within 1 to 100 nm. Under this definition one can classify metallic nanomaterials in to four main categories. They are, metallic nanoparticles (0D), metallic nanowires and rods (1D), metallic sheets and platelets (2D) metallic nanostructures (3D). The most extensively explored metallic nanoparticles are gold and silver. However, scientists have developed metal nanoparticles with almost all stable metals in the periodic table.

Metallic nanomaterials have attracted large number of scientists over the years and has grown in to a distinct stream of research. This interest is primarily due number of advantageous properties these materials have shown that may unlock new pathways in the field of nanotechnology. Among others, the field of biomedical science and engineering has heavily utilized metallic nanomaterials. This is primarily due to the fact that these materials can be synthesized and modified with appropriate functional groups that would allow them to bind with drugs, antibodies, ligands: substances of high interest in biomedical field (Sekar RP 2013).

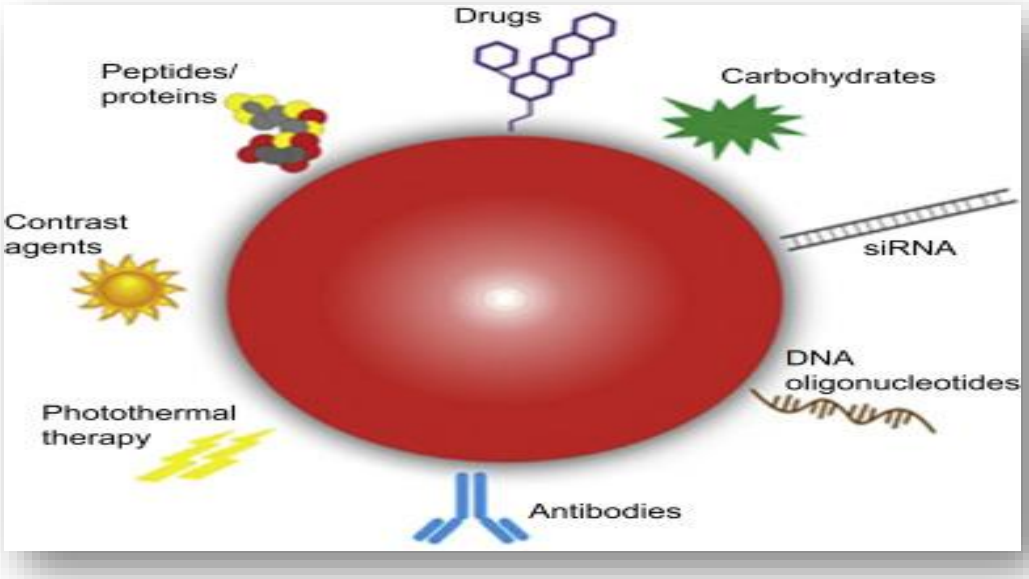


Image 2.1.1 Metallic Nanoparticles.

2.2 ***Types of Nanoparticles***

Nanoparticles can be classified into separate types based on size, morphology, physical and chemical characteristics. Some of these are carbon-based nanoparticles, ceramic nanoparticles, metal nanoparticles, semiconductor nanoparticles, polymer nanoparticles and lipid-based nanoparticles.

Carbon-based nanoparticles

Carbon-based nanoparticles contain two important materials: carbon nanotubes (CNT) and fullerenes. CNTs take the form of graph sheets that are wrapped in a tube. The use of the above materials is performed to strengthen the structure which has a strength of at least 100 times more than steel.

CNTs can be classified into two types, single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). CNTs have a uniqueness as they have thermal conductivity along the length and non-thermal conductivity along the tube (Khan, Ibrahim 2017).

The so-called fullerenes are allotropic coals which take the form of a hollow cage of sixty or more carbon atoms. The structure of the C-60 is called Buckminsterfullerene. The carbon units found in these structures have a pentagonal and hexagonal layout. These are applied in the commercial sector due to their electrical conductivity, as well as their structure, high durability and electron affinity.

Ceramic Nanoparticles

Ceramic nanoparticles are inorganic solids made up of oxides, carbides, carbonates and phosphates. These nanoparticles have high heat resistance and chemical inertness. They have applications in photo catalysis, photo degradation of dyes, drug delivery, and imaging.

By controlling some of the characteristics of ceramic nanoparticles like size, surface area, porosity, surface to volume ratio, etc., they perform as a good drug delivery agent. These nanoparticles have been used effectively as a drug delivery system for a number of diseases like bacterial infections, glaucoma, cancer, etc (Khan, Ibrahim 2017).

Metal Nanoparticles

Metal nanoparticles are made from a series of precursor metals. These nanoparticles can be combined with some chemical, electrochemical or photochemical systems. In chemical systems, the metallic nanoparticles are obtained by yielding the metal ion precursors in a solution comprising chemical reducing agents. These have the ability to draw small molecules and have a high energy surface.

As a rule, they are applied in research, for the monitoring and mapping of biomolecules related to the environment and biology. Giving an example, gold nanoparticles are used to process the sample before the SEM sensor. This is done to upgrade the electronic flow, which makes it easier for us to accept SEM images which are of higher quality (Khan, Ibrahim 2017).

Semiconductor Nanoparticles

Semiconductor nanoparticles have similar characteristics to those of metals and non-metals. They are located in the periodic table in a set of groups such as II-VI, III-V or IV-VI. The so-called particles have band gaps which are large in size, while in terms of coordination they have different characteristics. They are applied in photocatalysis, in electronic devices, as well as applications that separate water.

Some examples of semiconductor nanoparticles are GaN, GaP, InP, InAs from group III-V, ZnO, ZnS, CdS, CdSe, CdTe are semiconductors II-VI and silicon and germanium come from group IV (Khan, Ibrahim 2017).

Polymeric Nanoparticles

Polymeric nanoparticles are organic based nanoparticles. Depending upon the method of preparation, these have structures shaped like nanocapsular or nanospheres. A nanosphere particle has a matrix-like structure whereas the nanocapsular particle has core-shell morphology. In the former, the active compounds and the polymer are uniformly dispersed whereas in the latter the active compounds are confined and surrounded by a polymer shell.

The advantages of polymer nanoparticles are many, some of which are controlled release, prophylaxis of drug parts, the ability to combine treatment and imprinting, specific targeting and much more. These apply to the delivery of drugs and the diagnosis of diseases. Deliveries of drugs bearing polymeric nanoparticles are biodegradable and biocompatible (Khan, Ibrahim 2017).

Lipid-Based Nanoparticles

The diameter of the lipid nanoparticles is from 10 to 100nm and they have a sphere shape. It consists of a solid nucleus made of a portion of lipids and a matrix form which contains lipophilic molecules which have the property of being soluble. The nanoparticles carry an externally located nucleus and its stabilization is performed by surfactants and emulsifiers. They are applied in the biomedical field with the property of drug carrier and RNA release as a form of cancer treatment.

Thus, the field of nanotechnology is far from being saturated and it is, as the statistic says, sitting on the staircase of an exponential growth pattern. It is basically at the same stage as the information technology was in the 1960s and biotechnology in the year of 1980s. Thus it can easily be predicted that this field would witness a same

exponential growth as the other two technological field witnessed earlier (Khan, Ibrahim 2017).

2.3 The main characteristics of MNPs

- Large surface symmetry - to volume in parallel with the bulk equivalents.
- Huge surface reactions.
- The passage between molecular and metallic states offering a defined electronic structure (local density of LDOS states).
- Plasma stimulation.
- Quantum constraint.
- Short-range commands.
- Increased number of stings.
- A large number of low-resonance stops, such as angles and edges, that carry a large number of "hanging beams" and consequently defined and chemical characteristics with the ability to collect excess electrons (Blackman 2009).

2.3 Goals and Problems in Metallic Nanoparticles Synthesis

- Ideally, metallic nanoparticles should be prepared by a method which: (Burda, 2005).
- Is reproducible.
- May control the shape of the particles.
- Yields monodisperse metallic nanoparticles.
- Is easy, cheap.
- Use less toxis precursors: in water or more environmentally benign solvents (e.g. ethanol).
- Use the least number of reagents.
- Use are action temperature close to room temperature.
- With as few synthetic steps as possible (one-pot reaction).
  - Minimizing the quantities of generated by-products and waste (Blackman 2009).

2.3.1 Goals and Problems Regarding the Synthesis of Ag NPs

- With the traditional methods, the major problem is often a limited flexibility in the size of particles that can be produced and such methods are usually sold on their ability to make < 10 nm
- The major problems for the non-traditional methods are often a wide size distribution, lack of particle crystallinity, and the cost and scalability of the production
- Synthesizing uniform and stable silver nanoparticles with a controllable size is difficult
- The optimum synthetic method should address all of the above problems and additionally yield particles with no extraneous chemicals that can potentially alter the particle's optical properties and surface chemistry

2.5 Applications

a. Metallic nanoparticle such as magnetic nanoparticle and semiconductor nanoparticle are employed in biomedical prospects such as in diagnostic, targeting cancer, treatment of disease, anti-microbial' activity and tissue engineering etc (Li X, 2012).

b. Metallic nanoparticles have better penetration of therapeutic moieties and tracking within the body as compared to ordinary carriers (Weindling P, 2000).

c. Functionalized metallic nanoparticles are effectively utilized as therapeutics in cancer diagnosis as compared to ordinary chemotherapeutic agents (Apostolou P, 2013).

d. Metallic nanoparticles along with their oxides i.e. Ti, Zn, Ag, Au and Cu are involve in the inhibition of growth of different disease causing viruses, fungi and bacteria. This inhibition is caused by several factors such as the size of nanoparticles, their concentration used as well as their stability (Akbar Vaseghi, 2014).

e. Metallic nanoparticles serves as an important gene delivery vehicles for therapeutics purposes.

f. Presently silver nanoparticles are used in wound dressings because of their antiseptic and anti-microbial property (Li C, 2007).

g. Metallic nanoparticles can be used in the removal of heavy metals in drinking water and also be used in production of hydrogen fuel elements (Sinha A, 2015).

2.6 Advantages and Disadvantages of Metallic Nanoparticles

a. Strengthening the Rayleigh dispersion
b. Seemingly enhanced Raman scattering
c. State plasma assimilation
d. Imaging of a biological model
e. Determine the chemical knowledge for nanoscale metal substrate (Li C, 2007).

Disadvantages of metal nanoparticles

a. Particle uncertainty: Nanomaterials can undergo transformation, as they are thermodynamically variable and are located in the area of local high energy minima. This leads to a degradation of quality, insidious corrosion resistance and most importantly that the maintenance of the building is difficult.

b. Waste: When combining nanoparticles, nitrides, oxides, the molding may deteriorate due to the unclean environment. Due to the fact that the nanoparticles have c reactivity, they may also have the potential for large amounts of waste. When the nanoparticles are in solution form they need to be encapsulated. For this reason it is considered a challenge to solve the problem of nanoparticle waste.

d. Biologically harmful: nanomaterials are considered to be toxic, carcinogenic and stimulate an irritation when their form becomes transparent in the cell skin.

e. Sudden onset: Exothermic combustion may cause an explosion, as the fine metal particles they contain can be transformed into powerful explosives.

g. Obstruction in synthesis: When the synthesis of nanoparticles is performed, it is appropriate to encapsulate, due to the fact that there is a problem with the size of the nanoparticles when it is in solution (Sinha A 2015).

2.7 Therapeutic Applications of Metallic Nanoparticles

As anti-infective agents

The antiviral properties of AgNps are more active compared to chemically synthesize silver nanoparticles (Bowman MC, 2008). Research on metal nanoparticles has been shown to act as a preventative treatment for HIV (Mallipeddi R, 2010). In other studies, silver has been found to act as a virulence agent that acts directly on the virus

by blocking the gp120 glycoprotein (Taylor U, 2010). This binding in turn inhibits the coherence of the CD4 virus which drastically reduces HIV-1 infectivity while the metal nanoparticles have been found to be active antiviral agents against herpes simplex virus, influenza, and respiratory syncytial virus (Sun L 2008).

As an anti-angiogenic

It is common that angiogenesis is the spread of new blood vessels and occurs in the stage of normal development and in some cases of disease, an important role is played by many diseases such as cancer, rheumatoid arthritis. Under normal conditions, angiogenesis is closely regulated between various growth promoting factors (VEGF, PDGF and TGF-B) and anti-angiogenic factors (platelet factor 4, TSP-1). When diseases occur, angiogenesis acts. Some studies have shown that these agents carry a high degree of toxicity which in turn causes fatal bleeding, thrombosis and hypertension. The problem may be solved if these nanoparticles alone can be active as anti-angiogenic agents.

In Oncotherapy

Bare gold nanoparticles have been investigated to block the action of proteins that in turn bind heparin such as VEGF165 and bFGF in vitro and VEGF induced angiogenesis in vivo (Tsai CY, 2007). Additional studies in this field have reported that on the surface of AuNPs the heparin-binding proteins are absorbed (Tsai CY, 2004) and then denatured. Studies have also shown that surface size plays an important role due to the therapeutic effect of AuNPs. In a study by Mukherjee and colleagues, they found that gold nanoparticles affected angiogenesis with VEGF intervention using it as a mouse model injected with a VEGF adrenoviral vector (Ad-VEGF-representing) (Ramezani N, 208). One week later, the administration of Ad-VEGF to AuNPs-treated mice promoted less swelling than the treated mice themselves. Eom and his colleagues have released the anti-cancer effects of 50nm AgNps in vitro and in vivo.

In Multiple Myeloma

The studies (University of Washington School of Medicine in St. Louis, journal Molecular cancer Therapeutics) have developed a treatment based on nanoparticles that is effective in treating mice with multiple myeloma. Multiple myeloma is a form of cancer that affects plasma cells. Mukherjee and the team found

that a gold nanoparticle inhibits VEGF- and bFGF-dependent multiplication of multiple myeloma cells.

In Leukemia

B-chronic lymphocytic leukemia (CLL) is considered a disease that is incurable and is manifested especially by apoptosis resistance, and by co-culture with anti-VEGF antibody, which was found to cause more apoptosis in CCL B cells. In CLL treatment, use of gold nanoparticles to increase the effectiveness of these agents. The gold nanoparticles have been selected based on their biocompatibility, the very large surface area they have, as well as the way the surface works and the ease of characterization. In the gold nanoparticles, VEGF antibodies were ligated and determined their ability to kill CLL B cells.

In rheumatoid arthritis

Scientists from the University of Wollongong (Australia) have developed a new class of arthritis-related drugs that could be used by gold nanoparticles and thus have limited side effects. Rheumatoid arthritis as an autoimmune disease which burdens the immune system resulting in problems with its function and damage to the patient's joints. A new study has shown that gold particles can invade macrophages and prevent them from causing inflammation without killing them. A journal of Inorganic Biochemistry has reported that by limiting the size of gold to smaller nanoparticles (50nm) it was able to produce more gold in immunocytes with less toxicity.

In the photo heat therapy

Gold nanoparticles strongly absorb light as they change the effect of photons into heat quickly and dramatically. Photothermal therapy (PTT) is an invasive treatment in which the effect of photons is changed into heat to kill cancer.

In Radiation Therapy

Gold-laden tumors, this absorbs more X-rays as gold is a fine X-ray absorber. Thus, it deposits more beam energy and directs it to a local dose that is specifically enlarged in the tumor cells. Gold nanoparticles were more essential for the treatment of cancer (Chen W, 2014).

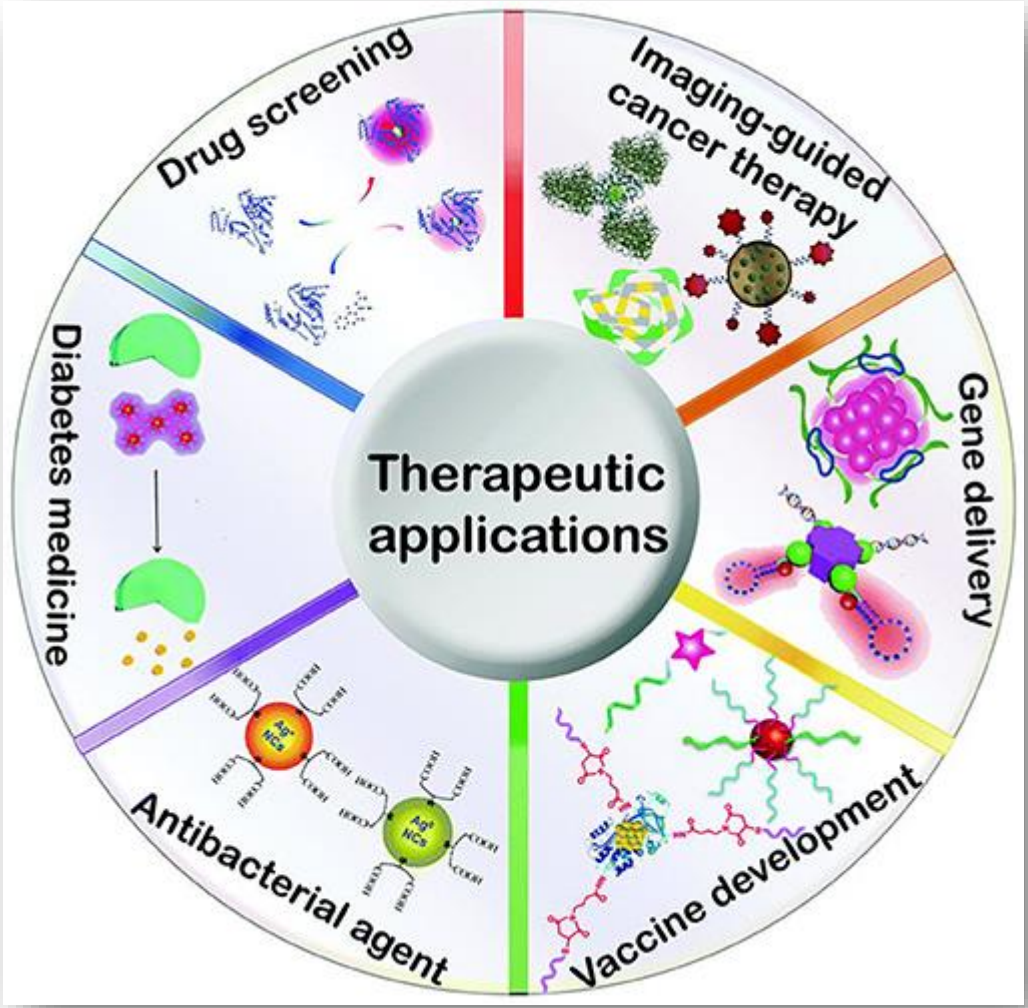


Figure 2.7.1. Schematic presentation of metal-based Nano clusters of therapeutic applications. Adapted from Tao et al. (2015), with permission from RSC.

2.8 Tissue Engineering

Nanoparticles have received particular interest in tissue engineering. The mechanical and biological principles were treated with practical substitutes for various types of damaged tissues. Nanoparticles that have low toxicity, feature adaptability, contrast actions, targeted delivery capabilities, and detailed behavioral control that make them suitable for use in tissue proliferation / regeneration (Corchero and Villaverde, 2009).

The nanoparticles have been made by treating different types of materials (composite materials) in order to enhance its activity and role in tissue regeneration. Adequate metal nanoparticles such as Ti, Au, Ag and various others have been synthesized, which have shown remarkable capabilities in this industry. κυττάρων (Lizundia et al., 2018; Bapat et al., 2019; Fathi-Achachelouei et al., 2019). Bioactive nanoparticles based on ceramic glass (BGC nanoparticles) are considered to be a material that has the potential to offer a lot and is therefore mainly used in bone tissue regeneration. Covarrubias et al. (2018) stated that the encapsulation of dense BGC nanoparticles in chitosan polymer gelatin has enhanced the alkaline phosphate effects always comparing other BPC intermediate nanoparticles. The sequence showed that the concentrated chitosan gelatin hydrogel with bioactive nanoparticles showed the highest rate of bone formation (80%) compared to others.

Li et al. (2018) investigated multifunctional BGC nanoparticles based on polycitrate-siloxane elastomer and applied it as an active material for bone tissue regeneration. Titanium nanoparticles have also been applied as a bone repair material. It is very often applied in the field of dentistry and orthopedics. It includes features such as greater fracture resistance, better ductility and a strong weight-to-strength ratio. Unfortunately, they brought lower bioactivity, as it does not maintain cell adhesion and growth. In conclusion, apatite coatings are considered to be preferred as bioactive materials. Therefore, many methods are commonly used to create the apatite coating around titanium. However, this coating has a number of limitations, such as disproportion in thickness, insidious adhesion and lower mechanical strength. Also, a stable absorption structure is necessary to enhance individual nutrients through cell growth. (Scott et al., 2013; Walmsley et al., 2015; Azizian et al., 2018; De-Witte et al., 2018

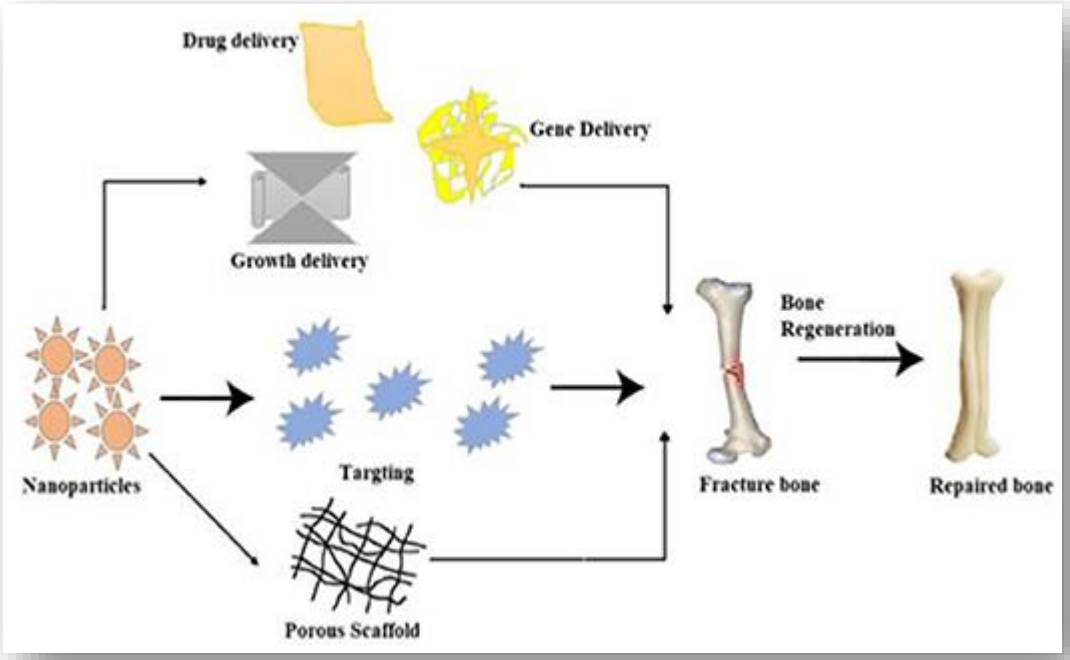


Figure 2.8.1 A schematic mechanism of the bone tissue engineering process by using nanoparticles and drug molecules.

CHAPTER 3 THE TOXICITY OF METALLIC NANOPARTICLES

3.1 What is the Toxicity of Metallic Nanoparticles

The release of dissolved metal from the region of MNPs may have toxic effects. A local point cause of large collection with slowly dissolving NPs is obtained. As a consequence, cells not far from the significant boundary source have a higher metal collection than always those in bulk solution (Apte et al., 2009). Small amounts of some metals may be extremely toxic. For example, copper and zinc, which in large quantities may present as a form of toxicity to aquatic organisms (Santore et al., 2001). Other studies have reported the rapid cleavage of ZnO and the cytotoxicity of Zn12 mesothelioma and fibroblast cell lines in rodents (Brunner et al., 2006).

3.2 Key factors affecting the neurotoxicity of NM

The physicochemical and biochemical properties of NPs are the main factors that interfere with their interactions with cells, including the reaction that cell stress will bring and the biological traits of defined cells. Also, the amount of supply, the mode of delivery and the time famous are exposed are important factors that affect the severe form of toxicity caused by any given NP (Yokel RA, 2011). There is ample evidence that cell uptake pathways, subcellular processing functions, and cytotoxicity are determined by the surface characteristics of the particles. Here, the most important factors influencing the toxicity of NPs in the CNS will be reviewed.

Size

The toxic behaviors of NPs differ from those of their counterparts (Kumar V, 2012). NP-induced cytotoxicity compared to tissue cells is greatly influenced by particle size. It is observed that the size of the particles should be taken as an important reason for the construction of NP which will be applied in the biomedical field. AgNPs (10 nm in size) that are smaller in size have been found to be more toxic than those with

larger AgNPs (50 and 100 nm) in causing necrotic cell death in PC12 cells (Kim TH, 2012). Sharma et al (2013) in a study showed that small NPs caused greater BBB division, brain edema and neuronal injury, regulation of glial fibrous acid protein and regulation of myelin in young animals. compared to the control group. These studies determine that the influence of NPs on neurotoxicity is inversely related to the size of NPs. It is necessary to give significant weight to the physicochemical features of NM when assessing their neurological effects.

Shape

Compared to a number of studies concerning the influence of different types of NPs on cell energies, very few studies report on the influence of the NP shape on cellular function. Different patterns of the same NM can potentially elicit different cellular responses with non-specific uptake into cells (Gratton SE 2008). Using spherical and rod-shaped mesoporotic silicate NPs (MSNPs), Meng et al (2011) showed that dimensional contrast differences in MSNPs could be detected by HeLa and A5 cells through an active sighting mechanism. The rate and abundance of MSNP uptake by the macro pinocytosis process were determined by the correspondence of MSNPs shapes to rod dimensions. Particle morphology appears to play a key role in both cellular interactions with NPs and the systematic division of NPs.

HAP NPs are very often used as bone covering material in the upper jaw and lower jaw. HAP NPs with needle and short rod dimensions have been found to cause greater cellular damage in primary cultured rat osteoplasts than spherical and long rod HAP NPs by stopping the spread and applying apoptosis (Xu ZL, 2012). Thus, the shape of HAP NPs may have a key influence on the apoptotic level, which may be attributed to mechanical stresses on the cell surface caused by particle-cell interactions (Jain TK, 2008).

Intermediate materials have started to be applied in the biomedical industry due to the large Brunauer, Emmett and Teller area and the large volume of resources. Rod-shaped MSNPs cause increased apoptosis of cells from short rod and spherical MSNPs with similar diameters, revealing that the shape of NPs affects NP uptake by cells through endocytosis (Huang XL, 2010).

Charge Charge

Recent research has shown that the load of NPs has a large effect on their cellular absorption in vitro (Asefa T, 2012). Due to the electrostatic attraction between the cell membrane and positively charged NPs, particles which in turn have positively charged surfaces are more easily absorbed on the cell surface and concentrated inside the cells than their negatively charged or neutral counterparts (Thorek DL, 2008). For NPs (2 nm), with a small size such as a positive charge, a positive charge may affect the capacity of the cell membrane, bringing in Ca2 + inflow into the cells and stopping cell proliferation.

Compared to anionic and neutral NPs, cationic NPs have been shown to have higher ROS-associated toxicity (Tarn D, 2013). Rejectively charged NPs do not easily hook to the cell surface due to repulsion with the negatively charged cell membrane and thus once appeared non-toxic. However, recent research has shown that even rejection NPs can cause a small amount of toxicity in their interactions with membrane proteins or lipid beams that form proteins or trans membrane channels (Lin JQ 2010). Membrane potential and membrane integrity appear to play crucial roles in proper signaling in neuronal cells. A disturbance in membrane potential and membrane utility could be caused by charged NPs, which may have a significant effect on normal CNS movement and thus be concluded to be one of the reasons for neurotoxicity.

Surface modification

For clinical applications, NPs need to have the ability to disperse in water and other hydrophilic media. Surface modification may be used to inhibit NP accumulation and improve the compatibility of NPs with solid matrices or biological environments. Surface change is considered to be one of the most effective ways to control and regulate cellular interactions with NPs and their subsequent biological sequences. Surface alteration may exceed or even reverse the biological penalties of NPs. For example, sulfur atom affinity is widely used in the surface variation of noble metal NPs. NPs coated with thiol noble metal consist in the yield of noble metals ion appearance thiol. While limited fine metal atoms accumulate to form NPs, thiol

molecules adhere to the surface of NPs to passivate the growing surface and minimize agglomeration (Templeton AC, 2000). Compared with unmodified fullerene NPs, which can construct ROS to damage cells, superficially modified fullerene NPs can selectively penetrate oxidized endothelial cells of the cerebral microvasculature, causing them to block ROS, such as F-actin DE polymerization (Lao F, 2009; Zhao F, 2011). Nanocrystalline TiO2 is widely used in biomedical ceramics and implant-associated biomaterials located in the brain of mice, causing pathological damage to the hippocampus and converting neurochemical levels in the brain after intranasal instillation J, 2008 W,). However, the neurotoxicity of nanocrystalline TiO2 could be affected by the surface change. For example, compared to hydrophobic NPs, hydrophilic Nano crystalline TiO2 NPs have a greater effect on 5-HT and 5-HIAA monoamine levels in the subcerebral regions (Zhang LL, 2011). Sousa et al (2010) varied the 15 nm gold NP region by overlapping functional layers of polyelectrolytes and human serum albumin and found that the modified gold NPs were able to cross the BBB and accumulate in neuronal or glial cells in adjacent glomerular cells. stem.

These studies suggest that physicochemical reform of NMs may affect or even reverse the neurotoxicity of mammals. Other important factors include 'protein crown', chemical traits, scrap metal and degradation characteristics.

Concentration and dispersion

The confluence and formation of a protein crown in the extracellular environment transforms the size, dimensions and surface characteristics of the particles, giving them a "biological identity" different from their original "synthetic identity". Interestingly, the subsequent interaction between NPs and cells is not determined by their "synthetic identity" but by their "biological identity" (Albanese A, 2014). Compared to organic NPs, inorganic NPs are more susceptible to confluence in biological environments. Sadhukha et al (2014) investigated the effect of agglomeration on NP-induced magnetic hyperthermia. Well-dispersed SPIONPs were found to cause less apoptosis than submicron-sized aggregates, with the latter causing lightning membrane damage and acute cell end. Their results indicate that the aggregation status of SPIONPs largely determines the proliferation and mechanism of cell terminus induced by hyperthermia.

In a complex nonlinear paradox, lower doses of NPs can sometimes increase, rather than decrease, the toxicity of a given source material as a function of the surface properties of the particles themselves. That is, higher concentrations or doses can favor NP agglomeration in the absence of surface modifications to prevent spontaneous agglomeration due to close physical interactions of highly reactive NPs in concentrated colloidal liquids (Sur I, 2012). The resultant Nano aggregates as a whole can then hide or quench the originally hyperactive surfaces of their smaller NP "parts" (Mudunkotuwa IA, 2011). Consequently, the specific larger agglomerated NP form is less toxic at a higher concentration than a lower dose of smaller but well-dispersed NPs, eg, NPs of PbS or copper (Bell IR, 2014).

3.3 Mechanisms of Metallic Nanoparticles Toxicity

Different mechanisms are involved in causing cellular toxicity including physical dam-age, toxic ions release, and reactive oxygen species generation.

3.3.1 Membrane Damage

The MNPs may cause acute toxic response directly at the cell membrane or by their cellular uptake. NPs possess several properties such as very high surface area and numerous morphologies (Klabunde et al., 1996). The nonspecific damage to cell membranes is mainly caused by the abrasive nature of MNPs, such as magnesium oxide NPs damaged bacterial cell membranes, and resulted in leakage of cell contents and ultimately cell death (Stoimenov et al., 2002). Other studies also reported bacterial cell membrane damage by silver and zinc oxide when used in high concentrations (Morones et al., 2005). The cell membrane damage is more pronounced in unicellular organisms (Wiesner et al., 2006).

3.3.2 Release of Toxic Dissolved Species

The release of dissolved metal from the MNPs surface may exert toxic effects. A localized point source of high concentration is obtained with slowly dissolving NPs. As a result, cells in close proximity to the main point source have higher concentration of metal as compared to those in the bulk solution (Apte et al., 2009). Minute amounts of some metals can exhibit extreme toxicities. For instance copper and zinc in minute amounts can be extremely toxic to aquatic organisms (Santore et al., 2001). Other studies have also reported the rapid dissolution of ZnO and cytotoxicity of Zn12onhuman mesothelioma and rodent fibroblast cell lines (Brunner et al., 2006).

3.3.3 Reactive Oxygen Species Generation

MNPs have a great ability to generate reactive oxygen species (ROS) such as peroxides, oxygen ions, and free radicals due to their physicochemical attributes including large surface area to volume ratio and high chemical reactivity (Oberdörster et al., 2005). ROS can interact with cells via different mechanisms leading to oxidative stress. It may damage several intracellular organelles. Oxidative stress results in loss of function of the main components of the body, such as fats, proteins, nucleic acids, etc., that are prone to oxidation (Fahmy and Cormier, 2009). Some of the MNPs have the tendency to induce oxidative stress. The main reasons for generation of oxidative stress include:

(1) The reactive surface of MNPs with prooxidant functional groups;

(2) Active redox cycling on the surface of MNPs due to transition metal-based NP; and

(3) Particlecell interactions (Risom et al., 2005; Manke et al., 2013).

3.4 Effect of Physicochemical Properties on Toxicity of Metallic Nanoparticles

Commercially, MNPs are prepared to impart certain physicochemical properties to the material that are relevant to the desired application. Given the diversity of products and their applications, one cannot generalize about the chemistry of MNPs. For instance chemical composition, sizes, shapes, and ability to disperse in solution can greatly affect the fate of MNPs in biological systems and environment. Therefore it is

worth discussing some of the fundamental concepts of physicochemical aspects of MNPs, and how this might apply to toxicity (Horie and Fujita, 2011).MNPs can exhibit various behaviors, such as agglomeration or aggregation, interactions with natural organic matter (NOM) in the water, and the adsorb ability particles onto surfaces. These behaviors are partly attributed to physicochemical properties. Therefore in the following section we attempt to discuss the key physicochemical properties of MNPs with respect to their toxicity (Apte et al., 2009).

3.4.1 Effect of Size

The particle size of manufactured MNPs is very small ranging from 1 to 100 nm as compared to the living cells sizes (typically 110μm in diameter). The nan scale confers different properties to MNPs such as larger surface area, different physical properties, and enhanced reactivity as compared to their bulk material. For instance when the size is reduced below 100 nm, the number of surface molecules increases exponentially (Fig. 3.4.1.1).

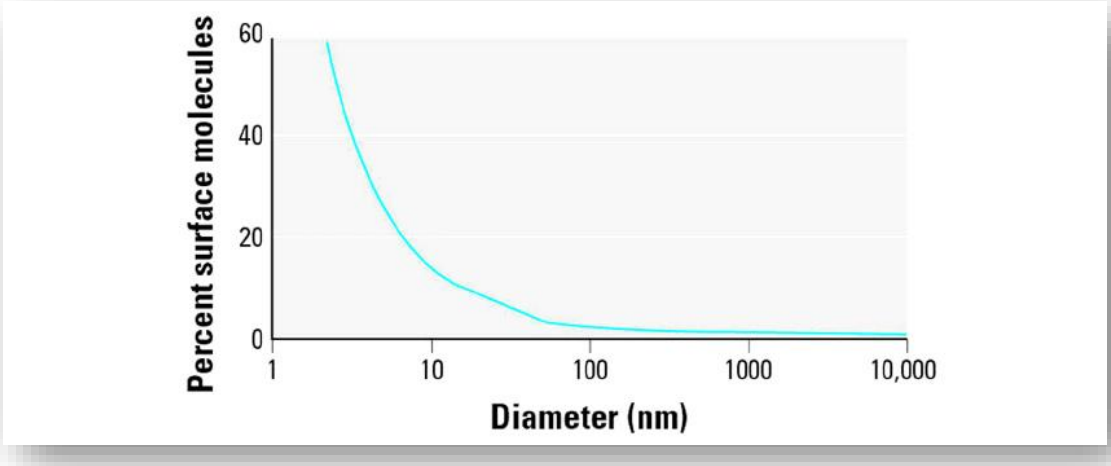


Figure 3.4.1.1 Increase in percent surface molecules as a function of particle size. Reproduced with per-mission from Oberdörster, G., Oberdörster, E., Oberdörster, J., 2005. Nano toxicology: an emerging discipline evolving from studies of ultrafine particles. Environ. Health Perspect, 113, 823.

For same mass of the same material, the number of molecules interacting with cells will be higher compared to its bigger particles. Thus the particle size is very critical in

determining the toxicity of materials where it is related to the surface area of the material such as ROS generation and release of toxic species upon dissolution. Toxicity studies of engineered NPs in cell cultures and animals have shown that various para-meters play a role in toxicity including size, surface area, surface chemistry, and solubility (Maynard et al., 2006). In vitro studies performed on NPs below 100 NM showed different degrees of proinflammatory and oxidative-stress-related cellular responses on different cell models (Oberdörster et al., 2005). Mostly, oxidative-stress-related changes of gene expression and cells signaling pathways are considered responsible for the toxicity of ultrafine NPs (Donaldson et al., 2002). Size-dependent cytotoxicity study of gold NPs suggests that bigger gold NPs are nontoxic in vitro compared to smaller counter parts, for example, 1.4 nm gold NPs were found to be more toxic as compared to 15 nm (Chen et al., 2009). Furthermore, no cytotoxicity of human retina micro vascular endothelial cells was observed for gold NPs of sizes 20 and 100 nm (Kim et al., 2008). Even if gold NPs show no effect on cell viability, it is important to note that genetic damage and transcription alterations can occur even in the absence of cytotoxicity. For instance no effect on the viability of MRC-5 human fetal lung fibroblast cells was observed when treated with 5 and20 nm gold NPs, but cell division stopped due to down regulation of cell cycle genes (Coradeghini et al., 2013). Similarly, Gutierrez et al. has reported that bactericidal property increased by 25-fold after reducing particles size from 80 to 20 nm (Martinez-Gutierrez et al., 2010).However, «the smaller the more toxic «cannot be generalized on the basis of in vitro data, since NPs of certain materials can be more toxic when the size is reduced, while in some cases the toxicity may increase with increase in size. For instance contrary to gold NPs, where bigger particles are considered nontoxic, 100 NM titanium dioxide (TiO2) are reported to induce apoptosis compared to 15 nm particles (Bauer et al., 2009). The data from in vitro toxicity studies of MNPs is hard to interpret since different investigators used different cell lines, different sizes of NPs, special surface modification, dissimilar amount, and time of NPs application onto the cells. Therefore it is important to obtain extensive data from in vivo experiments of expo-sure before any conclusion is drawn for therapeutic applications. For example, Chen et al. studied different sizes of citrate-capped gold Nano spheres of diameters 3, 5, 8, 12, 17, 37, 50, 100 nm in mice. The small size (3 and 5 nm) and the largest size (50 and100 nm) were found nontoxic in the used doses, while the intermediate size range837 nm showed severe toxicity on

mice including loss of appetite, weight loss, change in fur color, and shorter average life span. The toxicity of intermediate size range was considered to be due to their accumulation and in turn damage in liver, spleen, and lungs. Interestingly, in the same report in vitro study showed no toxic effects on HeLa cell lines (Fig. 3.4.1.2) (Chen et al., 2009).

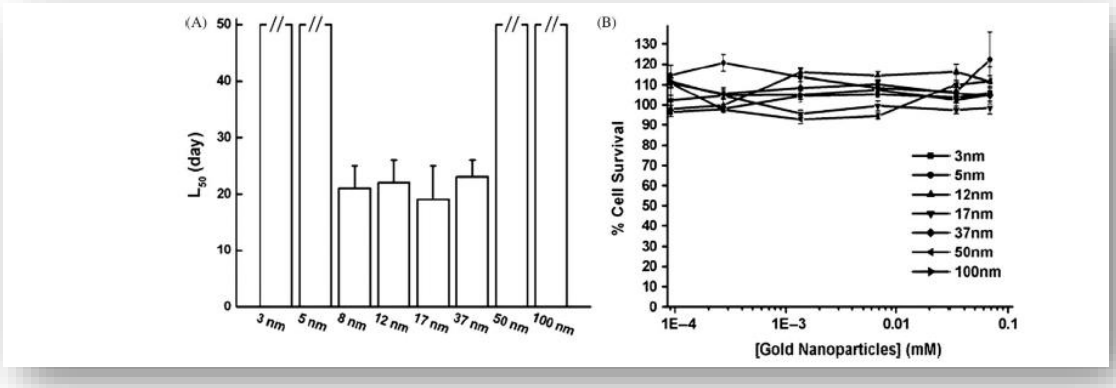


Figure 3.4.1.2 In vivo; average lifespan (the time beyond which half of the mice died) of mice receiving different sizes of gold nanoparticles (A), in vitro; cell viability of HeLa cells exposed to different sizes of gold nanoparticles (B).Reproduced with permission from Chen, Y.-S., Hung, Y.-C., Liau, I.,Huang, G.S., 2009. Assessment of the in vivo toxicity of gold nanoparticles. Nanoscale Res. Lett., 4, 858.Copy right: Springer science.

3.4.2 Effect of Surface Charge

NPs may form a stable suspension if they repel each other via having sufficient surface charge or if there is steric hindrance due to long polymer chains attached to particle surface. NPs stability in electrolyte solution is based on the interactions of two forces according to Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory: electrostatic repulsion, due to electrical double layer of the adjacent particles, van der Waals 'attractive forces. The surface charge of NPs is usually expressed in terms of zeta potential, that is «the potential difference between the mobile dispersion medium

and the stationary layer of the dispersion medium attached to the dispersed particle"(Lu and Gao, 2010)(Fig. 3.4.2.1).

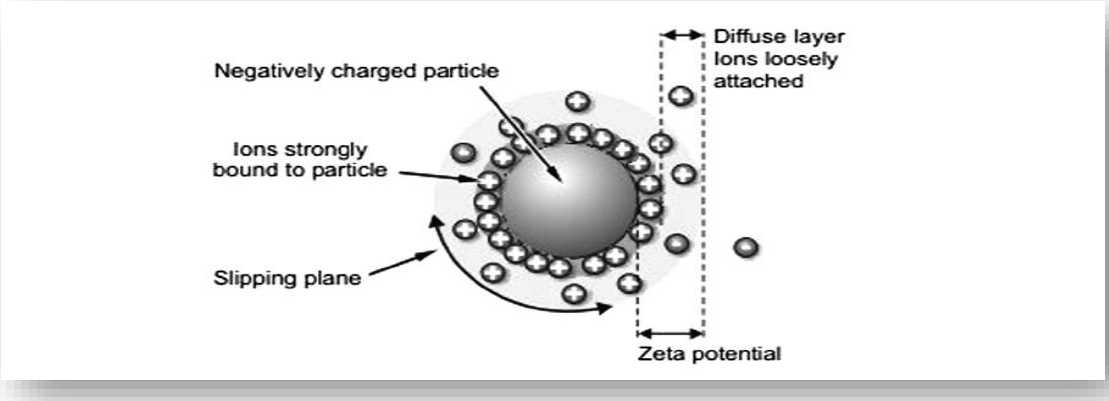


Figure 3.4.2.1 Schematic representation of the electric double layer surrounding the nanoparticles in aqueous medium. The potential at the slipping plane is known as zeta potential. Image taken from Zetasizer user manual (Malvern, Z.N.S.U.M., 2004. Malvern Instruments Ltd. Worcestershire, UK).

The particles movement within a fluid resulting in a net surface charge is measured via zeta potential. Thus the stability of particles dispersion can be depicted from zeta potential. NPs with a zeta potential value greater than130 mV or lower than230 mV are considered to be stable and unlikely to aggregate (Malvern, 2004).Contrarily, nanoparticle with low zeta potential value tends to stick to each other. This sticking cohesion of NPs is termed as "aggregation" when the particles are strongly bonded, or "agglomeration" if particles are loosely bonded due to van der Waal's forces. MNPs are usually stabilized by surface coating with certain polymers, in order to prevent aggregation by either steric hindrance or electrostatic repulsion, for example, citrate stabilized silver and gold NPs. These surface modifications affect the biological activity of MNPs. For instance El badawy et al. (2011) presented a direct correlation between the surface charge and toxicity of silver NPs; according to their report positively charged branched-polyethylenimine coated silver NPs were more toxic compared to negatively charged citrate-coated silver NPs (Fig. 3.4.2.2).

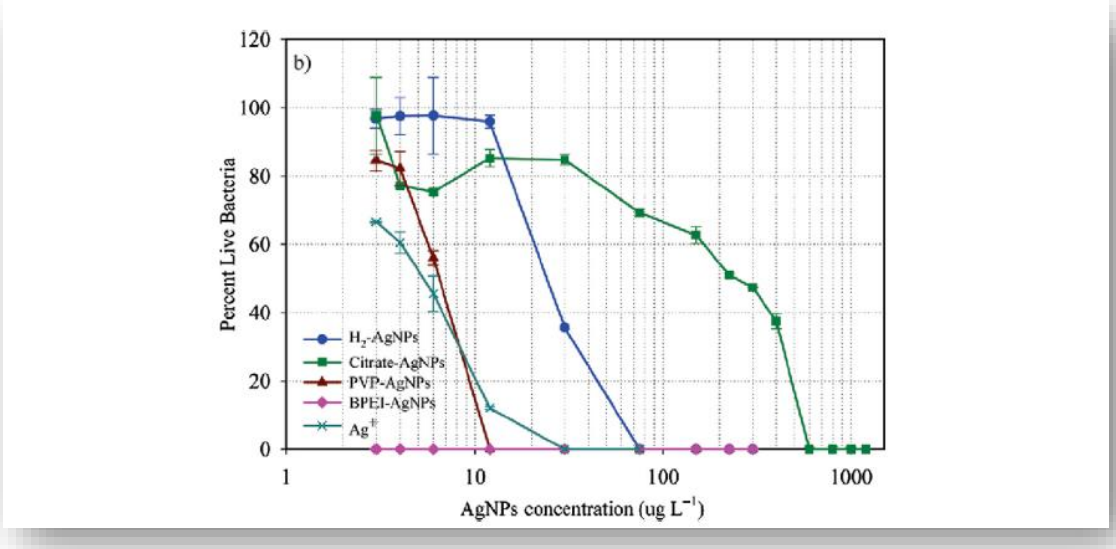


Figure 3.4.2.2 Effect of surface charge on bacterial viability after 5 days. Reproduced with permission from EL Badawy, A.M., Silva, R.G., Morris, B., Scheckel, K.G., Suidan, M.T., Tolaymat, T.M., 2011. Surface charge-dependent toxicity of silver nanoparticles. Environ. Sci. Technol., 45, 283287. Copy right: AC Spublications.

In similar studies by other researchers, TiO2NPs modified withOH andNH2showed increased cytotoxicity compared to unmodified TiO2NPs. On the other hand,COOH modified TiO2NPs did not affect the cell viability (Thevenot et al.,2008). The different cellular response is considered to be due to surface charge; negative charged particles such as COOH modified TiO2 has minimal effect on cells since they are taken up into the cells easily without membrane binding.

3.4.3 Effect of Shape

MNPs exists in many different shapes such as rods, spheres, ellipsoids, cylinders, cubes (Hamilton et al., 2009; Kong et al., 2011; Ispas et al., 2009; Favi et al., 2015; Acharya et al., 2018) triangular, hexagonal, etc. (Hamilton et al., 2009; Kong et al., 2011; Ispas et al., 2009; Favi et al., 2015; Acharya et al., 2018; Lei et al., 2013). The shape of the MNPs is also a critical factor that affects biological response. It is reported that the ability of NPs to cross biological barriers, the mechanism by which NPs enter the cells, cycling time, targeting effect, partly depend on geometry of NPs. For instance Jiang et al. found that spherical gold NPs had higher tendency to be taken up by HeLa cells compared to rod-shaped gold NPs of similar size (Jiang et al., 2008).

Moreover, spherical gold NPs have been proved less toxic compared to star-shaped counterparts, on human skin fibroblasts and rat fat pad endothelial cells (Favi et al., 2015).

CHAPTER 4 MECHANISMS OF METALLIC NPS FOR TOXICITY IN THE CNS

4.1 Potential Hazards of NMs to the CNS: Nano Neurotoxicity

For the past several years, nanotechnology has benefited humankind in scores of ways. With its increasing use in many aspects of human life, the potential hazards of NMs have begun to garner greater attention.

The effect of the NMs on bio systems is determined by various factors. Particle size, shape, and ability to interact with the surrounding tissue greatly influence the toxicity of NMs. NMs may cause phagocytic cells to "overload", leading to a defensive fever and reduced body immunity. NMs may be unable to degrade, and they may accumulate in the organs owing to their slow degradation rates. Because of their high surface area, NMs exposed to the organism will affect enzymes and proteins and may disturb biological processes in the body. Unlike other damaged tissues, neurons cannot be repaired via regeneration (von Bohlen, 2007). Moreover, most drugs cannot pass the BBB into the brain, which makes it difficult to mediate damage to neurons. Thus, a comprehensive and systemic evaluation of Nano neurotoxic effects is particularly important for reducing or preventing CNS damage.

An increasing amount of research on the toxicity of NMs is being carried out by researchers all over the world. Hirst et al (2013) concluded that the bio distribution and potential applications of CeO2 NMs are related to their hazard assessment. Silver NMs have been found to be transferred to the blood and distributed throughout the brain after subcutaneous injection in rats. Furthermore, swollen astrocytes, BBB destruction, and neuronal degeneration have also been observed in the same test rats (Tang JL, 2009). Ma et al (2010) reported that a high-dose administration of Nano metallic particulate anatase TiO2 could lead to brain injury and change some glias into filamentous shapes and others into inflammatory cells. The concentration of Nano metallic particulate anatase TiO2 in the brain was highly correlated with the Nano metallic particulate anatase TiO2 dosages used. In a study by Wang et al (2007) the fatty degeneration of the hippocampus and brain lesions were observed in ICR mice after the injection of a nanosized TiO2 suspension via the gastrointestinal tract.

Boyes et al (2012) suggested that the NMs could be 1) located in the luminal wall of brain micro vascular endothelial cells or the pericytes, 2) located in the astrocyte foot processes, or 3) maintained by the basement membrane.

In "Relationship between NMs and CNS dysfunction" section, we will review the possible relationship between NMs and CNS diseases and the possible mechanisms of Nano neurotoxicity.

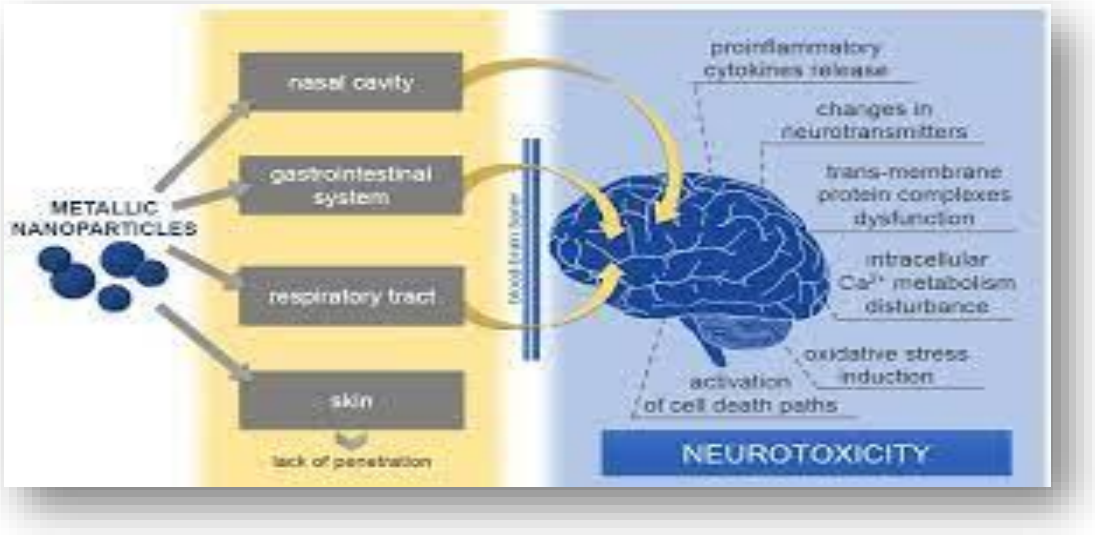


Image 4.1.1 Toxicity of metallic nanoparticles in the central nervous system.

4.2 Relationship Between NMs and CNS Dysfunction

An increasing number of people are reported to suffer from neurodegenerative diseases, such as Alzheimer disease (AD), Parkinson disease (PD), Huntington disease (HD), and primary brain tumors. The exact cause of these diseases is still unknown, but we suspect that NMs from both Nano drugs and environmental NP pollutants may play a role in aggravating these diseases (Ma LL, 2010). Although there is a lack of evidence regarding the relationship between NMs and CNS diseases in human studies to date, animal research suggests possible links. For example, Mirsattari et al (2004) reported a case of a 71-year-old man who developed myoclonic status epileptic us and went into a coma after daily ingestion of colloidal silver for 4 months. High levels of silver were found in the plasma, erythrocytes, and CSF of the patient. Unfortunately, plasmapheresis was not effective in this patient, and he persisted in vegetative state until his death 5.5 months later. Silver products may

induce irreversible neurologic toxicity, which is related to poor outcomes. Another study in mice indicated that TiO2 NMs accumulate in the mouse hippocampus by intragastric administration, leading to hippocampal apoptosis and impairment in spatial recognition. In vivo studies have shown that the neurotoxic effects of TiO2 NMs on mice are likely caused by the disturbed homeostasis of trace elements, enzymes, and neurotransmitter systems (Hu RP, 2010). In addition, silver NMs have been found to have the ability to induce damage to the BBB and astrocytes and cause neuronal degeneration in rats after subcutaneous injection (Tang JL, 2009).

The impact of NMs on offspring is another concern because the CNS exhibits considerable plasticity in the early stage of life and could be significantly influenced by environmental invasions encountered during the fetal period (Siddiqui MA, 2013). Unfortunately, studies have revealed that NMs entering the maternal body during gestation may harm fetal development through direct or indirect mechanisms. Even small amounts of particles in the maternal blood can translocate to the fetal compartment. Neurodevelopmental studies have observed that both male and female offspring exhibit differential phenotypes after prenatal insults by NMs. Furthermore, Hougaard et al (2010) found that the inhalation of TiO2 NMs led to long-term lung inflammation in mating adult mice, and their offspring exhibited abnormal neurobehavioral as a result of the gestational exposure to NMs.

One study showed that TiO2 NMs administered subcutaneously to pregnant mice translocated to the offspring and affected the genital system of the male offspring by reducing their daily sperm production. The cranial nervous system was also affected, and an increased number of apoptosis cells was observed in the olfactory bulb of the brain (Takeda K, 2009). A subsequent study by the same author showed that the mice fetuses that were exposed to TiO2 NMs prenatally exhibited an increased level of dopamine and its metabolites in the prefrontal cortex and neostriatum. This result highlights the possibility that prenatal exposure to TiO2 NPs might affect the development of the central dopaminergic system in mouse offspring (Takahashi Y, 2010). Shimizu et al (2009) also found that maternal exposure to TiO2 NMs influenced the expression of functional genes related to brain development in mice. Similar in vivo studies showed that prenatal exposure to TiO2 NMs affects the synaptic plasticity in the offspring’s hippocampal dentate gyrus area, revealing the

hidden toxicity of TiO2 NMs in CNS. These findings indicate that the developmental brain is vulnerable to TiO2 NP exposure, especially during the lactation period (Gao X, 2011). In addition, Mohammad pour et al (2014) observed reduced hippocampal cell proliferation and decreased spatial memory, inhibitory memory, and learning ability in rat offspring after the maternal administration of TiO2 NMs during pregnancy. In that study, rats were exposed to 100 mg/kg body weight of TiO2 NPs every day, which is equivalent to ~6,000 mg/60 kg of body weight for humans, far lower than the LD50 (dose required to kill 50% of a population of test animals) of TiO2 for rats (12,000 mg/kg body weight) per the 1969 guidelines of the World Health Organization (WHO). Although this dose was less than the dose reported by the WHO, it still had side effects on the hippocampus. Therefore, long-term applications of NMs, such as the use of TiO2 NMs in pregnant women in dentistry, must be considered with great caution. NMs may harm not only the mother but also the offspring. This type of effect is serious and may last for a lifetime.

4.3 Possible Mechanisms for NP Neurotoxicity: Nano neurotoxicity

Because of their small size, NPs can easily reach the brain and are taken up by the brain cells, such as neurons and glia. The well-accepted mechanisms of NP uptake by cells include pinocytosis, endocytosis dependent on caveolae and lipid raft composition, clathrin-dependent endocytosis and phagocytosis (Asharani PV, 2009). The intracellular sites of localization of NPs vary depending on the cell type and the uptake mechanism. NPs may enter the endothelial cell monolayer and accumulate along the endolysosomal pathway, affecting the normal morphology and function of the BBB itself. The intercellular interactions between NPs and biological molecules, such as DNA, proteins, and lipids, could have many consequences, including oxidative stress, conformational alteration, increased membrane permeability, mutations, signaling pathway activation, ionic exchange disorder, enzyme failure, and new protein epitope exposure (Xia T, 2009). After interacting with NPs, various mediators released from the microglia and astrocyte induce inflammation, apoptosis, and oxidative stress in the brain. Because nervous tissue regeneration is limited, most of the nerve damage is irreversible (Geraets L, 2012). From this perspective, it is critical to investigate the mechanisms of Nano neurotoxicity, and an increasing number of studies are being conducted to discover the underlying mechanisms. The

interaction between NPs and the bio system is also determined by the type of NPs. Soft NPs, such as liposomes, and hard NPs, such as metal oxides, disturb the bio system through different mechanisms. The possible mechanisms discussed in here are the most commonly proposed in the current literature and include oxidative stress, immune system dysfunction, and autophagy dysfunction.

Oxidative stress

Reactive oxygen species (ROS) are potentially highly reactive molecules that contain an oxygen atom; examples include the superoxide radical (O2−) and H2O2 (Rang FJ, 2014). Under physiological conditions, ROS are present in every cell, being produced by the mitochondrial and cytoplasmic oxidation processes. A low to moderate ROS concentration is essential to maintain normal physiological processes. However, an excessive production of ROS induced by oxidative stress would be extremely damaging. The CNS is particularly susceptible to oxidative stress because of its high oxygen consumption, its weak antioxidative ability, and the terminal-differentiation feature of neurons (Li J, 2013). Oxidative stress has been established as a crucial contributor to acute-CNS-injury-related neurode-generative diseases, such as DNA damage, which can impair the viability of cerebral cells (Smith JA, 2013). An increasing number of studies show that ROS play a critical role in neurodegenerative diseases, such as AD and PD. Moreover, ROS contribute to neuronal apoptosis, which is a key mechanism in brain development (Sorce S, 2009). Furthermore, ROS can regulate neuronal ion channels, kinases, and transcription factors (Schappi MG, 2008). ROS generated from the NADPH oxidase 2 (Nox2) system also contribute to long-term memory dysfunction (Massaad CA, 2011).

ROS are capable of inducing membrane damage that affects the structure and function of inner proteins, lipid denaturation, and structural alteration of DNA. DNA oxidation is of the utmost concern because it can lead to mutations and alterations in gene expression. Due to the lack of DNA repair enzymes, mitochondrial DNA appears to be more susceptible to ROS-induced mutations. Protein oxidation may cause the aggregation of insoluble protein, which serves as the molecular basis of some diseases, especially neuro-degenerative pathologies (Brieger K, 2012). There is an increasing amount of evidence indicating that NPs can cause cytotoxicity due to their

large surface area, which is capable of inducing ROS, a key factor that generates cellular stress and disease pathogenesis (Nel A, 2006). However, the impact of NP-induced ROS on the CNS is unclear. Several types of NMs, such as quantum dots and metal-oxide NPs, have been shown to cause the overexpression of intracellular ROS (Hanley C, 2009). NPs can provoke oxidative stress through multiple interactions, as reviewed in what follows (Figure 4.3.1).

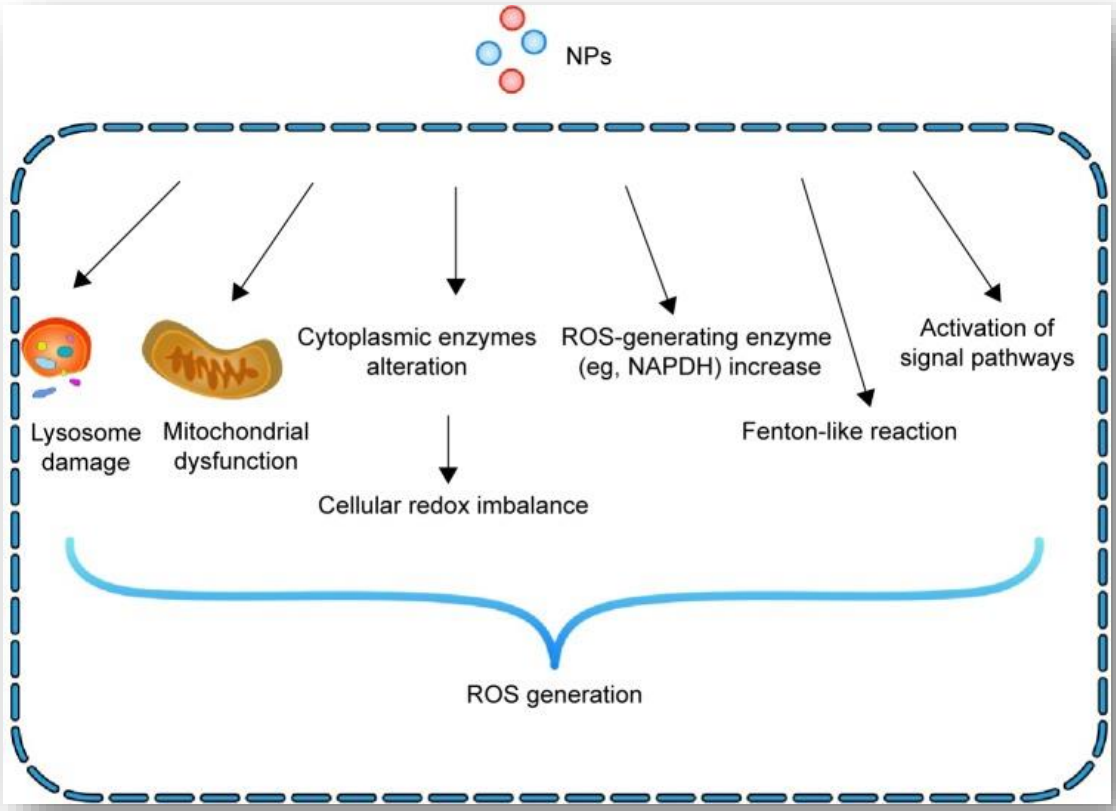


Figure 4.3.1 Mechanisms of NP-induced ROS generation in cells.

4.4 Interaction of NPs with Mitochondria

The mitochondria are the cellular energy house and play a significant role in all physiological and pathological processes via their ability to generate ATP. NPs of various sizes and shapes can accumulate in the mitochondria after interacting with cells. This accumulation can directly damage the mitochondrial membrane and

interrupt the respiratory chain function. As the latter is one of the main sites for ROS generation, any perturbation of this electron transport chain can result in increased ROS production. Oxidative stress and mitochondrial abnormalities have been reported to play a role in numerous neuropsychiatric disorders (Tobe, 2013). TiO2 NPs were shown to stimulate brain microglia to generate ROS and disturb the mitochondrial energy production (Long, 2006). Cellular alterations caused by TiO2 NPs, such as changes in the redox state, insufficient defense of antioxidant enzymes, and mitochondrial depolarization, may lead to cell apoptosis by activating apoptotic-signaling pathways. Siddiqui et al (2013) observed a decrease in the mitochondrial membrane potential followed by an increase in the Bax/Bcl-2 ratio, suggesting a mediator role of the mitochondria in ZnO NP-induced apoptosis. Shimizu et al found that prenatal exposure to anatase TiO2 NPs altered the expression of genes related to brain development, cell death, response to oxidative stress, and mitochondria in the brain during the prenatal period and the expression of genes related to inflammation and neurotransmitters in the later stage. Furthermore, Huerta-Garcia et al (2014) found that TiO2 NPs caused intense oxidative stress in both rat and human glial cells (C6 and U373 lines) by regulating changes in the cellular redox state and lipid peroxidation associated with a rise in the expression of glutathione peroxidase, catalase (CAT), and superoxide dismutase 2 (SOD2). TiO2 NPs also induced morphology changes, mitochondrial damage, and increased mitochondrial membrane potential, indicating a cytotoxic effect of TiO2 NPs on glial cells. In a subsequent study, the mitochondrial depolarization observed in C6 and U373 cells led to caspase-3 activation, chromatin condensation, and apoptosis (Zanotto, 2012).

The generation of mitochondrial ROS also plays a key role in several redox-associated signaling processes, such as the aging clock. Many studies have shown that mitochondrial integrity declines with increasing age (Balaban, 2005).

4.5 NP Interaction with Cytoplasmic Enzymes that act in Maintaining Cellular Redox Potential

After cellular uptake, NPs can directly interact with the cytoplasmic enzymes responsible for maintaining cellular redox potential, such as antioxidant enzymes and

NADPH. Free radicals are constantly generated in living cells and eliminated by antioxidant enzymes, which serve as the front line of defense against ROS in animal and plant cells. These enzymes include SOD, glutathione peroxidase, glutathione reductase, and CATs. Any damage to the antioxidant enzymes will lead to an increased ROS level in cells. Few studies have demonstrated that NPs can reduce the activity of antioxidant enzyme. Freyre-Fonseca et al (2011) found that cellular changes induced by TiO2 NPs included alteration in the redox state, reduction of the antioxidant enzymatic defense and mitochondrial depolarization, and activation of the apoptotic pathways. In addition, ceria NPs have been shown to be capable of altering brain oxidative stress indicators and antioxidant enzymes after intravenous administration in rats, demonstrating the ability of metal oxide NPs to produce neurotoxicity (Hardas, 2010) In their recent study, Hardas et al (2014) found that cytoprotective Phase II antioxidant activities were inhibited in the rat hippocampus after a single systemic infusion of 30 nm nanoceria. A subsequent study found that both the SOD activity and CAT activity in the liver cells were down regulated after oral administration of purified and functionalized MWCNTs suspended in water (Awasthi 2013). Reduced SOD and CAT activity have been observed in the digestive glands of mussels in Mytilus galloprovincialis after exposure to 10 μg/L Cu in the form of CuO NPs and Cu2+ for 15 days (Gomes, 2012). Furthermore, nanoiron induces a decrease in the SOD level and increases the malondialdehyde level in a dose-dependent manner in the medaka embryo. In adult med akas, a damaged ant oxidative balance occurs during the early exposure period, as indicated by monitoring the hepatic and cerebral SOD and reduced glutathione (Li, 2009).

ROS were once thought to originate nearly entirely from the mitochondrial metabolism. However, increasing evidence has demonstrated that cellular enzymes, such as NADPH oxidase, are also important sources of ROS in humans (Krause 2007). Wilhelmi et al (2013) demonstrated that ZnO NPs are able to trigger p47phox NADPH oxidase-regulated ROS formation in macrophages and induce rapid nuclear condensation, DNA fragmentation, and the formation of hypo diploid DNA nuclei and apoptotic bodies in the murine macrophage RAW 264.7 cell line.

Interestingly, in Culcasi et al (2012) after micro molar doses of nano-CeO2 were applied to human fibroblasts, the membrane NADPH oxidase activation occurred. The

cytotoxic effects may be caused by the activation of both the mitochondrial and Nox2- and Nox4-dependent NADPH oxidase complexes. These studies also suggest that the specific inhibition of ROS-producing enzymes may be a new approach promising clinical efficacy in treating ROS-related disease (eg, cardiovascular and neurodegenerative diseases), especially because the current widespread use of antioxidant supplementation has proven largely ineffective in treating diseases caused by a surplus of ROS (Thomas 2007).

Although oxidative stress caused by NMs was thought to be the main mechanism for NMs toxicity in different cell models, the exact role of ROS formation and degradation dysfunction in CNS toxicity caused by NMs is still unclear.

CHAPTER 5 TOXICITY ASSESSMENT AND RISK MANAGEMENT

5.1 Risk Management of NPs

The vast diversity of engineered NMs and their great potential for commercial applications have introduced significant challenges for risk assessment and management. While the risk management framework provides a systematic and scientific approach for risk characterization, application of such a framework to NMs often involves uncertainty factors significantly larger when compared to other chemicals or pharmaceuticals. The International Organization for Standardization (ISO) has established a number of technical reports to provide a framework for risk assessment of nanotechnologies such as ISO/TR12855 and ISO/TR13121 (ISO. ISO/TR 12885). The later technical report provides a framework for hazard identification, risk evaluation, decision options, and the risk communication of manufactured NMs in order to protect health and safety of exposed populations, including the general public, consumers and workers as well as the environment ISO. ISO/TR 13121). While the risk assessment framework is not unique to NMs, the process is focused on engineered NPs used in industrial settings, chemical manufacturing, and consumer product applications and on the potential risks related to the release of NMs during their life cycle. This technical report supplements issues specific to manufactured NMs and includes admitting where information is not complete, how to address information gaps, and the rationale behind risk management decisions and actions. The recommended process can be summarized in Table 5.1.1 (ISO. ISO/TR 13121, Australia 2011).

Table 5.1.1 Risk management framework relevant to nanomaterials.

Risk Management	Details of Each Step Relevant to Nanomaterials
Identify hazard	Describe nanomaterial & applications Determine nanomaterial profiles Physicochemical profiles Hazard profiles

	Exposure profiles
Evaluate risk	Based on the combination of: Identified hazards, exposure, potential risks Exposure patterns: including likelihood and severity
Control risk	Level 1: Eliminate the hazard Eliminating the nanomaterial Level 2: Substitute, Isolate and engineering controls Substitute to a safer material, product or process Apply process containment Use local exhausted ventilation systems equipped with efficient filters (e.g., HEPA) Level 3: Reduce exposure by Administrative controls (e.g., develop Safety data sheets and safe work procedures) Personal protective equipment (e.g., appropriate gloves, eye and respiratory protection)
Decide, document & act	Decide: Whether or in what capacity to continue development and production of the nanomaterial Sharing information with the stakeholders Further information to be collected
Review & adapt	Update the risk assessment process through: Regular reviews Reviews triggered by specific events

5.1.2 Neurotoxicity Studies of NP

The central nervous system is composed of two parts: the brain and the spinal cord. Both of them are delicate organs inhuman body which must be protected from the injury to xenobiotic. Several drugs could distribute into the CNS and thus cause

unwanted neurotoxicity by themselves (Chow et al., 2003; Screnci and Mc Keage, 1999). Also, recent observation suggests that several NPs, such as polysorbate 80-coated PBCA NPs and pegy laded PLA immune nanoparticles, are able to cross BBB (Olivier, 2005; Sharma and Sharma, 2007) through intravenous administration and followed by the accumulation in the brain. As these NPs have the capacity to penetrate the BBB, they may subsequently influence the BBB function and brain physiology and cause severe side effects. So far, there are already some reports, but not many, which observed the neurotoxicity of nanoparticles both in vitro and in vivo (Kim et al., 2008; Pisanic et al., 2007). In vitro studies Several laboratories have reported potential toxic effects of nanoparticles on different types of cells in vitro(Deng et al., 2009;Hussain et al., 2006; Long et al., 2007; Pisanic et al., 2007). The most widely used cell model for NPs neurotoxicity study is PC12cells which are a cultured neuronal phenotype and was used as a paradigm for neurobiological and neurochemical studies (Greene and Tischler, 1976). Changes in cellular viability after exposure to nanoparticles are assessed using MTT method. For instance, Pisanic et al. (2007) showed that exposure to increasing concentrations of anionic magnetic nanoparticles (MNPs), from 0.15 to 15nm of iron, resulted in a dose-dependent diminishing viability of PC12cells using MTT method. Also, the capacity of PC12 cells to extend neuritis was deceased in response to nerve growth factor (NGF). Hussain et al. (2006) reported that the exposure of PC12 cells to manganese oxide (Mn-40nm) particles increased the production of reactive oxygen species (ROS) and could deplete dopamine (DA), dihedronxy phenyl acetic acid (DOPAC) and homo vanillin acid (HVA) in a dose-dependent manner, while the Ag-15nm could produce cell shrinkage and irregular membrane borders. Wanget al. (2009) examined the expression changes of dopaminergic system-related genes in PC12 cells induced by manganese, silver, or copper nanoparticles, and found that Cu-90 nanoparticles induced dopamine depletion in PC12 cells, which was similar to the effect induced by Mn-40. The results suggested that Mn and Cu NPs could induce dopaminergic neurotoxicity and might share some common mechanisms associated with neuron degeneration. Besides PC12 cell lines, primary culture cell lines were also used to assess the neurotoxicity of NPs. To examine the possible neurotoxicity of TiO2, brain cultures of immortalized mouse microglia (BV2), rat dopaminergic neurons (N27), and primary cultures of embryonic rat striatum were exposed to Degussa P25, a kind of commercially available TiO (2) nanomaterial. BV2 microglia, which was exposed

to P25 (2.5–120ppm), responded with an immediate and prolonged release of ROS. Microarray analysis onP25-exposed BV2 microglia indicated up-regulation of inflammatory, apoptotic, and cell cycling pathways, and down-regulation of energy metabolism. These results indicate that P25 is nontoxic to isolated N27 neurons, but stimulates BV2 microglia to produce ROS and damages neurons at low concentrations in cultures of brain striatum, plausibly though microglial generated ROS (Long et al., 2007). Nano sized zinc oxide particles were examined for their neurotoxicity in mouse neural stem cells (Deng et al., 2009). It was found that ZnO nanoparticles induced cell apoptosis and this toxicity comes from the dissolved Zn (2+) in the culture medium or inside cells. Generally, the above mentioned NPs of different particle sizes, ranging from 20 to 300 nm, or produced by different materials could cause cell apoptosis in vitro, indicating there are potential harmful effects of NPs to human health, and further studies on the acute and long-term effects of NP sin vivo is both warranted and necessary.

5.1.3 The Mechanisms of Neuron Injury by Ambient and Metallic NPs

The mechanisms of neuron injury are diverse for such a wide variety of materials used (Donaldson, 2006). However, a common mechanism of oxidative stress (OS) caused by surfaces, organics and metals associated with the NPs has been identified (Nel et al., 2006; Oberdorster et al., 2004). This oxidative stress leads to inflammation (Donaldson et al., 2003) and then forms a link between the exposure to these particles and the types of adverse effects observed (Donaldson et al., 2005). As a number of manufactured NPs have already been demonstrated to cause oxidative stress, this maybe a common mechanism of NP toxicity (Donaldson, 2006). For example, it was suggested (Rahman et al., 2009) that Ag-25 nanoparticles have the potential to generate reactive oxygen species (ROS) via a metabolic pathway and induce oxidative stress, including oxidative DNA damage. Also, the elevated levels of oxidative stress (OS) were found in the brains of Apo E-deficient mice exposed to concentrated particles, indicating nanoparticle could cause OS in the brain (Veronesi et al., 2005).
OS caused by free radicals generated by the interaction of NPs with cells may result in cell death. Evidence of mitochondrial distribution and oxidative stress response after endocytosis of nanoparticles was noted (Chan, 2006; Oberdorster, 2004).

And it is thought that nanoparticles, such as 60nm NH2-labeled polystyrene (PS) Nano spheres, may injure cells by gaining access to cell organelles (such as mitochondria) while larger particles (200nm NH2-labeled polystyrene (PS) Nano spheres) may not (Xia et al., 2008). In the olfactory bulb, the particles were found to be located mostly within mitochondria. The mechanism for such an accumulation is unclear, but it is evident that the translocation of nanoparticles into the mitochondria can lead to cellular toxicity (Mistry et al., 2009; Xia et al., 2008).

ROS is reported to be associated with several neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, and Huntington's disease (Mates et al., 1999). Evidence for the involvement of ambient air NPs in these disorders was presented by studies on biopsies from city dwellers. Alzheimer's-like pathology was demonstrated in brain sections by increased markers of inflammation and AB42-accumulation in frontal cortex and hippocampus in the presence of nanoparticles (Calderon-Garciduenas et al., 2004). Also inhalation exposure of BALB/c mice to particulate matter showed activation of prion flammatory cytokines in the brain (Campbell et al., 2005). The brain is especially vulnerable to OS damage because of its high content of easily peroxidizable unsaturated fatty acids, high oxygen consumption rate, and relative paucity of antioxidant enzymes compared with other organs (Skaper et al., 1999). NPs possess unique physical and surface properties and may cause oxidative stress. Therefore, brain could be continuously exposed to ROS generated by the NPs. In the CNS, OS is largely mediated by the microglia, a macrophage-like, phagocytic cell that is normally inactive unless confronted by potential damage and exogenous stimuli (e.g., xenobiotic, chemicals, particles) (Fig. 5.1.3.1). Their immediate response to such stimuli is known as the "oxidative burst" (Colton and Gilbert, 1987; Segal and Abo, 1993) and involves a rapid sequence of events that includes an increase in metabolic activity, a change in cell shape and size, and cytoplasmic Fig. 1. The representative schematic of neuron injury caused by nanoparticles (NPs).

The exposure of quiescent microglia to NPs results in the elaboration of numerous pseudopodia which engulfed NPs, and followed by the generation of ROS, which caused oxidative stress and induced neuron injury. Engulfment (i.e., phagocytosis) of the offending stimuli (Long et al., 2006, 2007).

Kleinman et al. (2008) found that inhaled ultrafine particulate matter could affect CNS inflammatory processes and they also demonstrated that such effect may be

exerted via MAP kinase signaling pathways using Apo lipoprotein E knockout mice as a model.

Increased nuclear translocation of two key transcription factors, NF-kappa B and AP-1, being involved in the promotion of inflammation, was found in the brains of mice exposed to the nanoparticles. Furthermore, several MAP kinases involved in the activation of these transcription factors were analyzed and it was found that JNK in the active form was significantly increased in the animals receiving a lower concentration of concentrated ambient particles (CAPs).

Therefore, the neurotoxicity of ambient and mental nanoparticles may be induced initially via the generation of ROS, which could cause oxidative stress, and followed by the up-regulation of MAP kinases to activate the MAP signaling pathway. Then, the inflammation related cytokines are highly expressed in the brain to cause brain injury.

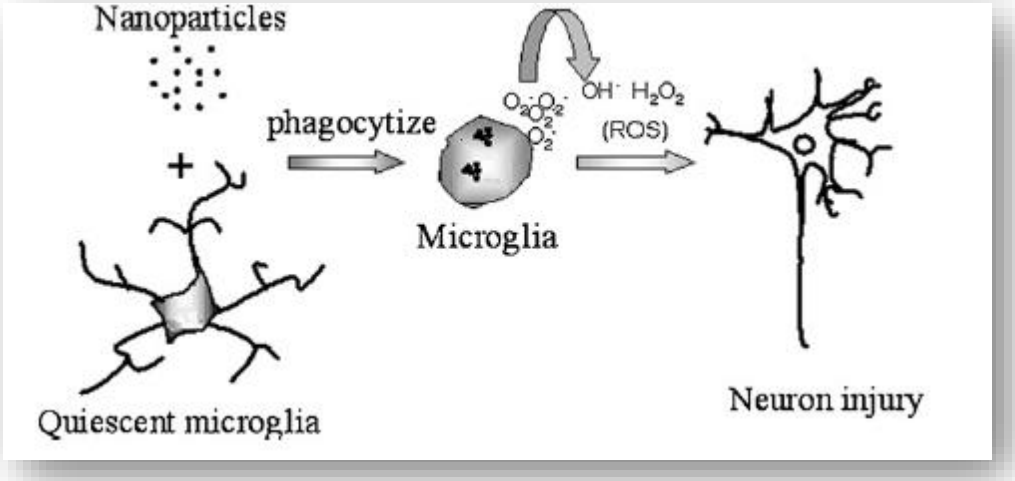


Fig. 5.1.31. The representative schematic of neuron injury caused by nanoparticles (NPs). The exposure of quiescent microglia to NPs results in the elaboration of numerous pseudopodia which engulfed NPs, and followed by the generation of ROS, which caused oxidative stress and induced neuron injury.

5.2 Mixed Silver Ion and Particle Toxicity

Exposure to 20 or 110 nm silver nanoparticles and their dissolution products caused loss of viability in RAW 264.7 cells and bone marrow derived macrophages, with significantly different dose-response curves (Fig. 5.2.1), particularly for SR-A(−/−) cells. We hypothesized that the form of silver causing toxicity could be identified by consistent LD20 values across particles and cell types. For example, if silver ions from cell culture medium entering cells were primary drivers of toxicity, similar LD20 values would be observed between particle uptake competent cells and SR-A(−/−) cells. LD20 values for all cell types and particles based on ISD3 calculated silver ion content ranged from 3.0–3.8 ng, demonstrating the most consistent dose metric measured by coefficient of variation (10%; Table 5.2.1). LD20 values based on other dose metrics including total silver nominal media concentration, cellular nanoparticle mass, and cellular nanoparticle surface area were much more variable (coefficient of variation: 67–86%; Table 5.2.1). These findings support our hypothesis that silver ions derived from media were driving toxicity of silver nanoparticles, even under conditions of combined particle/ion exposures.

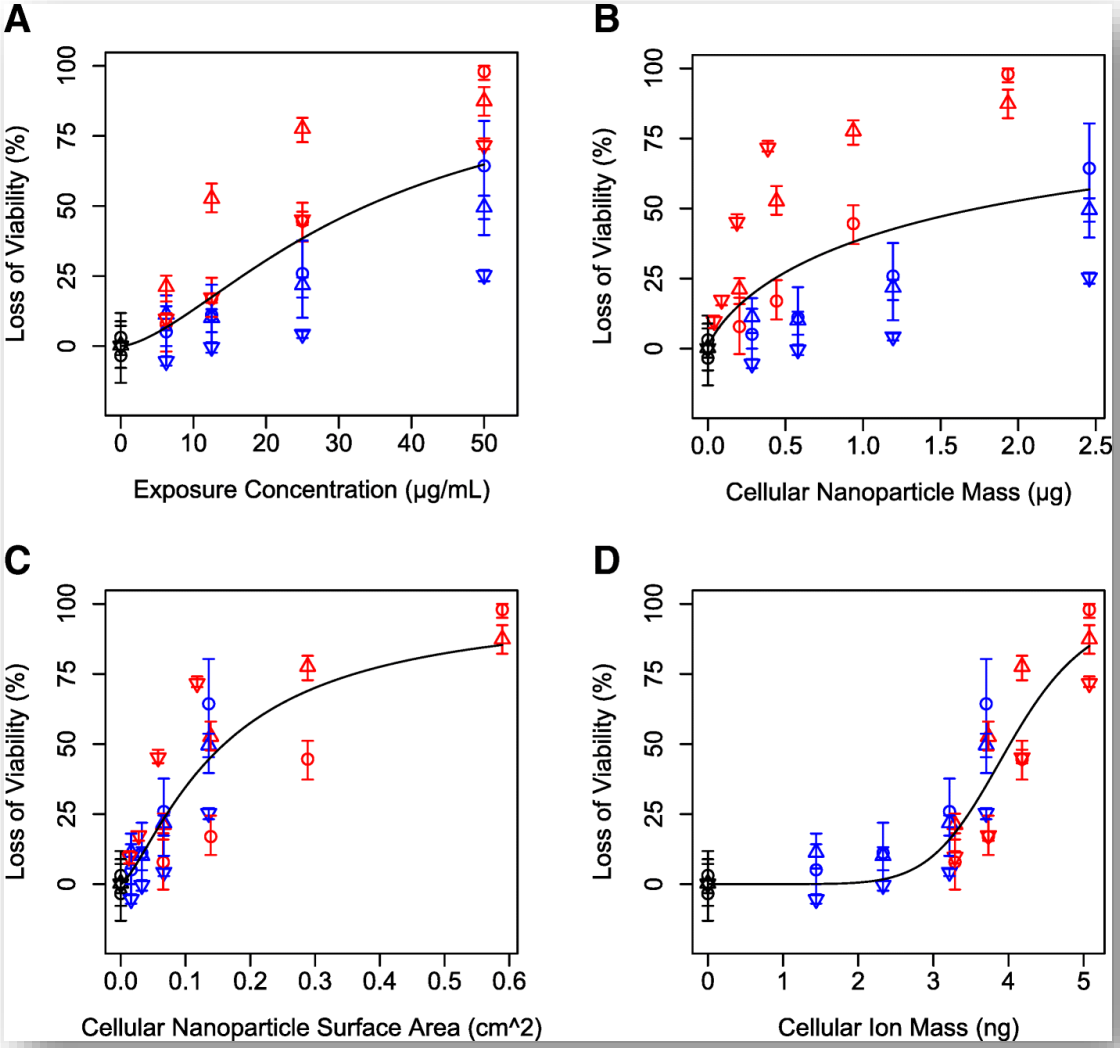


Table 5.2.1 Loss of viability in RAW 264.7 cells (circle) and bone marrow derived macrophages from wild-type mice (up triangle) and SR-A deficient mice (down triangle) exposed to various concentrations of 20 nm (red) or 110 nm (blue) silver nanoparticles (a) as a function of various dose metrics predicted by ISD3 including cellular nanoparticle mass (b), cellular nanoparticle surface area (c), and cellular ion mass (d). Lines are dose response model fits to the data.

5.3 Fresh and Aged Silver Ion Toxicity

Unpaged silver ions were 5–35 times more toxic on a nominal media concentration basis compared to silver nanoparticles (Figs. 2a, 3a). Freshly mixed silver ions produced a steep dose-response curve for cell viability in RAW 264.7 cells after 24 h of exposure (Fig. 5.3a). LD20 and LD50 values were very close (1.28 and

1.42 µg/mL), indicating a steep dose-response curve. Almost complete (98%) loss of viability was observed after 24 h of exposure to 2.4 µg/mL of freshly mixed silver ions. LD20 values from nanoparticle exposures ranged 6–45 µg/mL (Table 5.2.1).

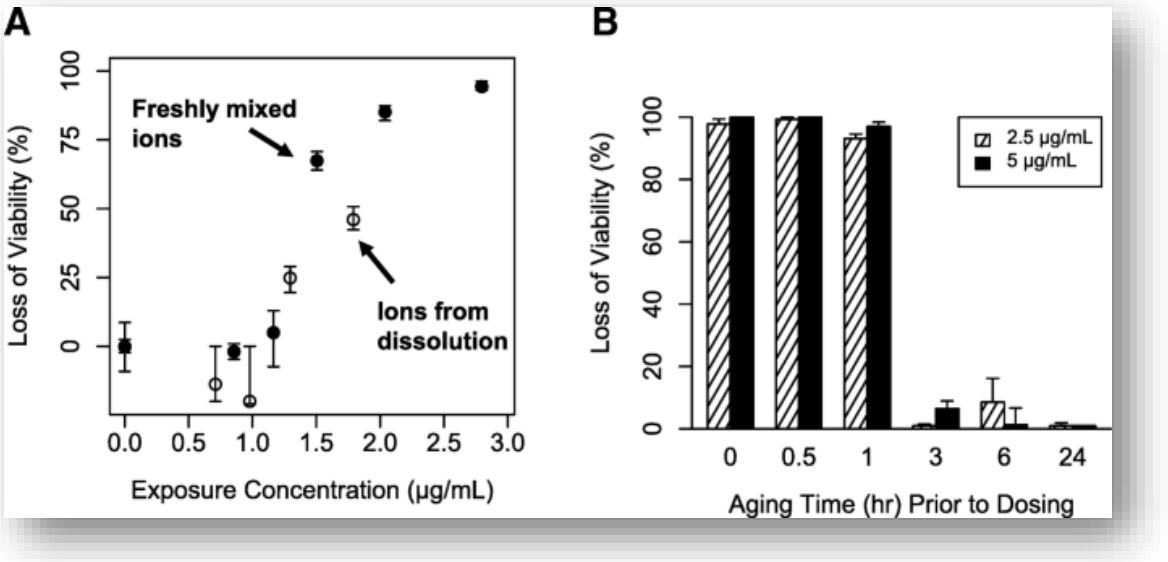


Fig. 5.3a Loss of viability in RAW 264.7 cells exposed to freshly mixed silver ions (closed circle) and silver ions formed from dissolution of 20 nm silver nanoparticles (open circle) (a). Loss of viability in RAW 264.7 cells exposed to silver ions (2.5 or 5 µg/mL) for 24 h aged in cell culture medium for various amounts of time before dosing to cells (b). Loss of viability was assessed 24 h post exposure for all experiments.

Aging silver ions (incubating in cell culture medium) for three or more hours before dosing significantly attenuated toxicity in RAW 264.7 cells. Silver ions aged for ≤1 h prior to dosing caused almost complete loss of cellular viability after 24 h of exposure at two exposure concentrations (2.5 or 5.0 µg/mL; Fig. 5.3b). Nearly no toxicity was observed after aging ions for ≥3 h (Fig. 5.3b) under the same exposure conditions. We hypothesized that during aging, silver cations (Ag+) bind to ligands available in cell culture medium (e.g. Cl−, SO4−, S−, proteins, etc.), and these bound silver complexes are less toxic than unbound silver cations. We hypothesize that the abrupt change in toxic to nontoxic is due steep dose response curve for silver ions (Fig. 5.3a).

Silver ions formed from nanoparticle dissolution were toxic to RAW 264.7 cells (LD50: 1.81 μg/mL, Fig. 5.3a). Two doses of ions formed from nanoparticle dissolution demonstrated toxicity. The highest dose of silver ions formed from dissolution (maximum ion concentration from ion harvesting conditions) were less toxic than freshly mixed ions (LD50: 1.42 μg/mL), while at lower doses, toxicity was similar (Fig. 5.3a).

5.4 Cellular Toxicity is Best Predicted by Cellular Silver Ion Mass

To further test our hypothesis that silver ions had the strongest influence of cellular toxicity, relationships between multiple dose metrics and viability were tested by fitting dose response models to data from all three cell types, both particles, and ion-only exposures combined into one dataset. ISD3 was used to calculate cellular dose metrics including intracellular particle mass, intracellular particle surface area, cellular ion mass, and associated AUCs. Intracellular nanoparticle mass poorly predicted loss of viability (highest AIC) (Fig. 2b). This finding is consistent with a hypothesis that complete nanoparticle dissolution within cells is unlikely and not controlling toxicity. Intracellular ion mass and intracellular ion mass AUC were the best predictors of loss of cellular viability (lowest AIC's). This finding is consistent with our hypothesis that silver ions are a primary contributor to cytotoxicity during exposure to silver nanoparticles in vitro, where ions and particles are both present. We found that the intracellular ion AUC was the best predictor of toxicity (AIC values: − 35.1 vs − 29.5, respectively; Fig. 4) when all dose-response data were included: ions only and mixed particle and ion exposures for 20 and 110 nm particles in three cell types.

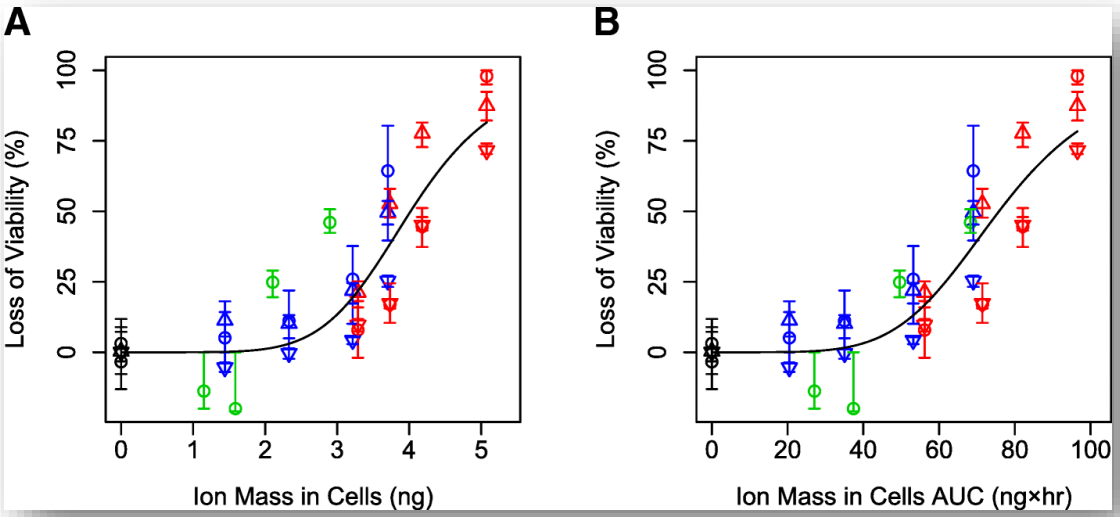


Fig. 5.4.1 Loss of viability in RAW 264.7 cells (circle) and bone marrow derived macrophages from wild-type mice (up triangle) and SR-A deficient mice (down triangle) exposed to various concentrations of 20 nm (red), 110 nm (blue) silver nanoparticles, or silver ions formed from dissolution of 20 nm silver nanoparticles (green) as a function of ion mass in cells (a) or ion mass in cells AUC (b) predicted by ISD3. Lines are dose response model fits to the data.

5.5 Release/Occurrence of Ag NPs into Environment

It is important to study the release of Ag NPs into environment as their production and ubiquitous use may potentially affect the health as well as environment. In order to foresee the occurrence of Ag NPs into the environment, we have to know their release routes such as during manufacturing, synthesis, use, and disposal of products (Benn and Westerhoff2008a,b; Benn et al.2010).The discharge via use and disposal of Ag NPs containing products needs more attention in risk assessment. Nano silver Centre for food safety is a product inventory authority which found products containing Ag NPs in(assessed on October 10, 2016) textile, food products, personal care products, medical aids, paints, etc. According to Project on Emerging Nanotechnologies (PEN) 2005,48% of nanomaterial-related consumer products in

the market claim to have Ag NPs. There will be even more number of products as many of the products may be missing in the list due to unavailability of information. Some of the important studies giving significant values regarding emission of Ag NPs have been reviewed, and it is found that a significant contribution of Ag NPs to water comes from textiles and personal care products during their use. Some other products are also contributing their share to the value to some extent. In 2008, Blaser et al.(2008) predicted that in 2010, 15%of the total Ag released into water in the European Union would come from biocidal plastics and textiles. Many reported results have justified the prediction. Benn and Westerhoff (2008a,b) found 15lgofAg?/L when Ag NP-containing sock fabric was washed once in tap water and confirmed the presence of Ag particles of diameter between10 and 500 nm. Geranio et al. (2009) measured 377lgofAg/g while washing with the detergent and concluded that dissolution was more in the presence of bleaching agents at neutral medium. It is also important to highlight the usage conditions which play a major role in the release of different forms of Ag into water. Benn et al. (2010)by assuming that all the released silver enters the sewage system calculated that 470lg/day of Ag NPs is released per capita from the use of daily use products (shirt, shampoo, detergent, toothpaste, etc.). The calculation used the wash water data for toothpaste and shirt and assumed that the shampoo and detergent release 100% of the silver content. It has been further elaborated that If 10,000 people released 470lg/day of Ag NPs per capita into 3785411.8liters per day capacity WWTP, the bio solid silver con-centration's may increase by 0.7 mg kg-1(Benn and Westerhoff 2008a,b). Echegoyen and Nerı́n(2013) considered three products to monitor the migration of NPs into food items and found NPs of concentration in the range of1.66–31.46 ng/cm2in all samples when analyzed through ICP and SEM-EDX, which was well below the permissible limits. Previous reports have found that increases in storage time and temperature (Alfadul and Elneshwy2010;Bouwmeester et al.2009; Huang et al.2011) result immigration of NPs. Huang et al. (2011) worked on commercial food container (polyethylene plastic bags) at temperature (25–50°C) and time intervals (3–15 days). SEM and AAS confirmed the migration of Ag NPs into food-simulating solutions. Similarly, the Ag NP products used in medical/healthcare sector also raise the issue of the negative impact of Ag NPs during or after usage. Benn et al.(2010) assessed the potential of Ag NP-based products to release NPs. Medical

mask was one such product which contained approximately 27% silver by weight, and released\0.01% (15.8lg) of its silver into the environment. These types of results may be used to estimate the concentration of Ag NPs that may enter sewage system though the usage of different products. However, for all commercialized products, such data are not available, and more studies are required in this context under different sets of usage conditions.

5.6 Silver Nanoparticles

Size

Exposure to SNPs and their persistence in the body can trigger undesirable effects (Hadrup N, 2014). However, it is still controversial if toxicity is associated with the nanoparticle itself or with the release of silver ions (Nel A, 2006). In this sense, Dąbrowska-Bouta et al (2016) assessed prolonged and low-dose exposure to SNPs (10 nm, citrate-stabilized) or ionic silver (Ag+) in male Wistar rats. Slight differences in behavior were found, but rats treated with SNPs increased their body weight and temperature, whereas after Ag+ exposure, animals presented a weak depressive effect and hyperalgesia. Pathological changes in myelin were also observed in brain tissues from Ag+ and SNPs-treated groups, compared to controls, and the levels of all examined proteins significantly decreased in the rats treated with Ag+ and SNPs, suggesting that both treatments produce a neurotoxic effect. Hadrup et al (2012) carried out a similar study, but compared the neurotoxic effects of 14 nm SNPs coated with polyvinylpyrrolidone (PVP) and ionic silver (Ag+, the form of silver acetate). SNPs, and a filtered fraction (containing ionic silver, 12kDa), showed equal toxicity, whereas ionic silver was toxic at doses two or tenfold lower than those of SNPs. Female Wistar rats orally treated with SNPs-PVP or Ag+ for 28 days did not show general appearance or behavioral changes, but presented alterations in neurotransmitter levels. The authors found a dose-response effect on neurotransmitter levels at the highest SNP concentration and the equimolar concentration for Ag+ in the treatment. Both SNPs and Ag+ also increased 5-hydroxytryptamine, noradrenaline and dopamine levels (Powers CM, 2010, Powers CM, 2011). Moreover, the addition of SNPs to PC12 cells led to alterations in the mRNA levels of dopamine-related

genes, (Wang J, 2009) confirming that SNPs or ionic silver induce disturbances of neuronal cell differentiation and concomitant ability to produce dopamine. The results suggest that SNPs and ionic silver exert similar neurotoxic effects.

Soria et al investigated the toxic effects of commercial 10 nm SNPs on wild type and transgenic Caenorhabditis elegans (C. elegans) strains expressing two prototypic amyloid genic proteins: b2-microglobulin and Aβ3–42 peptide. The toxicity of SNPs could be detected in C. elegans at lower concentrations than in in-vitro studies. In the N2 wild type C. elegans, the exposure to a range of SNP concentrations significantly affected the worm's body bends in a dose-dependent manner. In the absence of nanoparticles, both transgenic strains exhibited a significant defect in their locomotory function, compared to the ancestral nematodes, but SNPs severely worsened their motility. A dose-dependency was also observed with the highest concentration tested (5.2 μg/cm2), which reduced body bends by almost 50% in the transgenic strains; 50% in the strains expressing b2-m, and 30% in those expressing the Aβ peptide (versus 20% in N2 wild type C. elegans) (Soria C, 2015).

The aforementioned studies suggest that small silver nanoparticles ranging from 10–20 nm possess in vivo toxicity effects, probably due to the induced production and release of silver ions from the nanoparticle's surface.

Functionalization

Surface modification of SNPs has been carried out for targeting and to improve their colloidal stability. Some studies, such as that of Khan et al, (2019) have leaned towards a green synthesis of nanoparticles by studying the in vivo toxicity of Rumex acetosa SNPs of 20 to 30 nm (SNPs-RU) in a zebrafish model. Zebrafish embryos orally treated with SNPs-RU showed embryonic head and pericardial malformations, yolk sac edema, and a bent tail. Increased exposure to SNPs-RU also increased mortality and produced severe toxicity in a dose-dependent manner. SNPs-RU at 50 and 100 μg/mL concentrations produced pericardial edema and bent tail formation, indicating that SNPs-RU mediate apoptosis in a dose-dependent manner.

The previous report described an oral administration route. However, other vias, such as intravenous (IV) or intranasal (IN), may lead to distinct accumulation, retention, and elimination profiles and therefore, different toxic outcomes. Chrastina et al (2010) developed radiolabeled 12 nm SNPs stabilized with polyvinylpyrrolidone (SNPs-PVP) and iodine-125 for in vivo tissue uptake tracking after IV administration in Balb/c mice. Bio distribution analysis revealed the highest uptake in the liver and spleen (24 and 41% ID/g, respectively), and minimal brain uptake (< 0.1% ID/g). Even though the authors indicated that SNPs strongly tend to produce hepatotoxic effects due to their higher accumulation in the liver, long-term exposure may trigger higher brain accumulation and retention, as described by Antsiferova et al,(2015) who used a similar, but larger (34 nm) nanosystem. Bio distribution, accumulation, and excretion after oral administration of SNPs-PVP in SHK mice and the ability of SNPs to penetrate the brain after a single- or multiple-administration (100 μg of SNPs) was reported. The highest concentrations were observed in the liver and blood in one-time exposure experiments (Figure 5.6.1Aa). However, redistribution was observed at two months, with the highest silver concentration in the brain and liver (Figure 5.6.1Ab). Even though the silver concentration was reduced in all organs after one month without treatment (Figure 5.6.1Ac), a shallow level of SNPs excretion from mouse brains (only 6% per month) was detected, compared to that observed in blood and the liver (about 80%), which may be due to accumulation of SNPs (Antsiferova A, 2015). Similar results were described by Wen et al,(2016) who studied intranasal instillation of SNPs-PVP (26 nm) or Ag+ in neonatal Sprague-Dawley rats treated during a short-term study (4 weeks) or a long-term study (12 weeks + 4 weeks of recovery). No physiological alterations or behavioral changes were observed in rats treated with SNPs or Ag+ at lower concentrations (0.1 mg kg−1 day−1). However, at 1 mg Kg−1 day−1, Ag+ exposure decreased body weight gain and caused 18.2% of mortality. The in vivo distribution and retention of SNPs or Ag+ were evaluated by measuring total silver concentrations, which were found predominantly in the liver (Figure 5.6.1Ba) for both Ag+ and SNP treatments after four weeks of exposure, similar to previous reports (van der Zande M, 2012). Interestingly, in the Ag+ group, the second-highest silver level was detected in brain samples. The silver distribution analysis in specific brain regions, including the cortex, cerebellum, hippocampus, medulla, and olfactory bulb, is shown in Figure 2Bb, which depicts that all five regions contained equally high silver content in the Ag+ and SNP groups. The tissue distribution profile varied

distinctly after 12-weeks of exposure. The highest silver content was observed in brain tissue for both Ag+ and SNPs, with a significant decrease in liver silver concentrations (Figure 5.6.1Bc). Finally, the silver retention study revealed that the elimination ratios of silver in serum and liver after 1- and 4-weeks of recovery were approximately higher than 60% for both treatments. However, and similarly to the study by Antsiferova et al, (2015) silver concentrations in the brain remained unchanged or slightly elevated.

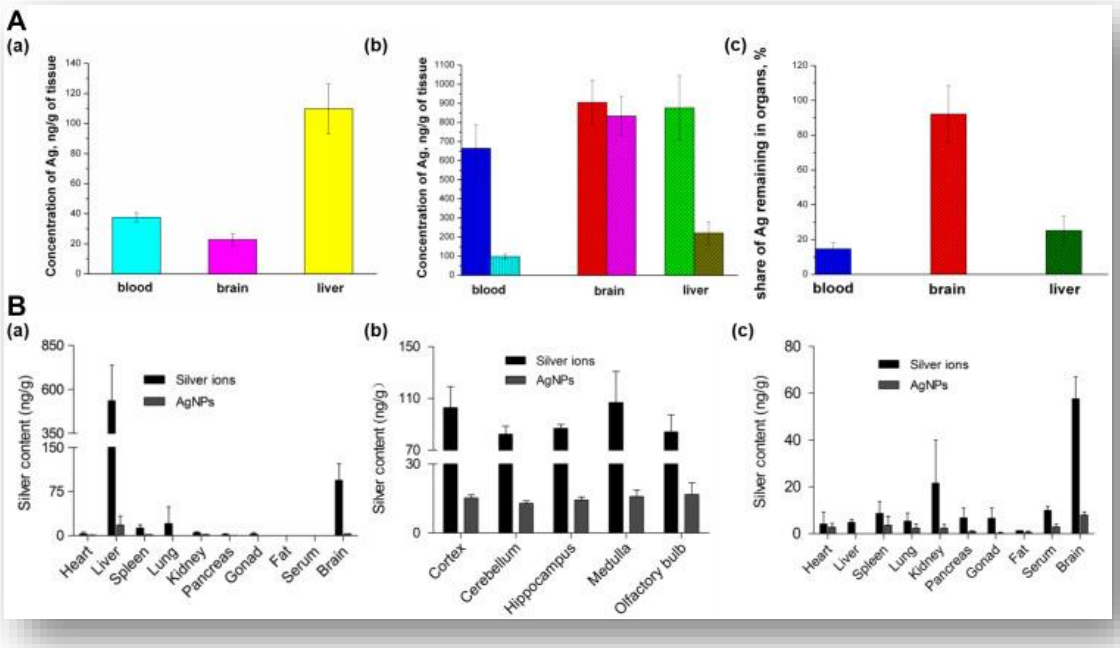


Figure 5.6.1 Biodistribution and toxicity of SNPs. (A) Silver concentrations in different organs (a) after one-time exposure to SNPs of size 34 nm, stabilized with PVP (b) after long-time exposure, (c) Fractions of silver remaining in mouse organs after 1 month of washing up. Adapted with permission from. Antsiferova A, Buzulukov Y, Demin V, Kashkarov P,Kovalchuk M, Petritskaya E. Extremely low level of Ag nanoparticle excretion from mice brain in in vivo experiments. IOP Conference Series. 2015; 98:012003. Creative Commons Attribution 3.0 licence (https://creativecommons.org/licenses/by/3.0/). (B) Silver concentrations in main rat organs with daily intranasal administration of silver ions and SNPs of size 26 nm, at a dosage of 0.1mg kg−1 body weight day 1 (n = 4). (a) 4-week exposure (b) Silver distribution in different rat brain regions after 4-week exposure. (c) 12-week exposure. Adapted with permission from Wen R, Yang X, Hu L, Sun C, Zhou Q, Jiang G. Brain-targeted distribution and high retention of silver by chronic intranasal instillation of silver

nanoparticles and ions in Sprague-Dawley rats. J Appl Toxicol. 2016;36(3):445–453. Copyright 2016 John Wiley and Sons (Wen R, 2016).

Therefore, despite the incorporation of molecules on the surface of 10 to 30 nm SNPs, the reported studies revealed the presence of toxic effects under the conditions studied (Wang J, 2009. Khan I, 2019) suggesting that SNP hazard might be associated with the release of ionic silver from the nanoparticle's surface and its action. As described, SNPs exhibit a longer half-life in the brain than in other organs. Given this tendency, SNPs and the related dissolved ions exert considerable neurotoxic effects, (Zhang X-F 2016) leading to an inflammatory response and oxidative stress, of which, the latter has been reported as one of the most important toxicity mechanisms related to silver exposure (Zhang X-F,2016, Strużyńska L. 2017).

5.7 Gold Nanoparticles

Size

Nanoparticle size may significantly influence the toxicological profile of gold nanosystems. Lee et al (2016) determined the cytotoxicity of 5 and 100 nm GNPs in human neural precursor cells (NPCs) and Sprague Dawley rats via unilateral injection, stereo tactically administered in the right cerebral cortex. The dose-dependency study on NPCs showed that 5 nm GNPs were more cytotoxic than 100 nm GNPs, reducing cell viability by up to 50%. Toxicity in the brain was determined in vivo by measuring nest in, whose expression is related to CNS injury. 5 nm GNPs showed higher nest in expression than 100 nm GNPs in immunoreactive cells, confirming that smaller GNPs were more toxic. On the other hand, other reports have demonstrated that the use of GNPs, with sizes ranging from 2 to 50 nm, does not produce significant neurotoxic effects (Rocha A, 2011). Naz et al (2016) reported a long-term in vivo study (90 days) of biokinetics, redistribution, and urinary excretion of 2 nm amine-terminated, citrate-capped 5 nm and 10 nm GNPs, administered by single IV injection in swiss albino mice at the dose of 1250 μg/Kg. The retention of GNPs in vital organs, such as the liver, spleen, lungs, heart, kidney, and brain, was estimated at different time points using inductively-coupled plasma atomic emission spectroscopy (ICP-AES). The

maximum amount of GNPs found in the brain was 2.0, 2.2, 2.3 μg/g for 2, 5, and 10 nm GNPs, respectively, and corresponded to lower concentrations than those found in other organs. The concentration of GNPs gradually decreased 1-day post-injection, and low concentrations were detected after 30 days. Brain tissue images showed occasional gold clusters in neurons and glial cells and confirmed very low sequestration of GNPs in the brain parenchyma. Moreover, the long-term study showed no effects of 2, 5, and 10 nm GNPs on mortality or abnormality of behavior, weight, necrosis, or fibrosis, compared with normal control mice. The authors indicated that ultrafine GNPs did not produce systemic toxicity and may be considered safe for drug delivery.

Moreover, 50 nm GNPs obtained from phytosynthesis showed to be biocompatible (Pannerselvam B, 2020). In this study, Wistar rats were intravenously administered with GNPs every alternate day for 28 days. Brain sections of animals showed a normal cerebellum, no alterations in cerebrospinal fluid, and the brain tissue evidenced normal axons. No detectable abnormalities in the experimental animals were found, compared to the control group, and no significant differences were noticed in body weight or hematological and biochemical parameters. Rocha et al used 50 nm GNPs, but capped with citrate, to evaluate the daily activity and survival rates during two months, as parameters of neurotoxicity in cockroaches (Blaberus discoidalis) (Rocha A, 2011). GNPs affected insect behavior, but had no major impact on their life expectancy, which may be attributed to the encapsulation of GNPs inside the insect's brain (day 17).

Lasagna-Reeves et al evaluated the bioaccumulation and sub-acute toxicity of 12 nm GNPs in C57/BL6 mice (Lasagna-Reeves C, 2010). Mice received daily intraperitoneal (IP) injections at doses of 40, 200, 400 μg/kg/day for 8 days. Results indicated that GNPs reached the CNS, and tissue accumulation was proportional to the administered doses (Figure 5.7.1Aa). At any of these doses, there were no alterations of behavior, animal weight, organ morphology, blood biochemistry, nor in histology examinations of the brain (Figure 3Ab) and other major organs.

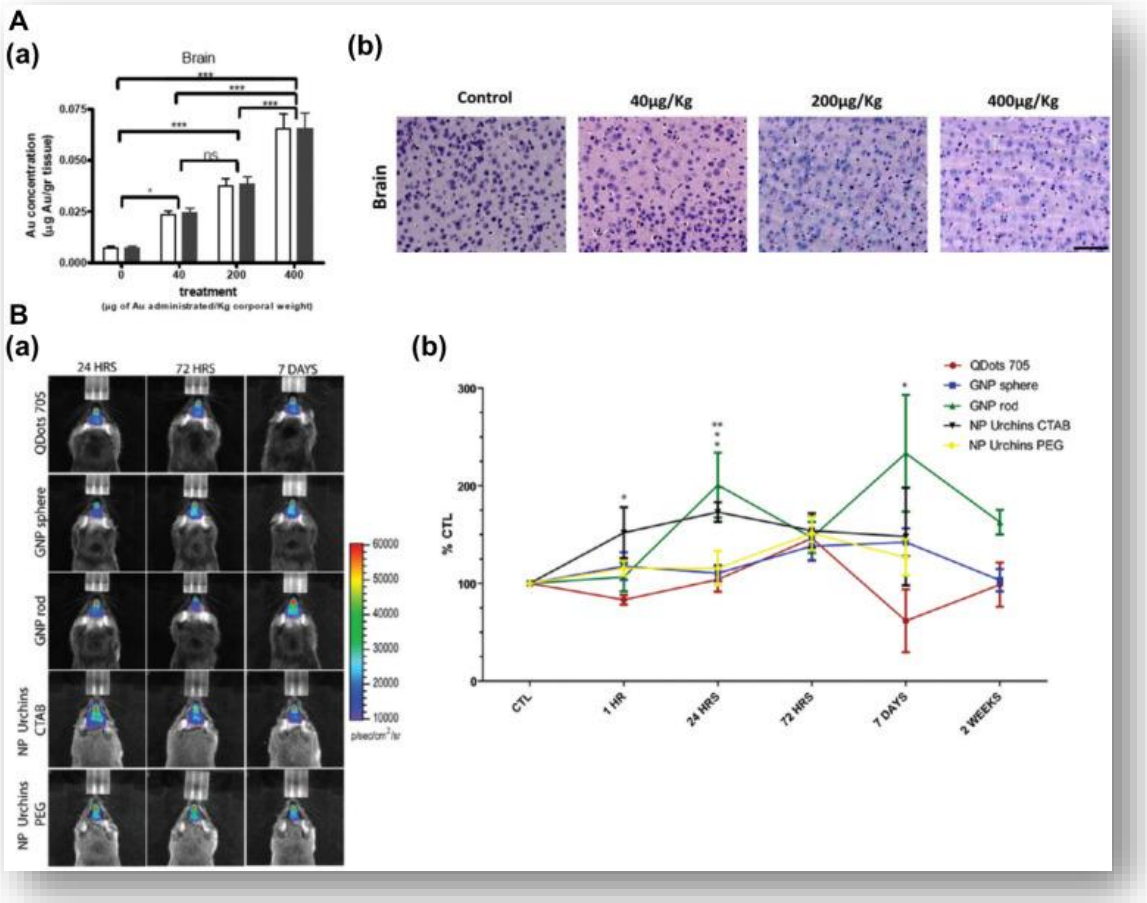


Figure 5.7.1 Distribution and toxicity of GNPs in the CNS. (A) Gold accumulation in the brain after intraperitoneal injection of GNPs (40, 200, and 400 µg/kg) for 8 consecutive days. (a) Gold accumulation was analyzed by GF-AAS (white bar) and ICP-MS (black bar). Bars represent mean ± standard error; data were analyzed by ANOVA with Bonferroni's post-test; ns: no significant difference, *$p < 0.05$, ***$p < 0.001$. (b) Histological analysis of the brain after treatment with GNPs to evaluate morphology and cellular damage. Tissues were stained with hematoxylin/eosin. Size of the bar corresponds to 75 µm. Adapted form Lasagna-Reeves C, Gonzalez-Romero D, Barria MA, et al. Bioaccumulation and toxicity of gold nanoparticles after repeatedadministration in mice. Biochem Biophys Res Commun. 2010;393(4):649–655. Copyright (2010), with permission from Elsevier.59 (B) In vivo bioluminescence imaging of microglia in transgenic mice after intranasal administration of GNPs. (a) Representative pseudocolor bioluminescence images (24 h, 72 h, 7 days, and 14 days) are shown for Quantum Dots 705, PEGylated gold spheres, rods, and urchins, and urchins capped with

CTAB. At right, quantification scales of the total photon emission signal (photon/s/cm2/sr). (b) Time course of the change in bioluminescence signal intensity in mice. Note a biphasic activation of microglia caused by rod GNPs, and a small, but significant microglia response to gold spheres. Mean values and standard error of the means from four animals per group per time point are shown. Significant differences: *p < 0.05; **p < 0.01. Adapted with permission from Hutter E, Boridy S, Labrecque S, et al. Microglial response togold nanoparticles. ACS Nano. 2010;4(5):2595–2606. Copyright © 2010, American Chemical Society.65; Images were with permission from Refs.

Another interesting study evaluated the interaction of uncoated GNPs (40 nm) and gold micro particles (GMPs, 637 nm) with endothelial cell barriers (Li C-H, 2015). Neither were genotoxic, mutagenic, nor affected the daily mortality and clinical behavior of ICR mice. However, when the authors examined the extravasation of Evans Blue dye in mice treated with GNPs, blue staining was evident in the brain at 24 and 48 h after dosing. These results suggested that GNPs alter the components of tight endothelial junctions, addressing the double-edged-sword effect of GNPs. Further studies are needed to investigate the direct/indirect toxicity caused by the opening of endothelial tight junctions induced by GNPs.

Functionalization

The incorporation of molecules on the nanoparticle's surface may improve biocompatibility of gold-based Nano systems and increase their delivery to the CNS. In this sense, 20 nm citrate GNPs were functionalized with a mixture of PEG and insulin (GNPs-INS) to determine their bio distribution pharmacokinetics for 48 hours (Shilo M, 2014). GNPs-INS and control GNPs were injected into the tail vein of male BALB/c mice. Higher accumulation of GNP-INS was found in the liver and pancreas, whereas the %ID in the brain was 0.64%. Toxicological analysis revealed that the maximum injected dose (6 mg Au per mouse) had no toxic effects in vivo. Later, the same group explored the brain distribution of GNPs-INS. Ex vivo and in vivo CT imaging and gold quantification studies revealed that particles migrated to the cerebral cortex, cerebellum, and hippocampus, which are structures involved in neurodegenerative and neuropsychiatric disorders. The authors mentioned that GNPs-INS were cleared from the brain by 48 h post-injection.

Improving the delivery of GNPs to the CNS is one of the main goals for the development of treatments or diagnostic tools. However, long retention in the brain may trigger toxic effects. Spinelli et al (2019) studied the kinetics, distribution, and stability of Cy5.5-labeled GNPs in CD1 mice. Although GNPs showed low cytotoxicity in cultured hippocampal neurons, in vivo pharmacokinetic distribution in the brain showed particle retention up to 25 days after intravenous injection. Therefore, in order to assess the biocompatibility of these gold-based nanosystems, long-term studies should be carried out. Sousa et al (2010) coated 15 nm citrate-GNPs with polyallylamine hydrochloride (PAH), polystyrene sulfonate (PSS), and human serum albumin to study the in vivo distribution of GNPs up to 7 days. The peak concentration in the head was detected between 19 and 24 h. GNPs were preferentially found in the hippocampus, thalamus, hypothalamus, and the cerebral cortex, brain areas of particular interest because they are related to Alzheimer´s (cerebral cortex), Parkinson´s (substantia nigra), and prion disease. No alteration in animal behavior was found over the complete experimental frame of 7 days.

Gold Nano clusters of about 3 nm have been radiolabeled with 64Cu (64Cu-GNCs) and complemented with the FUSIN technique to evaluate the ex-vivo spatial bio distribution in C57BL/6 mice (Ye D, 2018). Intranasal delivery of 64Cu-GNCs and FUSIN to the brainstem enhanced the delivery to the brain region by 2.72-fold, compared with intranasal administration only, and histological studies indicated that these nanoparticles did not produce tissue damage in the nose, trigeminal nerve, nor in the brain. Other radioisotopes have also been used to modify GNPs and monitor their bio distribution (Guerrero S,2012).

Even though most in vivo studies have used spherical nanoparticles, only a few reports have explored how shape might affect brain toxicity in vivo. Hutter et al (2010) reported the interactions of microglia and neurons with GNPs of three different morphologies (spheres, rods, and urchins) and different coatings (PEG and CTAB). After intranasal administration of these GNPs in transgenic mice (Figure 5.7.1Ba), a real-time study showed a transient up-regulation of toll-like receptor 2 (TLR-2) in the microglial cells located in the olfactory bulb (Figure 5.7.1Bb). In conjunction with the

in vitro results, the study demonstrated that the morphology and surface chemistry of GNPs strongly influence microglial activation and potentially produce toxic effects.

The in vivo toxicity and bio distribution studies of SNPs and GNPs have shown on one side, that small SNPs of 10–30 nm generate toxicity, probably due to the release of silver ions, which trigger biochemical alterations, abnormities in behavior, and neurotoxic effects. Moreover, although the functionalization of SNPs diminishes their toxicity and they exhibit a more powerful radio sensitizing ability than GNPs, long-term accumulation of SNPs in the brain might be counterproductive. These results may be the cause of the limited number of articles on SNPs in the last decade.

On the other hand, the tendency to use GNPs has increased. While it is difficult to establish a correlation between size and toxicity for small GNPs ranging from 2 to 10 nm, it is clear that larger GNPs (from 5 to 100 nm) are less toxic. Moreover, the functionalization of GNPs plays a fundamental role, since it increases biocompatibility and exerts minimal or no abnormalities on animal behavior after the administration of GNP-based nanosystems. Figure 5.7.2 illustrates a general conclusion of the effect of size and functionalization on toxicity; while small and uncoated nanoparticles increase toxicity, modified nanoparticles decrease it. However, there is a marked difference between the toxicity generated by GNP and SNP, since SNPs produce silver ions and generate a long-term accumulation in the brain and therefore, higher toxic effects than GNPs.

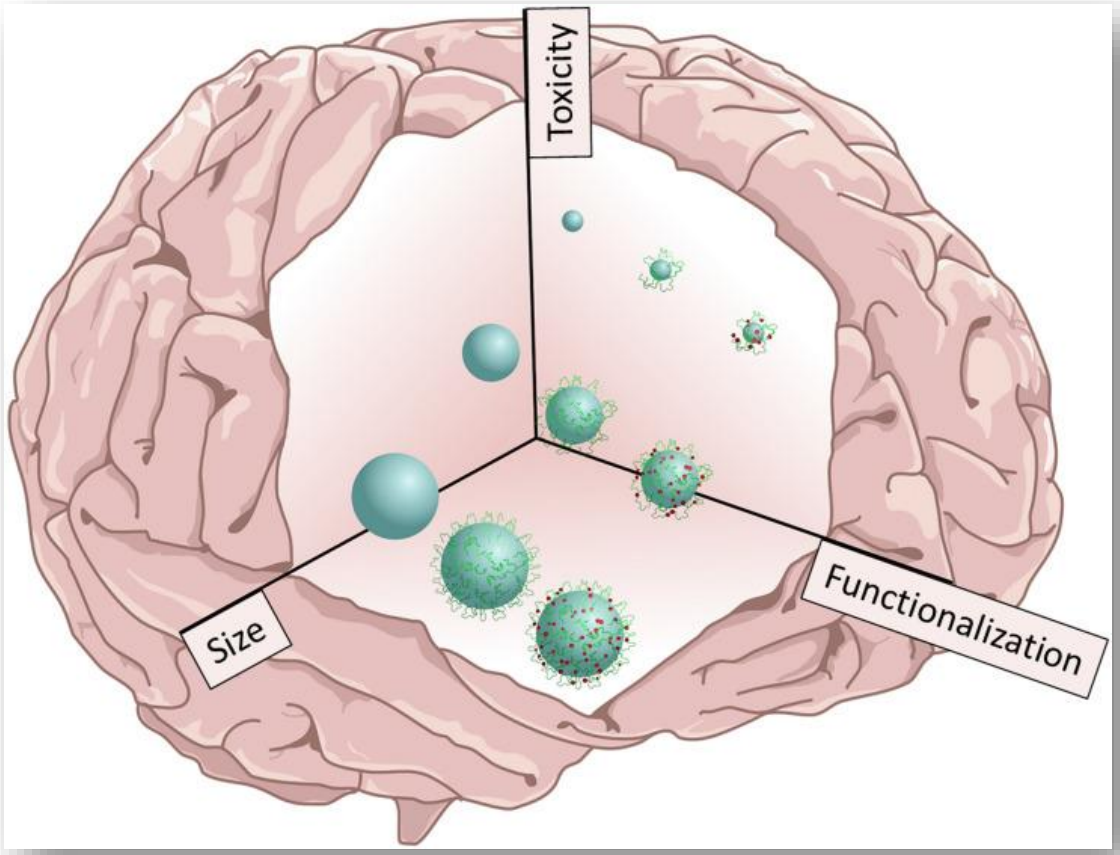


Figure 5.7.2 Effect of size and chemical composition on toxicity. Schematic illustration representing the size and chemical composition of either functionalized silver or gold nanoparticles as selected factors that produce toxicity effects in the CNS.

CHAPTER 6 DISEASES

6.1 Nanoparticles' Interaction with the Central Nervous System

Nanoparticles have shown biological effects such as fighting pathogenic bacteria and viruses (eg flu), but many studies have shown that nanoparticles may have a negative effect (in terms of dosage) on human. Human nerve cells, such as hippocampal cells located in the CNS, are considered to be the most vulnerable cells in the body and are responsible for the actions and emotions experienced by the brain. It is unsafe for ischemia, insufficient oxygen and various external factors. Nevertheless, science is raising concerns about technological development, even though we are in the twenty-first century in terms of nanoparticles that can cause potential toxic effects on human nerve cells because of their ability to pass through biological environments. 2001).

The blood-brain barrier (BBB) is the division of blood located in and the cerebrospinal fluid (CSF) maintained by the choroid plexus in the CNS, which arises from the selectivity of the connections made between its endothelial K cells that mitigate the passage of dissolved substances. In the interface that occurs between blood and the brain, the endothelial cells and the related astrocytes are connected to each other by tight links. Endothelial cells attenuate the release of small and large objects or hydrophilic molecules in the CNS, while approving the release of small hydrophobic molecules (eg O2, hormones, CO2). BBB-binding cells transmit active metabolic components such as glucose located beyond the barrier with defined proteins (Seidner et al. 1998).

The nanoparticles exposed (such as Ag) in the body are also becoming increasingly known through antibacterial fabrics and coatings. However, the effects of the appearance (or even confluence) of metal nanoparticles on the brain and through the BBB have not been adequately investigated. Particles that are small in size have improved mobility and it is imminent that the movement of nanoparticles through BBB can be accomplished either by poppy release or by vector-induced endocytosis (Hoet et al. 2004). In addition, nanoparticles can be absorbed directly into the brain by

transynaptic movement (Oberdörster 2004). For example, it is reported that Ag nanoparticles can penetrate through the BBB (Panyala et al. 2008) and concentrate in separate parts of the brain (Rungby & Danscher 1983), which may be useful for drug delivery, but may in addition to posing a serious threat to the patient (Sarin et al. 2008; Muthu & Singh 2009). It has also been found that the exposed nanoparticles may cause damage to neurons that are normal (Tang et al. 2008), microglia (Au et al. 2007) and not only that but worsen the process of brain pathology (Sharma & Sharma 2007). Ion channels play an important role in cell viability and function, especially in the CNS, which are needed because they are considered to be a fine indicator of cell status and viability.

Sodium currents determine a number of neuronal energies, such as energy in the formation of potential effects and the spread of effect abilities at synapse terminals. Localized neuronal depolarization may also be influenced by the presence of nanoparticles. However, what plays a major role has not yet been determined in the transport of amine (DA), norepinephrine and serotonin). In addition, mutations in neurotransmitter amino acids (eg aminobutyric acid (GABA)) and monoamines (i.e. doped to cause changes in Na + channels having a predisposition, which is associated with a sequence of neurological diseases, including and are involved in various psychiatric disorders (Meisler & Kearney 2005; Guo et al. 2008).

The frequently applied nanoparticle degradation content (0.1-1% w / w) may be below the toxicity dosage limit until the nanoparticles appear in the CNS from the point of contact. This fact is estimated that the free nanoparticles / ions are required to enter the body, and then it is appropriate to pass the BBB with the final destination the CNS. Part of the percentages of nanoparticles that are free from work applied to composite solid particles - particles (i.e. epoxy resins, as shown in Figure 4) show the ion / particle collection range where its dimensions approach from 10 −5 g ml - 1, which is considered the lowest non-constructive dosage for all neuron cell controls in the CNS (drug toxicity tests are approximately 10−6 g ml - 1). These nanoparticle neuron tests also take into account various types of nanoparticles, e.g. Ag, CuO, ZnO, TiO2 (Xu et al. 2009; Zhao et al. 2009; Liu et al. 2010). Due to the fact that metal particle systems are very often applied to computers, paints and clothes, it is appropriate to pay special attention to this industry and further parametric variables (eg exposure, contact time, bond strength and weight loading).

Sodium stream is considered to be a neurologist for the modulation of neuronal cell stimulation and neuronal effect and function in the CNS. In conclusion, a possible modification of the current by the Nano metal particles is imminent, bringing about changes in the function. Some studies have shown that nanoparticles can impair cell function and have the potential to cause certain cell death (Shin et al. 2007; Cha et al. 2008; Tang et al. 2008). In recent research on the neurotoxicity of metal nanoparticles, a neuroendocrine cell line (PC-12 cells) is exposed to nanoparticles such as Ag (5 × 10−5 g ml - 1), which in turn reduced the DA level. It was also found that Ag nanoparticles had higher toxicity than manganese (Mn) nanoparticles in designated cells (Hussain et al. 2006a). These findings suggest that Ag and other nanoparticles may have significant pathological effects on the mammalian brain while helping or interrupting certain functions.

6.2 Cancer Detection

Early stage cancer or tumor sighting may reduce the chances of the tumor progressing and enhance successful treatment to provide quality life to patients. In order to detect or diagnose the tumor in the early stages, it is necessary to first detect some tumor markers, macrophages or tumor cells that are in the circulation so that we have data for the examination and diagnosis of cancer. In the early stages of the disease, the explanation of cancer biomarkers is very low and requires a number of strategies that take into account the management of various biosyntheses that carry a high content of defined and sensitive visual signals for signal tracking (Du et al. 2010).). Nanotechnological approaches must be appropriate for the study of certain functions of Nano-scale materials in order to play an important role in the diagnosis of cancer compared to conventional chemotherapeutic agents.

Metal NPs have been found to be considered amazing nanomaterials in terms of cancer detection due to their size, shape, composition, ease of preparation, light assimilation and diffusion capacity and size-dependent optical characteristics. Metallic NPs such as Ag and Au have the ability to diffuse light at perceived

wavelengths and NIRs when overstimulating their SPR. The tone of the propagating light is very vulnerable in terms of the size and concentration of NPs. Much effort has been made to treat SERS-dependent AgNPs for the investigation and testing of human blood plasma for the spread of an easy-to-use blood-free esophageal cancer detector, which has been shown to be successful of AgNPs for the development of cancer detection and screening (Duo et al. 2014). Another study by Lin et al. (2011) promoted a non-invasive tool that relies heavily on detecting and screening for malignant cancer cells by mixing whole serum proteins in isolation with blood plasma with SERS-effected AgNPs. Another new functional carbon nanotube (CNT) / AgNP Nano hybrid has been shown to be a probe in detecting tumor markers that have a sensitive single-vessel treating site of AgNPs deposited on carboxylic CNT et al. . 2011).

The sphere-shaped AuNPs, hence the name, are known as optical detectors for the detection of prostate cancer biomarkers using immunoassay. AuNPs carrying 37 nm in diameter have been conjugated to anti-prostate specific antibodies used as probes (Liu et al. 2008). AuNPs (~ 35 nm) have been conjugated to monoclonal anti-epidermal growth factor (EGFR) receptors to detect oral epithelial live cancer cells. The proliferation and assimilation imaging of SPR carries significant differences between cancer and non-cancer cells have marked the technique useful in the diagnosis of cancer (El-Sayed et al. 2005). Also, citrate-coated AuNPs have been used as candidates for the detection of early-stage cancer after a blood test. AuNPs when mixed with the blood serum of cancer patients have formed a protein crown on their surface and this is due to the adsorption of proteins by the serum found in the blood. Quantification of immunoglobulin G (IgG) present in the coronary protein associated with the surface of AuNPs has been shown to produce IgG confluence which has also been shown to induce IgG in cancer patients as a self-defense mechanism. . This careful blood test through the use of AuNPs which is simple has negligible cost, and most importantly helps in tumor tracking and risk assessment at an early stage (Zheng et al. 2015). New Ag-Au bimetallic nanostructures that are synthesized by the abduction of deoxyribonucleic acid (DNA) which acts as an active SERS substrate and at the same time showed significant signs of amplification to respond to the detection of Y-embryo cancer (2009).

The 000A assay based on chromatography based on Cu -Au NPs iodide responses has been formulated for extremely vulnerable recognition of cancer cells. The developed bimetallic platform NPs has the ability to chemically degrade iodine as well as the ability to modify biomolecules developed nanobodies can search for and alienate cancer cells even when they are still in a small time interval 25 minutes with great sensitivity and specificity at an early stage (Song et al. 2011). Yuan et al. (2013) have investigated that it is possible to enhance the colorimetric detection of target cancer cells by causing a change in the confluence of Cu -Au NPs (Ye et al. 2015). MNPs are considered vulnerable and selective at the same time for diagnostic purposes. In addition, the difficulty presented in the magnetic background does not exist with the use of these NPs as the biological indications do not provide any virtual magnetic background. The monoclonal antibody together with the fluorescent-magnetic-bio-targeting which refers to multiple functions of the nanobiomass has advanced in terms of the diagnostic part as well as for the alienation of a type of cancer cell for the purpose of a complex exhibit. These Fe3O4 @ TiO2-coupled nucleotide NP photoreactors conjugated to EGFR for high resolution NP X-ray imaging for cancer detection in HeLa cells. A fast and vulnerable nano-sensor based on the binding of biotin-labeled aptamer to streptavidin-coated iron oxide MNPs has been developed to detect cancer. Aptamer is the one that has the ability to distinguish and decisively block the target cancer cell, while the huge surface area of MNPs can happily accommodate a large amount of aptamers which have the ability to help clinicians determine the cancer cells at the unicellular or molecular level (Bamrungsap et al. 2012).

The proliferation of a new QDs protein microarray system has become known to be used in the detection of cancer markers. QDs provide the characteristic of photostability and luminosity while protein microarrays approve the quantification of defined proteins which are negligible, fast and carry a small sample volume, leaving the complex assay to be used as an important of cancer as well as the investigation of biomarkers (Zajac et al. 2007). Semiconductor NPs have been used to investigate cancer in particular because of the principle of electron transport and energy transfer. (Hu et al. 2010). Another very sensitive electrochemistry (ECL) approach has been devised based on AuNPs ECL-enhanced CdS nanocrystalline film, which was further supplemented by magnetic separation for the detection of HL-60 (human progenitor cell proliferation). was used to investigate HL-60 cancer cells in the range of 20-1.0 × 106 cells / mL. High biocompatibility and QD luminaires of stable CuInS2 / ZnS @ SiO2 NPs were incorporated into silane micelles to grow CuInS2 / ZnS @ SiO2 NPs with NIR emission and 30-50% quantum yield. Holotransferrin has also been bioconjugated to these NPs for targeted imaging of live cancer cells by these NIR emitters and potent NPs (Foda et al. 2014).

6.2.1 Anti-microbial Activity

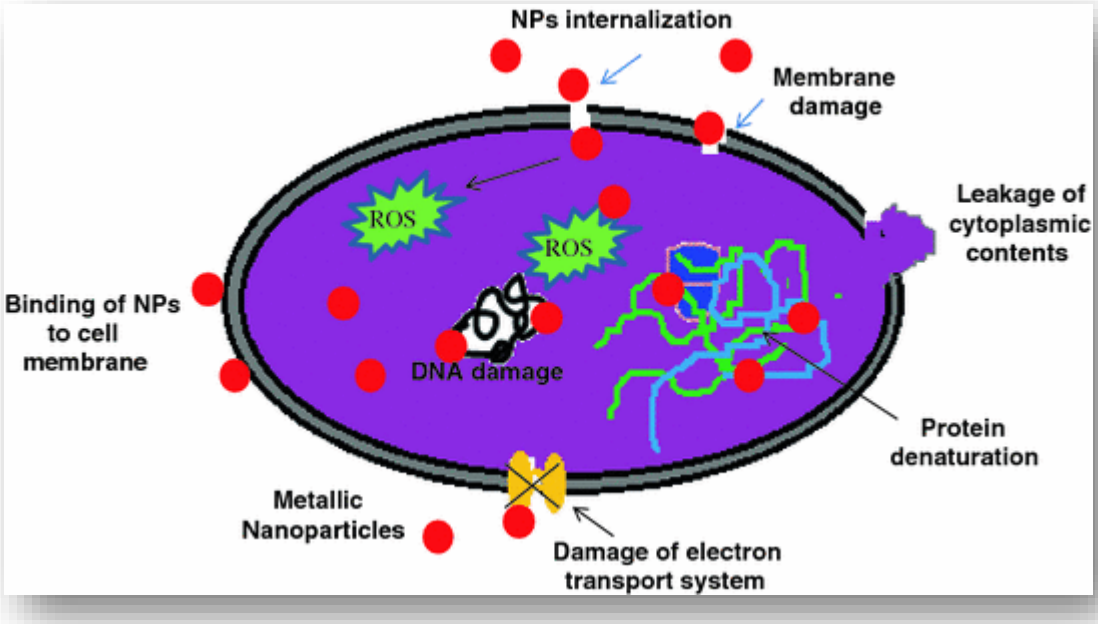
Antimicrobial agents are those that kill or counteract the spread of germs such as bacteria, fungi, algae, etc. The presence of infectious diseases poses a serious risk to human health due to microorganisms which show antibiotic resistance. Due to the overdose of antibiotics, the microorganisms acquire a resistance to various antimicrobial agents. Nanotechnology is evolving and is now applied to the analysis of antimicrobial NPs that do not have the same shape and size to examine the spread of bacteria at a reference level. NPs require great caution in individual areas with particular emphasis on drugs as the function of NPs depends on the size of the particles. The skill of easily manipulating or examining NPs schemes has created opportunities for the spread of new antimicrobial agents based on nanotechnology. NPs that provide antimicrobial activity are quite commonly used in various biomedical uses, such as their use as a material in ulcer dressings as well as to prevent microbial infections that result in the aggravation of deadly diseases.

A wide range of NP ions and metal oxides including Ag, Au, Cu, Ti and Zn are widely used to inhibit the spread of many varieties of bacteria, fungi and viruses. Delaying the spread of bacteria is influenced by factors such as the size of the NPs, the confluence and the stability they bring to use. Smaller NP sizes have been distinguished for greater bacterial delay (Kumari et al. 2014a). The size of a bacterial cell is in the micrometer range and their membrane pores are in nanometric shapes. It is well known that treating NPs that are smaller in size than bacterial pores is preferred as an option for what is considered best to kill them. Mineral NPs that are small in size can cross the bacterial cell membrane and enter the cell, killing the proteins, thus inhibiting the growth of bacteria (Prabhu and Poulose 2012).

Krishnaraj et al. (2010) examined the antimicrobial effect of biosynthetic AgNPs from Acalypha indica leaf extract and found that synthetic NPs measuring 20-30 nm in partial inhibitory confluence (MIC) showed a against E. coli and Vibrio cholera. Similarly, AgNPs of variable regimens synthesized by L. camara LE showed a resistant antibacterial effect against E. coli at a MIC of 50 μg / ml (Kumari et al. 2014a). Hydrophobic and cationicized AuNPs have been shown to have strong potent bactericidal activity compared to both Gram-positive and Gram-positive bacterial drugs (Li et al. 2014). Ligament adhesion and surface chemistry have been found to play an important role in the antimicrobial effect of these AuNPs. AuNPs and AgNPs and has also been shown to have an antibacterial effect against E. coli and BCG. Changes in the surface characteristics of AuNPs showed a change in the inhibitory action of AuNPs. These AuNPs and AgNPs may act in the form of anti-TB compounds for potential uses in the biomedical field (Zhou et al. 2012). CuNPs have many potentials to act as antimicrobial agents. The antimicrobial effect of CuNPs synthesized by the polyol system has been evaluated against bacteria such as Micrococcus luteus, S. aureus, E. coli, K. pneumonia and P. aeruginosa, and against fungi such as Aspergillus flavus, Aspergillus niger and Candida albicans (Ramy et al. 2012). MNPs and QDs have also been shown to act as antimicrobial agents with some modifications to the surface.

The mechanistic effect of the antimicrobial action of NPs is not clearly understood. NPs are thought to accumulate at close range from the microbial cell membrane and penetrate inside through membrane damage or through the formation of pits. Once inside the bacterial cell membrane, NPs have the property of generating some free radicals or interacting with many proteins that are located inside the bacterial cells

and inactivate enzymes. These events cause a distortion of the microbial cell environment internally and are inferred to be responsible for cell killing (Prabhu and Poulose 2012). The pictorial description of the antimicrobial function of NPs is shown in Fig. 6.2.1.1



;;;;;;Fig. 6.2.1.1 Mechanistic action of anti-bacterial activity of metallic NPs.

6.3 Silver and Gold Nanoparticles for Neurodegenerative and Other Brain Diseases

Nanoparticles have also been studied as potential tools for treating and diagnosing neurodegenerative diseases, (Li Y, 2018) such as Alzheimer´s disease (AD), ischemic stroke, (Kim J-Y, 2015, Yoon H-J,2015) prion disease, perinatal asphyxia, and herpes-associated amyloid-β secretion (Serramia MJ,2020).

Neuronal death in AD is triggered by protein aggregates composed of the Aβ1-42 peptide, its main therapeutic target. The use of nanosystems, which allow Aβ disaggregation or destruction, has emerged as a possible treatment for AD (Prades R 2012, Morales-Zavala F, 2017). Morales-Zavala et al demonstrated that gold nanorods functionalized with the D1 peptide -which specifically recognizes the Aβ peptide- decreased in vivo toxicity of Aβ in the C. elegans model of AD. Moreover, a study in the mouse AD model that used IV injection of 50 nm GNPs functionalized with anthocyanins proved a reduction of neuroinflammation and neurodegeneration induced by Aβ1-42 (Kim MJ, 2017). Lai et al developed in-situ gold nanoclusters

(GNCs) of 3.3 nm for in vivo fluorescence imaging after IV injection of HAuC solution. Since the redox environment of the AD brain is completely different from that of the normal brain, under these conditions HAuCl4 forms Au salts, which subsequently self-assemble to form GNCs. While the presence of GNCs was confirmed in the brains of mice with AD, no GNCs were found in the brains of normal mice. After 4~6 weeks, neither AD nor normal mice showed histopathological abnormalities nor changes in their physical behavior.96 In the case of SNPs, Zhang et al reported that IP administration of 12 nm SNPs obtained by green synthesis produced positive effects in preventing and reducing deficits in recognition and spatial memory in a rat model of sporadic AD (Zhang X, 2020). However, the toxicity of the nanoparticles used in this study and their effect was not described.

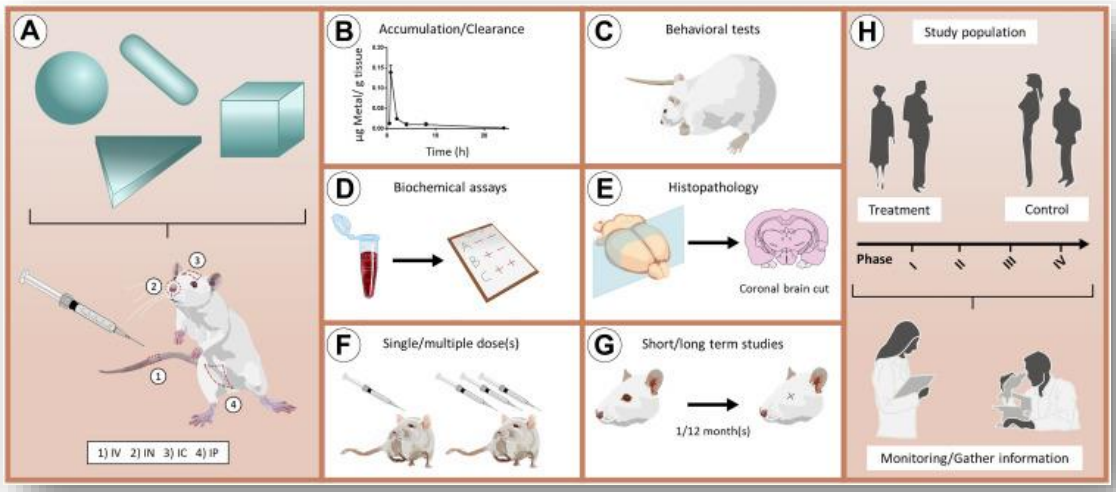


Figure 6.3.1.1 Ideal methodology to assess the toxicity of GNPs/SNPs for applications in brain-related disorders. (A) Schematic representation of in vivo administration of nanoparticles with different shapes through the main routes of administration. (B–G) Main in vivo experiments that can be performed to obtain a complete toxicological profile of nanoparticles. (H) Representation of a clinical trial that includes treatment and control groups. During the different phases of the clinical trials (I–IV), it is relevant to monitor individuals to gather essential information.

6.4 Nano neurotoxicity of Nanoparticles and Alzheimer's Disease

Silica nanoparticles (SiNPs) are among the more commonly used nanoparticles. They have been well studied about their toxic effects, such as increasing cell apoptosis and intracellular production of the reactive oxygen species (ROS) and decreasing overall cell viability (Yang X. 2014). In order to determine their neurotoxic effects, human SK-N-SH and mouse neuro2a (N2a) neuroblastoma cells exposed to SiNPs have been studied (Yang X. 2014). It was found that the cells exhibited increased amounts of both Aβ1–42 peptide and enhanced phosphorylation tau at Serine 262 and Serine 396, two AD-like hallmarks (Yang X.2014). There were also an increase in the number of round cells, as well as a decrease in dendrite-like process, and a decrease in cell density.

Silver nanoparticles (AgNPs), which are found in many daily-use products, are also related to the development of neural disorders (Lin HC 2016). A study looking at levels of gene expression in mouse neurons found that the AgNPs were able to enter the mouse neuronal nuclei after 24 hours of exposure and exposure ultimately induced the deposition of Aβ amyloid in mouse N2a cells. This change came with other variations that interfere with neuron growth and differentiation (Lin HC 2016). Another study looking at gene expression in murine brain astrocytes, microglial and neuron cells that were exposed to the AgNPs, found an increase in the expression of inflammation related genes as well as induced APP expression which may ultimately result in an increase in Aβ amyloid protein, allowing for the formation of the Aβ amyloid plaques that are one of the AD hallmarks (Huang CL 2015). Additional studies examining the effects of silver, cerium oxide and cadmium telluride nanoparticles found that both silver and cerium oxide nanoparticles severely hampered the cells' Aβ uptake. All three inhibited microglia growth by arresting cell division (Sikorska K., 2019).

While potential mechanisms-of-action may not have fully been explored, many metal nanoparticles, such as aluminum, mercury, lead, iron and copper are found in abnormal abundances in individuals with AD. This indicates that there is a correlation between exposure to the metal nanoparticles and the development of the disease. It is

thought that the tendency for metals to increase the rate of Aβ amyloid aggregation, thereby promoting AD pathology (Liu G.2006).

Not all engineered and environmental borne nanoparticles can be directly correlated with Ad pathology. However, there are many that interfere with neural processes in such a way that they may exacerbate or enhance the disease progression. Copper oxide nanoparticles (CuONPs) are notably toxic and are known to decrease cell viability and trigger DNA fragmentation in high concentrations (Perreault F. 2012). When human brain neuroglioma cells are subjected to CuONP exposure at a concentration of 100 μM, it causes significant amounts of cell death, indicating a dose-dependent toxic response to the nanoparticle.

A study observing the effects of CuONPs on hippocampal CA1 neurons suggests that they inhibit voltage-gated sodium currents, prolonging the peak rise time of the action potential and reducing the overall amplitude of the current, both of which are detrimental to neuronal functions (Liu Z.2011). Indeed, various studies attest to the neurotoxic effects of copper in the brain. Nanoneurotoxicity of CuONP has been investigated in vivo in which learning and memory was weakened (An L. 2012). CuONP neurotoxicity also suggested a slop decline in the LTP test as compared to control group rats, which, in turn, suggested an impairment of synaptic plasticity (An L 2012). CuONPs may induce oxidative damage as well, as the levels of ROS and malonaldehyde were increased in rat brain exposed to CuONPs (An L. 2012).

In vitro studies also suggest significant neurotoxic effects of CuONPs. One study compared genotoxic effects such as DNA fragmentation, DNA methylation, chromosomal damage and lipid peroxidation in addition to cytotoxic effects measured through mitochondrial reduction. CuONPs may impact rat hippocampus potassium currents by blocking them upon initial exposure, which may interfere with functioning of CA1 pyramidal neurons (Xu L-J., 2006). Moreover, some studies indicate that CuONPs induce potent in vitro neurotoxicity (Chen J., 2008).

Iron oxide particles are widely used in the field of biomedicine, primarily due to their magnetic properties; they are frequently used as a contrast in magnetic resonance imaging, as well as cell-tracking and other treatment purposes (Petters C 2014). While

they are considered to be safe in low enough quantities, some studies suggest that they have toxic effects. Studies performed on dopaminergic rat neurons exposed to iron oxide nanoparticles which have remained in the striata suggest an increase in oxidative stress, decreased neuron viability and an activation of pathways involved in apoptosis; all of these effects can lead to neurodegenerative disease (Durga M 2015). Observing mice exposed to iron oxide nanoparticles over a period of 30 days found that memory and motor skills deteriorated with exposure. This was due to a variety of different effects, ranging from axon demyelination to alteration of neurotransmitter concentrations (Manickam V., 2019). The apparent neurodegenerative effects of the iron oxide particles could indeed contribute to the AD progression as well.

6.4 Potential Nanotheranostics for Alzheimer's Disease

While various nanoparticles may be neurotoxic and have the potential to exacerbate or hasten the AD development, there are some that are well studied to be fairly safe and many that are medically useful. The use of nanoparticles as drug delivery mechanisms has been mentioned previously, but nanoparticles can be useful for enabling drugs to pass through the blood-brain barrier, which poses a considerable hurdle for drug treatment of brain-based disorders (Karthivashan G., 2018). In addition, administration of so-called "protective complexes" alongside nanoparticle exposure have been shown to mitigate the cytotoxicity and genotoxicity of CuONPs, one of the most well-studied and potently toxic nanoparticles. Among those more benign variants are some super paramagnetic nanoparticles (Fe3O4) which, with the proper coatings and probes, have great potential as molecular imaging agents. In a recent study, a novel nanoparticle containing a variety of metals, dubbed MZF-PiB, was fitted with a probe designed for Aβ amyloid plaques. The nanoparticle was proven to be both nontoxic and effective in detecting Aβ amyloid plaques, making it a potential early-diagnostic tool for AD (Zeng J., 2017).

Despite the potential effects of various types of nanoparticles in exacerbating AD progression, recent research has suggested that certain sizes, shapes and types of sulfur nanoparticles (SNPs) can have the mitigating effect upon AD pathology (Sun J., 2018). Three kinds of brain-targeting sulfur nanoparticles (RVG@Met@SNPs) with novel morphologies: volute-like, tadpole-like and sphere-like have been designed

and their different effects on Aβ aggregation, their ability to cross the BBB and their overall neurotoxicity were determined. Results suggest that smaller sized nanoparticles, such as sphere-like SNPs, may reduce Aβ peptide aggregation (61.6%) and increase cell viability (92.4%), contrary to other nanoparticles mentioned that may promote Aβ peptide aggregation and worsen AD pathology (Sun J., 2018).

Some protein coated metal oxide/sulfide nanoparticles, such as Fe3O4 or CdS have also proven to be potent inhibitors of tau protein aggregation, while having no measurable toxic effects on neuroblastoma cell lines. As tau protein is the basis for NFTs that form as part of AD these particles, alongside the appropriate protein coat, are a potential avenue for AD treatment development (Sonawane S, 2019). Another potential treatment involves Fe3O4 super paramagnetic nanoparticles, some variations of which are proven non-toxic that can be modified with negatively charged ions. These modified nanoparticles have been shown to decrease the rate at which Aβ amyloid-fibrils form, while remaining non-toxic; this has some potential in slowing down the progression of the disease (Javdani N, 2019). AgNPs were found to improve spatial learning and memory in rats through the inhibition of Aβ amyloid fibril-induced neurotoxicity (Ramshini H. 2017). As a final example, cerium oxide (CeO2) nanoparticles may be able to both reduce Aβ amyloid peptide aggregation, as well as protect against neurotoxicity in the form of oxidative damage by blocking the production of ROS and scavenging free radicals (Zhao Y. 2016).

6.5 Nano medicines for Alzheimer's Disease with Specific Reference to the Metallic Nanoparticles

The therapeutics of AD mainly targets the aggregation of theβA, metallic chelation neurotransmitter modulation and inhibition of inflammation. TargetingβA seems to be the most popular approach for treating AD. In a recent study, monoclonal antibody functionalized PEGylated NPs have been directed toward βA which showed better results in binding by binding as well as clearing βA in mice, followed by the restoration of memory (Carradori et al., 2018). In another research, it was demonstrated that the PEGylated NPs can adsorb the toxicβA in the systematic circulation which can improve the condition (Brambilla et al., 2012). Nano liposomes with cur cumin expressed on the surface demonstrated specific affinity to stainβA in

vivo and can be investigated further for theranostics of AD (Lazar et al., 2013).MNPs are used for diverse applications. However, their use in AD appears as distant or requires further research for a clear picture of the AD etiology. After administration, the MNPs are absorbed in the body and appear in other organs such as brain. Brain appears to be the most delicate organ which can be adversely affected due to the accumulation of such metallic entities leading to neurotoxicity (Feng et al., 2015).Oxidative stress is considered to be one of the major causes of the progression of AD, and metals can induce neurotoxicity. In some studies, neuronal cells have been treated with MNPs together with antioxidants. Results indicated decreased metal-based neurotoxicity by reducing ROS. MNPs such as Ag, TiO2, ZnO, etc. can be translocate and accumulated in the brain where they can cause a permanent damage (Wen et al., 2016). Conversely, selenium NPs have attracted many researchers because of being less toxic and ant oxidation properties. Selenium is found as a trace element in the body which can ameliorate the oxidative stress. Selenium NPs are proposed to have a neuroprotective effect in AD (Yin et al., 2015). In a recent study, a B6 peptide coated selenium NPs functionalized with sialic acid demonstrated excellent results in crossing the BBB. In light of the converging evidence of the metal-based neurotoxicity, their therapeutic role remain distant and must be subjected to further research for more clarifications (Yin et al., 2015). Other researchers targeted theβA and metal mediated fibrillation process using L-Cys modified Se, Ru, and Se-RuNPs. Their results were auspicious due to the high affinity of Se-RuNPs in binding and preventing the metal induced fibrillation process, and hence could reduce the disease progression (Yanget al., 2014). Epigallocatechin gal late (EGCG) stabilized SeNPs coated with Tet-1 also demonstrated promising potential in hindering theβA aggregation (Zhang et al., 2014). Another study was carried out on the biogenic synthesis of gold NPs using a medicinal plant Terminal a arjuna, used for diverse health purposes in folklore medicines. The synthesized gold NPs were not only effective in inhibiting the fibrillation process ofβA but also destabilized the mature fibrils. Furthermore, the AuNPs significantly inhibited the cholinesterase (ChE). The inhibition of ChE is considered a pop-ular strategy to ameliorate the AD condition by increasing the acetyl choline (neurotransmitter) content. While studying their toxicity in zebra fish, the T. Arjunamediated gold NPs were found to be biocompatible and nontoxic (Suganthy et al., 2017). Medicinal plants not only provides valuable chemical entities that can be of significant interest in AD and

cancer (Ovais et al., 2018b; Ullah et al., 2016; Ayaz et al.,2019; Zohra et al., 2019), but can be used in the biosynthesis of NPs (Ovais et al.,2018d). Considering, the aforementioned results of the possible applications of the biogenic gold NPs, further research on biogenic Se, Ru, ZnO, etc. should be carried out in which therapeutic plants for applications can be used. Such green chemistry-based methods are not only economical but also eco-friendly (Hassan et al., 2018; Ovais et al., 2018a) and provide platforms for the synthesis of NPs with miscellaneous properties (Khalil et al., 2018). The converging evidences of MNPs demands more research for its potential use in AD and to study in detail their safety and toxicity in the brain.

6.6 Biogenic Nanoparticles as Promising Alzheimer's Disease Theranostic Agents

Recently, green synthesized NPs have demonstrated potential in vitro and in vivo activities against different pathological disorders including AD (Ovais et al., 2017, 2018a,g, Nasar et al., 2019). The interface of nanotechnology and green chemistry has paved way for the development of more biocompatible and nontoxic theranostic agents (Ovais et al., 2018d). There are various disadvantages (toxicity and cost) associated with physicochemical methods of NPs synthesis, which hinders their way to be used as a potential Nano medicine (Ovais et al., 2016; Emmanuel et al., 2017). It is worthy to note that in the chemical synthesis method chemical substances utilized remain attached on the surface of NPs which causes potential toxicity (Barabadi et al., 2017; Khalil et al., 2017b). Hence, the green synthesis of NPs is emerged as a novel method in which pure phytochemicals or medicinal plants are utilized for chelation and stabilization of biocompatible NPs (Khalil et al., 2018, 2017c,d).Utilizing. T Arjuna bark extract was used for the synthesis of biocompatible gold NPs with potential neuroprotective activity (Suganthy et al., 2017). The gold NPs inhibited ChE enzymes, destabilized the mature fibrils and reduced the Ab fibrillation process. In another study trehalose functionalized gold NPs demonstrated significant inhibitory potential for protein aggregation and mature fibril disintegration (Mandalet al., 2017; Ovais et al., 2018c). Not only the green synthesis of NPs but the functionalization of chemically synthesized NPs with antiamyloidogenic molecules can result in enhanced neuroprotective effect. Currently EGCG functionalized selenium NPs were synthesized as potential neuroprotective agents for AD treatment (Zhang et al.,2014).

EGCG which is a polyphenol have potential to inhibit various amyloid forming proteins (transthyretin,α-synuclein, and hunting tin) involved in the progression of AD.Tet-1 proteins with potent neuronal affinity were further coated on EGCG-stabilized Se NPs for targeted delivery. As evident in Fig. 3.2Tet-1-Se@EGCH Nano system effectively inhibited the Ab fibrillation process along with disintegration of matured fibrils. Moreover, Tet-1-Se@EGCH Nano system inhibited DNA fragmentation and ROS generation at very low concentrations. Beside EGCG other polyphenols can also be utilized as potential functionalization agents for AD theranostics.

CHAPTER 7 FUTURE FOR METAL NANOPARTICLES

The metal-based nanoparticles seem to have a commanding role in the twenty first century because of their vital role in Nano medicine and other biological applications. The nanoparticles can be fabricated by using several synthetic routes and effectively used in various Nano medical and biological applications. But there is still a need to prepare these nanoparticles on a commercial scale to reduce cost. The natural resources for the preparation of these nanoparticles should be sustainable, inexpensive, eco-friendly, and free from toxic chemical. It is important to produce monodispersed nanoparticles for future research. However, the mechanism to synthesize nanoparticles has not been completely explained at present. Therefore, future research should address the mechanism by which the shape and size of nanoparticles can be controlled. Another major challenge is the need to expand the use of nanoparticles in therapeutic applications and to reduce the level of toxicity. New strategies are being introduced with potential development in Nano science to overcome the challenges by using noble metal nanoparticles. Prior to extensive use, impact on anthropological health factors needs to be considered.

Nano science can play a major role in modified biomedicine. It needs to be explored much more compressively in the future. The noble metal nanoparticles can serve as active agents in diagnosis and other therapeutic applications as they exhibit novel properties at atomic level, as well as at the supramolecular level. At present, there is a high demand for different types of nanomaterials and their composites in the health care, medical, and biological industries. Therefore, an emphasis must be made on the safety precautions to safeguard human health. In-depth studies on safety profiles are required specifically in the case of using metallic nanoparticles in health care. Future research should be directed to investigate the use of specific metal nanoparticles for individual applications.

The preparation of noble metal nanoparticles needs to be up-scaled from laboratory size to commercial medical size in the future. Metallic nanoparticles have been developed and are in an advanced state of testing in multidirectional applications. Currently, it is mainly used for the treatment of cancerous cells. Metal nanoparticles

highlight their effectiveness as novel agents for future tumor therapeutic modalities. A question that needs to be answered is: Are the noble metal nanoparticles cytotoxic or biocompatible? Therefore, it is necessary to examine the nanoparticles interactions at laboratory scale and modify the nanoparticles for better biocompatibility with cells (cancerous) to remove injury to normal cells. Modern investigation should focus on the potential applications of nanoparticles at the commercial level, because in general, at present, all research is being conducted at a laboratory scale. Exploration at the commercial level may bring a revolution in the life of human beings.

Nano carrier-based drug delivery systems have the potential to be applied in the treatment of various brain diseases. Despite their high stability, target specificity, nontoxicity, Nano carriers have some limitations. In that a drug-loaded Nano formulation has a chance to be captured by the reticule endothelial system and eliminated from circulation. For brain-specific targeting, larger than 100 NM size particles may be stuck at brain extracellular site due to the narrow width (3565 nm) of space (Thorne, 2006). Receptor-targeted Nano carriers are able to deliver sufficient drug molecules to the brain which may be higher in amounts as compared to other drug delivery vehicles. In spite of higher drug delivery systems, receptor targeted Nano carriers may also deliver bulk amounts of drugs to the nonpathogenic sites due to the presence of targeted receptor at that nonpathogenic site. For example, In the case of glioblastoma, there is overexpression of transferrin receptors and these receptors are targeted by Nano carriers. At the same time, there is a chance of accumulation of drugs to other organs of the body that generally express transferrin receptors. There is another hypothetical concern that over whether brain receptor-targeted delivery with Nano carriers performed in animals for treatment of certain brain associated diseases will be efficiently translated in humans. Or will there be expression differences of receptors taking place in different species that limits the translation of receptor-targeted clinical studies. In addition to limitations, one site-specific targeted strategy for particular brain diseases may or may not be useful for another type of brain associated disorder (Costantino 2012). There have been numerous studies to gain understanding of NPs, their engineering, their fates after degradation and their long-term effect, yet no adequate understanding has been acquired regarding NPs and their biological interactions with organs other than those

targeted organs. In in vivo studies of brain tumors, there are high chances of accumulation of Nano carriers at the BBB site due to limited specificity for the targeted site. In respect to challenges in the use of NPs-based delivery system, neurotoxicity is also a matter of concern. NPs with less than50 nm size may be accumulating in other no targeted healthy organs. Although they are nontoxic, they may elicit some long-term effect via modulation of some metabolic path or may interfere with other biological interaction. There is enormous versatility in structures, compositions, and modifications of Nano carriers existing. These kinds of versatile and engineered NPs may remain in circulation for a long time and elicit minute changes to the functions of cells that may be at some point after an extended period lead to either immortality or death of the healthy cells. In respond to challenges associated with NPs, researchers have synthesized PEG-coated NPs that have less interaction with body fluids which enhances the nontoxic potential of Nano carriers in circulation. To over-come challenges with NPs restriction at the extracellular space of brain, NPs with less diameter then 100 nm have been developed. In contrast to NP-induced toxicity, some NPs have been reported to be neuron protective, including ceria NPs and water-soluble fullerene. NPs has been of great interest for research, but the subject is not yet fully understood. Thus, it is very difficult to answer questions about the safety of NPs if they are used in commercial life. They may be nontoxic to the human body but maybe they will have an adverse effect on the environment. According to the recent knowledge of NPs, there are two perspectives, some are in favor of their nontoxicity and some are in favor of their toxicity. Hence, further clinical studies and investigations are required to reach a consensus. In a similar way, future strong evidence is needed to arrive at the conclusive answer about the neurotoxicity of NPs (Costantino, 2012).

The mechanism of NM Nano toxicity is still not clear; for example, many studies attribute it to oxidative stress or ROS, but some NMs, such as CNT fullerene, are very good free radical scavengers and antioxidants. Therefore, the mechanism of Nano toxicity is still an important topic for future research.

A lack of common standards is one of the limitations of the current studies of NP cytotoxicity. Different cell lines, exposure times, and colorimetric assays are used in different studies, which make it difficult or even impossible to compare cytotoxic

effect among these results. No single method meets all conditions for obtaining information on NP cytotoxicity. Because different NPs exhibit different biological responses, a combination of assays is often suggested to study the mechanisms underlying cytotoxicity. A set of sensitive cell lines would also be required to accurately identify NM cytotoxicity (Kroll A, 2011).

Another limitation is that the NP dose used in in vitro studies often exceeds a realistic relevant dose. Moreover, these in vitro models cannot accurately reproduce various cellular interactions present in the body. Therefore, it is difficult to predict the toxicological behavior of NPs in living organisms by in vitro models alone (Kim JS,2013, Adamcakova-Dodd A, 2014).

There are still many unanswered questions concerning nanoneurotoxicity. For instance, after bypassing the BBB, where do NPs go? How do they leave the brain? The degradation of NP coatings and NP cores inside the cell environment is an important issue that deserves serious consideration when designing safe and functional NMs. No results have been reported on this issue to date.

When NPs enter the body, the surface properties of NPs may change by adsorbing proteins from biological fluids (such as blood, plasma, or interstitial fluid), leading to a distinct new epitope, for example, and protein corona exposure in the biological microenvironment. Furthermore, serum protein binding to the NPs can alter the surface charge and accelerate the cellular uptake of NPs through receptor-regulated endocytosis. However, so far, studies addressing the cell surface protein corona interactions with NPs remain limited.

Data regarding the distribution of metal-based NPs in the brain parenchyma are scarce, including data regarding the disruption of the BBB and adverse brain alterations caused by metal-based NPs. The effects of the persistence of poorly soluble metal-based NPs are of particular concern, and few studies have considered the effect of NPs on the CNS (Yokel R, 2013).

Nano toxicity research can be applied to a number of applications, such as determining composition levels in coatings for medical devices, medical-grade sheet

molding compounds for hospital equipment, aircraft filter fabrics, printing-coat films/inks and compositions for high-performance aviation gas turbine lubricants.

The direction provided from previous work on functionality/Nano toxicity of nanoparticles on CNS cells should be focused towards further understanding the mechanism of action, and their neurological and circulatory effects using animal and in vitro models. Rat models, such as ischemia, vascular dementia, epilepsy and diffuse axonal injury, are the first step for further assessment of functionality. Methods to address current problems in such tests also need to be developed. The existing problems in biological tests include nanoparticle agglomeration and aggregation within both liquid and airborne forms. In particular, nanoparticle dispersion in air with different sizes, materials and morphologies with controlled agglomeration involving aerosol delivery for in vivo and in vitro studies is the most challenging work in the field of nanoparticle toxicology due to difficulties in nanoparticle measurements, generation and observation (Kim et al. 2010), although some technological advances have been made on the proof of concept stage.

Also, the current knowledge on engineered nanoparticles and their interactions with the CNS cells is extremely limited and traditional drug toxicology studies may not be ideal models to draw comparison with due to the special Nano functions and features. Further research on Nano toxicity as well as functionality will allow the expansion of the much needed understanding in this area, with a build-up of the physical and chemical properties of nanostructures influencing in vivo and in vitro behaviour towards CNS neural cells. In the CNS, microglial cells are a type of macrophage found in the brain, and they may be involved in handling any nanoparticles that reach the brain, and these cellular responses to nanoparticles should be investigated. Biological (CNS cells) interactions linked to particle size, surface energy, composition and aggregation will form a focal point of some future studies. Many biological properties of nanoparticles (i.e. Ag, Cu, Fe2O3, Al2O3, ZnO, SiO2, TiO2, CuO, Cu2O, and WC, etc.) have been investigated in terms of the etiology, pathology, physiology and epidemiology; however, no report has been obtained on CNS neurons owing to the complexity and high costs associated with assessments.

Conclusion

For appropriate utilization of MNPs on industrial scale and to decide whether to opt for its use or not, its risks and benefits both must be evaluated via using appropriate experimental systems. The risks evaluation and underlying effects determination is also a necessary parameter when these NPs are intended for administration into the body. Potential for bioactivity is greater among NPs as compared to fine particles but the exact mechanism of increased toxicity of NPs is still unclear. However, the changes in physical properties and higher uptake due to size, charge, or composition lead to some adverse effect that may not be possible for bulk material. Improved uptake at one side is beneficial, but at the same time it may contribute to toxicity. For instance NPs having less than 12 nm diameter may cross the blood brain barriers, while molecules having 30 nmor below may be endocytosed by cells. The size-dependent toxicity is mainly related to inverse relationship of size with surface area, the smaller the particle the greater the number of molecules exposed at the surface. Moreover, the increase in solubility for MNPs is considered the critical factor for their toxicity, that is, due to the increase in surface area per unit weight of the NPs. The biological systems can be affected by eluted metals from MNPs, thus apoptosis and inflammation can occur as a result of increased oxidative stress.

In conclusion, we observed silver nanoparticles and freshly mixed silver ions were toxic to cells, while aged silver ions were not toxic, implicating the unbound silver cation is the ultimate toxicant to cells after exposure to nanoparticles. ISD3 quantitatively described particle and ion dose metrics after nanoparticle exposure in cells with competent and deficient nanoparticle uptake. Dose response modeling with ISD3 simulated dose metrics suggest that amount of ions in cells and area under the curve (AUC) of ion amount in cells are the most predictive of cell viability after nanoparticle and combined nanoparticle/dissolution-formed-ions exposures, respectively. This observation implicates silver ions formed extracellular drove toxicity after exposure to nanoparticles in this study. Applying computational modeling (ISD3) to better understand dose metrics for soluble nanoparticles allows for better interpretation of in vitro hazard assessments.

A large number of synthesis methods are followed for the formation of NPs which include physical, chemical and biological (green) methods. Generally in the recent times, researchers are more convinced by biological approaches for the development of nanostructures as compared to the conventional physical and chemical methods because biological approaches are eco-friendly, inexpensive and lesser time and energy consuming. These nanomaterials possess unique physical, chemical, optical, mechanical and electrical properties making the utility of such NPs in wide spheres of life especially biomedical field. The size, shape, morphology and surface chemistry of NPs have tendency to alter the properties of NPs and ultimately their behavior in the biological systems. NPs play a deterministic role in disease diagnostics, therapy, tissue engineering and the agnostics. Apart from innumerable benefits of NPs, there are certain toxicity concerns behind the use of these metallic NPs under in vitro and in vivo conditions which need to be minimized to realize the full potential of nanomaterials for the betterment of human life.

Nanoparticles have a wide variety of consumer and industrial uses that should not be understated. Their small size and reactivity make them ideal for certain applications. They are also a somewhat inevitable byproduct of industry and an additive in a variety of consumer goods. However, largely due to their small size, nanoparticles are prone to disperse into the air, where they can be inhaled by the individual working with them or become part of air pollution that has hazardous effects upon the general population. Not only are many of these nanoparticles toxic, but many are able to enter the brain, where they contribute to or exacerbate the symptoms of neurodegenerative disorders such as AD. Given these risks, it may be wise to conduct further study on the different varieties of nanoparticles, both in current use and in development, ideally before they become too widespread. With that being said, they may also serve as starting materials for developing potential AD theranostic agents, as they can also inhibit some of the processes thought to be responsible for AD etiopathology or detect AD lesions such as Aβ amyloid plaques.

Phyto therapeutics and Nano medicine based approach to treat AD is most recent and potential research area for the targeted delivery of drugs. A large number of phyto-chemicals have been reported to exhibit considerable efficacy in AD therapy. The efficacy of these phytochemicals can be augmented via the use natural products based

utilization of nanotechnology (green synthesis) for more effective delivery of drugs to the target sites. The presence of metallic NPs in the brain areas can exhibit beneficial as well toxic effects. For instance, several metallic NPs including Au, Ag, TiO2, and ZnO can initiate oxidative degeneration in the brain. Yet some of the NPs including selenium NPs are known to exhibit significant antioxidant potentials in the brain areas. As majority of the NPs synthesized till now utilize chemistry techniques which are associated with these side effects, whereas, green chemistrybased NPs can be a more effective yet safe. However, the science of green nanotechnology is in its embryonic stage and further research in the area of green chemistry can open new avenues in the management of AD.

During the past decade, a dynamic development of nanotechnology has been observed. Products containing metallic NPs in their composition can be found everywhere in a wide range of commercial products commonly available to virtually everyone. In addition, NPs have excellent applications in medicine, in medicaments and diagnostics. NPs, due to their small sizes, have remarkable properties which determine their widespread use and immense bioavailability in the environment.

However, the metallic NPs, in addition to their many advantages, unfortunately also have drawbacks. Figure 1 summarizes the present stage of knowledge about toxicity of metallic NPs in CNS reviewed in this work. Studies have shown a greater toxicity and reactivity of metallic NPs compared to their bulk counterparts. There are several main routes of exposure to metallic NPs, of which perhaps the most dangerous in the context of neurotoxicity NPs are those suspended in the air. It was found that NPs easily penetrate the human body, as well as rodents and aquatic organisms. In addition, researchers have observed that some of them rapidly spread throughout the body and accumulate in various organs. Numerous studies confirm that NPs can penetrate into the brain by means of a fixed route (olfactory bulb - olfactory nerve-brain), thus bypassing the BBB. On the other hand, some NPs which enter the body can pass through the BBB, and circulate in the blood vessels in the form of ions released from the surface of the NPs, as well as through damage to the integrity of the BBB which increases its permeability. Of course, it is a serious consequences to bring on the influx of unauthorized substances into the brain, and violation of the delicate homeostasis of the microenvironment. The brain, due to its sensitive structure, is

particularly vulnerable to the adverse properties of metallic NPs. Numerous studies performed both in vitro and in vivo show that NPs are toxic to neuronal cells. It has also been demonstrated that NPs can penetrate into the cells, mainly through the mechanism of endocytosis, although there are other possibilities, such as transport facilitated by some membrane receptors. The primary mechanism to induce neurotoxicity can be attributed to the generation of free radicals and induction of oxidative stress, which is known to damage the cell elements (proteins, lipids, nucleic acids). In particular, oxidative damage to DNA is dangerous due to its nature and mediation of mutation in cancer formation. Although metallic NPs have not yet been directly linked to the etiology of any neurode-generative diseases, more and more studies are being conducted which provide new evidence in support of this thesis.
In conclusion, most studies on the interaction between CNS neuronal cells and nanoparticles have used metal or metal oxides (including Cu, CuO, Zn and Ag) with selected neuronal cell lines (PC-12, CA1 and CA3). Neurologists have an interest in both functionality and toxicity with regard to the effects of nanoparticles, with the more recent studies focusing on the interaction with hippocampal cell membranes, as carried out for CNS drug toxicity. The effects of nanomaterial on ion channels within neurons may specifically relate to Na+ [INa (A)] and K+ [IK (A)] channels, as shown by a number of studies. It is possible that such ion channel effects may not apply to some other nanoparticles such as gold, which has been reported to possess unique biological properties and positive functionalities.

Neurologists have an equal interest in both areas of positive functionality and negative toxicity of nanoparticles on the human neuron cells as well as the interactions when passing through the BBB. Studies now have focused on biological membranes on the hippocampal cells, as carried out previously for drug toxicities within the CNS.

The range of applications continues to grow for nanomaterials at a rapid rate. The potential of individual nanoparticles and carbon nanotubes as constituents of toothpastes, beauty products, sunscreens, coatings, drug delivery systems, sensors, building materials, and textiles are being explored. Thus, a complete understanding of the mechanisms of interaction between nanoparticles and target cells that may lead to local and systemic effects within the CNS is required.

SNPs and GNPs have provided endless opportunities for in vivo biomedical applications. However, like other xenobiotics, they are usually redistributed and accumulated in some vital organs and produce toxic effects, a crucial issue to consider for potentially advancing clinical trial designs. In the case of SNPs, a limited number of in vivo toxicity studies related to neurodegenerative and other brain-related diseases were found, probably due to their potential and major toxicity, especially in the case of bare SNPs, compared to GNPs. Currently, there is a consensus that the toxicity of SNPs is associated with the release of silver ions, which trigger biochemical alterations, abnormalities in behavior, and neurotoxic effects. As discussed previously, although in vivo functionalization of SNPs clearly diminishes their toxicity and SNPs exhibit a more powerful radio sensitizing ability than GNPs, long-term accumulation in the brain might be counterproductive.

In contrast, a clear tendency for the use of GNPs has been observed during the last decade. While it is difficult to define a correlation between size and toxicity for small GNPs ranging from 2 to 10 nm, it was clear that larger GNPs (from 5 to 100 nm), are less toxic. Moreover, functionalization of the GNP surface increases biocompatibility, facilitates crossing the BBB, and enhances delivery to the brain, improving potential treatments and diagnostic methods. Moreover, all the studies that were reviewed showed minimal or no abnormality in animal behavior after administering GNP-based Nano systems. Therefore, GNPs are definitely less toxic than SNPs.

It is important to note that clinical trials lacked complete in vivo toxicity studies, and that most of the studies with SNPs and GNPs used spherical nanoparticles. It is well known that anisotropic shapes, such as rods, stars, and prisms, have interesting physicochemical properties; for example, plasmons centered at the near-infrared region can be exploited for biomedical research and clinical applications. In the coming years, more studies should thoroughly evaluate the in vivo toxicity of these nanosystems. As an approach, 3D human CNS in vitro models can be used as a perspective, especially when using human neural precursor cells (NPCs) derived from human-induced pluripotent stem cells (hiPSCs).108 Depending on the developed 3D model, it is possible to mimic specific aspects of human brain physiology, including the development of brain-related disease models. These 3D models could accelerate data acquisition related to SNP and GNP neurotoxicity, allow the development of

high throughput screening methodologies, and reduce the time-consuming animal experimentation.

Figure 6.3.1.1 illustrates the main toxicity studies that should be performed to obtain a complete toxicological profile of SNP- and GNP-based nanosystems. The use of in vivo experiments, such as accumulation/clearance studies, behavioral tests, biochemical assays, histopathology examination, administration of single/multiple doses and short/long term studies will allow many of the interesting studies described in this review to get closer to the next step: clinical research. We hope that this review will stimulate further development of SNPs and GNPs of different sizes and shapes, and we encourage the scientific community to continue with in vivo experimentation, especially regarding the CNS and brain-related diseases.

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