REVIEW

A review of nanoparticle functionality and toxicity on the central nervous system

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Although nanoparticles have tremendous potential for a host of applications, their adverse effects on living cells have raised serious concerns recently for their use in the healthcare and consumer sectors. As regards the central nervous system (CNS), research data on nanoparticle interaction with neurons has provided evidence of both negative and positive effects. Maximal application dosage of nanoparticles in materials to provide applications such as antibacterial and antiviral functions is approximately 0.1–1.0 wt%. This concentration can be converted into a liquid phase release rate (leaching rate) depending upon the host or base materials used. For example, nanoparticulate silver (Ag) or copper oxide (CuO)-filled epoxy resin demonstrates much reduced release of the metal ions (Ag⁺ or Cu²⁺) into their surrounding environment unless they are mechanically removed or aggravated. Subsequent to leaching effects and entry into living systems, nanoparticles can also cross through many other barriers, such as skin and the blood–brain barrier (BBB), and may also reach bodily organs. In such cases, their concentration or dosage in body fluids is considered to be well below the maximum drug toxicity test limit (10^{−5} g ml^{−1}) as determined in artificial cerebrospinal solution. As this is a rapidly evolving area and the use of such materials will continue to mature, so will their exposure to members of society. Hence, neurologists have equal interests in nanoparticle effects (positive functionality and negative toxicity) on human neuronal cells within the CNS, where the current research in this field will be highlighted and reviewed.

Keywords: nanoparticles; central nervous system; functionality; toxicity

1. INTRODUCTION

The advent of nanoparticle systems has had a major impact in a host of scientific areas, opening up new capabilities and functionalities across a wide range of applications. The properties of nanomaterials can differ from those demonstrated by their bulk forms and, in some cases, give completely unexpected physical and chemical properties. For this reason many industries and manufacturers are now introducing nanomaterials and nanotechnologies in their mainstream products so as to exploit these new capabilities.

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Many types of nanomaterials are also flourishing in medical science and technological areas while related research and applications are exploring potentials in biosensors, biomaterials, tissue engineering, DNA modification, drug and drug-delivery systems (Chen *et al.* 2006; Lu *et al.* 2008; Kim *et al.* 2009; Sun *et al.* 2009; Kirkpatrick & Bonfield 2010). Another area which has benefited from these advances is microbiology, where the inhibitory effect of nanoparticles on microbes can be seen as a tool to combat and control outbreaks of disease. However the effect on microbes must also be viewed carefully as it demonstrates the potential effect nanoparticle systems can have on living systems; the relatively modest information on nanoparticle toxicity in various human systems means the issue of safety still remains

Figure 1. Scanning electron micrograph (SEM) showing multi-wall carbon nanotubes.

incomplete. Several studies have highlighted this issue further. For example, it has been shown that man-made nanomaterials possess highly activated surfaces which are capable of inducing carcinogens, mutagens or cytotoxic activity (Seemayer *et al.* 1990; Seaton *et al.* 2010). Coupled to these surface properties is the direct size comparison; for example, nanoparticles are 100 times smaller than normal red blood cells, which increases the potential for interaction, and there is evidence that nanoparticles interact with proteins, DNA (Seeman 2006), lung cells and viruses. Hence, understanding nanoparticle interaction with living cells and other biological systems is critical, especially as the potential and exploitation of such technologies is rapidly gathering pace, requiring healthcare professionals and the public in general to be in closer contact with such materials. For example, metallic nanoparticle systems are now readily used for their anti-microbial properties in everyday products ranging from deodorants to personalized computer devices (Allaker & Ren 2008).

As was the case with nuclear technology, the personal computer boom and GM crops/animals, more than 68 per cent of people now believe that nanotechnology 'would make things better in the future' (Royal Academy of Engineering 2004).

One class of material which has had such impact is carbon nanotubes (CNTs). Single-wall (approx. 10 nm in diameter) or multi-wall CNTs with extremely high aspect ratios (as high as 1000) have a much lower percolation threshold. They are composed of many nanotubes and are chemically bonded together, possessing a highly activated surface and exhibiting superior strength, rigidity and electrical conductivity. Figure 1 shows standard industrial grade multi-wall CNTs used in high-performance conventional batteries (large scale).

Biocompatible and biomedical materials such as hydroxyapatite (HA) and carbon-coated titanium alloys for bone and hip replacements release debris of nanometre- and micrometre-scaled particles from metallic implants owing to host environment friction, and several studies have pointed out the potential risks in such bone repair and replacement (Moore *et al.* 2001). Nanoparticles such as SiO_x and CNTs may also act similarly with neurotoxins which affect the central or peripheral

nervous system. Although the most common neurotoxin is alcohol, others include heavy metals, organic solvents and rarer 'designer' drugs (Cole & Sumnall 2003). As nanoscaled substances have an active surface, they may cause or produce acute neurological complications, or subacute or chronic illnesses. Neuron synaptic transmission and neuron cell membrane with the ionic channels for Ca^{2+} , Na^+ , K^+ and Cl^- may also provide a route of entry for CNTs or smaller nanoparticles.

Although there are numerous biological systems which can be investigated for such nanoparticle interactions, this review focuses on studies related to the functionality and toxicity of anti-viral/anti-microbial nanoparticles in the central nervous system (CNS). The current findings show that both negative and positive effects are observed by using selected nanoparticles, typically deployed as anti-bacterial/viral compositions (0.1–1.0%w/w; Xu *et al.* 2009). Figure 2 shows typical copper oxide (CuO) nanoparticles (figure 2a) and agglomerated silver nanoparticles (figure 2b) that are used in such studies as model anti-microbial nanomaterials, which provide better anti-bacterial capabilities than their bulk material (Raffi *et al.* 2008; figure 3).

1.1. Important issues of nanoparticle toxicity in general

A selection of findings on nanoparticle toxicity on a host of living systems can be illustrated to elucidate these points, and also advocate the need to understand these interactions in greater detail. For example, some studies on rats have shown that 15 per cent of the sample population died within 24 h due to blockage of the airways as a result of carbon nanotubes being injected into their lungs (Lecoanet *et al.* 2004; Warheit *et al.* 2004). More of a concern is the effect observed from micro- and/or nanoscaled debris of artificial hip replacements as there is a growing demand for such biomaterials (e.g. implantable devices). These loose particulates arise as a result of friction, and travel into the blood stream and eventually lead to the formation of a thrombus (De Jong & Borm 2008). There is also evidence to suggest migration of particles into organs (liver and the spleen) from similar prostheses (Gatti *et al.* 2004). Moving away from implantable devices, there is a risk posed from inhalation. Research has demonstrated that radio-labelled nanoparticles can reach the blood stream within 60 s via inhalation; and the liver within 60 min (Chunfu *et al.* 2004).

The current assumption is that smaller sized and highly activated nanoparticles (such as silica featured as hydrophilic, hydrophobic or even amphiphilic) can be taken up by human membranes. However, no response or signal is initiated that leads to the rejection of the particles, and these pass through the membrane passively. Potential health hazards related to such particles are the adsorption and enrichment of various poisonous substances (metals, dioxins, combined with hydrogen chloride (HCl), hydrofluoric acid (HF) on the particle phase which possess a much larger surface area (Robichaud *et al.* 2005)). Tetra-ethyl lead

Figure 2. Transmission electron micrograph (TEM) of (a) CuO nanoparticles (QinetiQ Nanomaterials Ltd) and (b) agglomerated silver nanoparticles.

Figure 3. Neuron synaptic transmission and neuron cell membrane with passes for Ca^{2+} , Na^{+} , K^{+} and Cl^{-} (interactive physiology), where carbon nanotubes or smaller nanoparticles might be able to pass through easily.

($\text{PbC}_8\text{H}_{20}$), generated by lead petrol (4 Star) from car exhausts, once inhaled could be accumulated in the human brain.

This review considers three key elements of the toxicity screening methodologies or strategies covering physico-chemical characteristics, *in vitro* assays (cellular and non-cellular), and *in vivo* assays relevant to CNS cells. In particular, the review intends to concentrate on introducing the current techniques of drug *in vitro* and *in vivo* toxicology test methods into nanoparticle toxicology test methods by using CNS cells. These could be considered to determine proposed possibilities that the biological activity of nanoparticles can depend on physico-chemical parameters; however, they are not routinely being considered in toxicity screening studies because of the complexities of the tests of physico-chemical properties of nanoparticles, as recognized by many leading toxicity researchers

(Oberdörster *et al.* 2005; Wang *et al.* 2009). Although the functionality of nanoparticles is closely linked to their physical status and properties such as the particle morphology, particle interactions of agglomeration and aggregation, this paper is mainly concerned with the identified results of biological interactions between the current industrial nanoparticles and CNS cells.

The general interaction between physical properties and biological functionalities have been investigated and clearly highlighted by Oberdörster *et al.* (2005) on the basis of physico-chemical interactions with biological cells such as liver, blood, lung, macrophages, spleen and the immune system, CNS and neurons and skin, etc. The importance of physico-chemical properties has been emphasized again in the understanding of the toxic effects on biological cells, which include particle size and size distribution, agglomeration state, shape, crystal structure, chemical composition, surface area, surface chemistry, surface charge, and porosity.

While the size aspect of nanoparticles attracts considerable and rapidly growing attention to several industries, the chemical aspect of the materials should not be overlooked as this has been shown to be an important aspect in several nanomaterial–cell interactions (Thian *et al.* 2008). This review focuses on nanoparticle functionality with a broad view on materials falling into the nanomaterials range. However, materials chemistry will also have an impact; for example, it has been shown that silver nanoparticles are more anti-bacterial than copper nanoparticles.

2. NEURON CELLS AND CENTRAL NERVOUS SYSTEM

Neurons are nerve cells that, together with neuroglial cells, constitute the nervous tissue making up the nervous system. A neuron consists of a nerve cell body

(or soma), axon and dendrites. Neurons receive nerve signals (action potentials), integrate action potentials, and transmit the signals to other neurons. Although the human nervous system is much more specialized and complicated than that of lower animals, the structure and function of neurons is essentially the same in all animals. *In vitro* systems to study the effects of particles on the nervous system have included neuron and nanoparticle cultures to determine the effects on neuronal functions (Oberdörster *et al.* 2005).

Ion channels are transmembrane proteins that mediate passive transport of ions, and the channels underlie a broad range of the most basic biological processes, from excitation and signalling to secretion and absorption. Studies of ion channels provide useful and informative clues for understanding the biophysics and pharmacology of these important and ubiquitous membrane proteins. There are many kinds of ion channels, such as sodium channels, calcium channels and potassium channels in rat models e.g. CA1 hippocampal neurons. Voltage-gated potassium (K^+) channels can play crucial roles in regulating a variety of cellular processes in both excitable and non-excitable cells, such as setting and re-setting membrane potential, action potential duration, the delay between a stimulus and the first action potential and discharge patterns.

Further research has been carried out using metal nanoparticles such as Ag, Cu and Mn on P12 brain cells to investigate potential neurotoxicity (Wang *et al.* 2009).

2.1. Nanoparticles' interaction with the central nervous system

Nanoparticles have shown biological functions such as killing pathogenic bacteria and viruses (e.g. flu), but research has also shown that nanoparticles may produce adverse effects (dose related) in human cells on contact. Human neural cells, such as hippocampal cells in the CNS, are the most sensitive and delicate cells in bio-organisms, and are responsible for brain functions and emotions. They are vulnerable to ischaemia, oxygen deficiency and external factors. One of the great concerns in science and technological development in the twenty-first century is that nanoparticles may produce potential functional and toxicity effects on human neural cells owing to their ability to pass through biological membranes (Brooking *et al.* 2001).

The blood–brain barrier (BBB) is a separation of circulating blood and cerebrospinal fluid (CSF) maintained by the choroid plexus in the CNS, which results from the selectivity of the tight junctions between endothelial cells in CNS vessels that restrict the passage of solutes. At the interface between blood and brain, endothelial cells and associated astrocytes are stitched together by tight junctions. Endothelial cells restrict the diffusion of microscopic objects and large or hydrophilic molecules into the CSF, while allowing the diffusion of small hydrophobic molecules (e.g. O_2 , hormones, CO_2). Cells associated with the BBB actively transport metabolic products such as glucose across the barrier with specific proteins (Seidner *et al.* 1998).

Exposure to nanoparticles (such as Ag) in the body is also becoming increasingly widespread through antibacterial fabrics and coatings. However, effects from the presence (or even accumulation) of metal nanoparticles in the brain and through the BBB have not yet been fully studied. Small-sized particles have better mobility and it is expected that the transportation of nanoparticles across the BBB is possible either by passive diffusion or by carrier-mediated endocytosis (Hoet *et al.* 2004). In addition, nanoparticles may be taken up directly into the brain by *trans*-synaptic transport (Oberdörster 2004). For example, Ag nanoparticles can enter via the BBB (Panyala *et al.* 2008) and accumulate in different regions of the brain (Rungby & Danscher 1983), and this may be beneficial for drug delivery, but may also pose a risk to the patient (Sarin *et al.* 2008; Muthu & Singh 2009). It has also been reported that nanoparticle exposure can induce impairments to normal neurons (Tang *et al.* 2008), microglia (Au *et al.* 2007) and even aggravate the process of brain pathology (Sharma & Sharma 2007). Ion channels play an important role in cell viability and functionality, especially in the CNS, which serve as a subtle indicator of the condition and viability of the cells.

Voltage-gated sodium currents determine a large number of neuronal properties, such as influencing action potential generation and the propagation of action potentials to synapse terminals. The local depolarization of neurons may also be affected by the existence of nanoparticles. However, what plays a key role remains to be determined in the transportation of amino acid neurotransmitters (e.g. aminobutyric acid (GABA)) and monoamines (i.e. dopamine (DA), norepinephrine, and serotonin). In addition, mutations may also cause changes in voltage-gated Na^+ channels, which are associated with a number of neurological diseases, including spontaneous epilepsy and pain conditions, and have been implicated in various psychiatric disorders (Meisler & Kearney 2005; Guo *et al.* 2008).

The effective nanoparticle content used in applications (0.1–1%w/w) could be well below the toxicity dosage limit by the time nanoparticles reach the CNS from point of contact. This estimation takes into account the fact that released nanoparticles/ions also need to enter the body, then cross the BBB and finally reach the CNS. Most of the nanoparticle release rates from work carried out on solid matrix–particle composites (i.e. epoxy resins, as shown in figure 4) display the release range of ion/particle concentration to be less than $10^{-5} \text{ g ml}^{-1}$, which is the minimum non-effective dosage for all the CNS neuron cell tests (drug toxicity tests are around $10^{-6} \text{ g ml}^{-1}$). These nanoparticle neuron tests also take into account several types of nanoparticles, e.g. Ag, CuO, ZnO, TiO_2 (Xu *et al.* 2009; Zhao *et al.* 2009; Liu *et al.* 2010). Since metallic particle systems are being used in a host of contact applications such as computers, paints and clothing, research into this area and further parametric variables needs to be considered (e.g. exposure, contact time, strength of binding and weight loading).

Voltage-gated sodium current is responsible for modifying the excitability of neuronal cells and neuronal activity and function in the CNS. Therefore, potential

Figure 4. Scanning electron micrograph (SEM) of carbon nanotubes reinforced with epoxy resin (scale bar, 5 μm).

modulation of the current by nanometal particles would be expected, leading to alterations in functionality. Some reports have shown that nanoparticles can impair cell function and even induce certain cell death (Shin *et al.* 2007; Cha *et al.* 2008; Tang *et al.* 2008). In recent studies on the neurotoxicity of metallic nanoparticles, a neuro-endocrine cell line (PC-12 cells) was exposed to nanoparticles such as Ag ($5 \times 10^{-5} \text{ g ml}^{-1}$), which reduced the level of DA. It was also found that Ag nanoparticles were more toxic than manganese (Mn) nanoparticles to particular cells (Hussain *et al.* 2006a). These findings suggest that Ag and other nanoparticles might have significant pathological consequences for the brain of mammals while enhancing or inhibiting some particular functionality (figure 5).

Nanoparticles have potential functionality and toxic effects on human neuron cells since they can pass through biological membranes (Brooking *et al.* 2001). It is known that the biological half-life of silver in the CNS is longer than that in other organs, suggesting that there may be some significant physiological functions, consequences and risks to the brain due to prolonged exposure.

However, the effects from the presence (or even accumulation) of such particles, especially Ag, in the CNS are not very well documented.

3. CURRENT RESEARCH ADVANCES IN NANOPARTICLE AND NEURON CELL INTERACTION

3.1. The neurotoxicity research of nanoscaled materials *in vivo*

To investigate the potential effects of nanomaterials on the brain, some *in vivo* tests have been carried out on different animal models. Nanoparticles (50 nm) of silica-coated cobalt ferrite were found in the brain after being administered via an intravenous injection

Figure 5. Schematic of hippocampal pathways.

in mice (Kim *et al.* 2006). In another study, F344 female rats received single or multiple exposures to 20, 100 and 1000 nm latex fluorospheres by intravenous injection or oral pharyngeal aspiration into the airways. In this instance, the 20 nm spheres were not detected in the brain; however, the 100 nm spheres were detected in the CNS 24 h after administration. The 1000 nm spheres were detected for up to 28 days and were no longer found in the brain after this time point (Sarlo *et al.* 2009). Although this study utilized the same material (latex) for the various particle sizes, modest consideration was granted for material physico-chemical properties and it would not be representative if other materials, e.g. other polymers, metals, metal oxides, ceramic composites, CNTs, etc., were to be used in the same tests.

In addition, maternal exposure of mice to TiO_2 nanoparticles may affect the expression of genes related to the development and function of the CNS. Analysis of gene expression using gene ontology indicated that gene expression levels associated with apoptosis were altered in the brain of newborn pups, and those associated with brain development were altered in early age. The genes associated with response to oxidative stress were changed in the brains of two- and three-week-old mice. Changes to gene expression associated with neurotransmitters and psychiatric diseases were found (Shimizu *et al.* 2009). The results suggest the potential toxicity of nanoparticles on the development of newborns. Nano- TiO_2 has also been shown to induce an increase in glial fibrillary acidic protein (GFAP), producing positive astrocytes in the CA4 region, which was in good agreement with higher Ti contents in the hippocampus region. This resulted in various types of oxidative stress in the brain of exposed mice such as lipid peroxidation, protein oxidation and increased activities of catalase, as well as the excessive release of glutamic acid and nitric oxide (Wang *et al.* 2008).

Nanotoxicology studies on the brain have also focused on fish. For example, in the brain of juvenile largemouth bass, a significant increase in lipid peroxidation was observed due to exposure to fullerenes (C60; $0.5 \times 10^{-6} \text{ g ml}^{-1}$; Oberdörster 2004). In addition, it is conceivable that colloidal fullerenes need to be transported to lipid-rich regions (e.g. brain) before the colloid dissociates and frees individual redox-active fullerenes. It is also possible that there may be an inflammatory response creating reactive oxygen species (ROS) or that a reactive fullerene metabolite is produced. The actual mechanism still needs to be

Figure 6. Confocal image of PC-12 cells used in toxicity studies. The cell lengths are approximately 25–30 μm under the confocal microscope before cell-line proliferation. However, after the cell division their lengths could grow up to several hundred micrometres (scale bar, 10 μm).

determined, and future research will focus on this question. The depletion of glutathione (GSH) is used as an indication of oxyradical scavenging ability, showing that the antioxidant defence system is overwhelmed by ROS (Oberdörster 2004).

Recent research has focused on the effects of nanoparticles on the BBB. According to a study by Sharma *et al.* (2010), administration of Ag, Cu or Al/Al₂O₃ nanoparticles showed disrupted BBB function and induced brain oedema formation. Moreover, silver nanoparticles induced BBB destruction and astrocyte swelling, and caused neuronal degeneration (Tang *et al.* 2009).

3.2. Neurotoxicity research of nanoscaled materials *in vitro*

Several studies have focused on PC-12 cells, a neuroendocrine cell line with the capability to produce the neurotransmitter DA and contain functional DA metabolism pathways (figure 6). Normal PC-12 cells are around 25–30 μm ; after cell division this could increase to several hundred micrometres. However, with exposure of PC-12 cells to Mn nanoparticles (40 nm), or Mn²⁺ (acetate), or Ag nanoparticles (15 nm) for 24 h, the cells showed contrasting results. Phase-contrast microscopy studies show that exposure to Mn particles or Mn²⁺ does not greatly change the morphology of PC-12 cells. But exposure to Ag particles caused cell shrinkage plus irregular membrane borders compared with the control cells. Further microscopic studies at higher resolution microscopy revealed that Mn nanoparticles and agglomerates were effectively internalized by PC-12 cells (Hussain *et al.* 2006a,b). Mitochondrial reduction activity, a sensitive measure of particle and metal cytotoxicity, showed only

moderate toxicity for Mn compared with similar Ag and Mn²⁺ doses. Mn particles and Mn²⁺ ions depleted DA (dose dependent) and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Ag particles significantly reduced DA and DOPAC only at concentrations of 50 mg ml⁻¹. Therefore, DA depletion due to Mn particles was most similar to Mn²⁺ ions, which is known to induce concentration-dependent DA depletion. The significant increase in ROS with Mn particle exposure also suggests that the increased ROS levels may participate in DA depletion (Hussain *et al.* 2006a). In another study, the expression of 11 genes associated with the dopaminergic system was examined using real-time reverse transcription polymerase chain reaction (RT-PCR). The results indicated that the expression of Txnrd1 was upregulated after the Cu-90 treatment and the expression of Gpx1 was downregulated after Ag-15 or Cu-90 treatment. These alterations are consistent with the oxidative stress induced by metal nanoparticles. Mn-40 induced a downregulation of the expression of Th; Cu-90 induced an upregulation of the expression of MAOA. Mn-40 also induced a downregulation of the expression of Park2; while the expression of SNCA was upregulated after Mn-40 or Cu-90 treatment (Wang *et al.* 2009).

PC-12 cells have also been treated with different concentrations of TiO₂ nanoparticles (1, 10, 50 and 100 $\times 10^{-6}$ g ml⁻¹) and the viability of these cells was significantly reduced, showing a significant dose- and time-dependent effect (Liu *et al.* 2010). In agreement with earlier findings (Hussain *et al.* 2006a,b), the flow cytometric assay gave an indication that the TiO₂ nanoparticles induced intracellular accumulation of ROS (as shown in figure 7) and apoptosis of the PC-12 cells with increasing concentration of TiO₂. Interestingly, pre-treatment with a ROS scavenger could inhibit PC-12 apoptosis induced by the particles (Liu *et al.* 2010). Similar findings have been reported by Long *et al.* (2006), where TiO₂ stimulated immediate ROS production.

Zinc (Zn) and Iron (Fe) nanoparticles have also been assessed for their cell interactions using a glioma cell line, A-172. Zn (300 nm), Fe (100 nm), Si (10–20, 40–50, 90–110 nm; 0.24–2400 $\times 10^{-9}$ g ml⁻¹) and a micro-sized (45 μm) Si (control) were analysed and used in cell cytotoxicity (Cha & Myung 2007). Fluorescence was absent inside the glioma cell line A-172, suggesting that nanoparticles did not alter the membrane permeability and the cytotoxicity of nanoparticles *in vitro* was low, and it was not dependent on the types and sizes of nanoparticles, showing a low level of toxicity *in vivo* (Zhao *et al.* 2009). Here, the toxicity was due to material chemistry rather than size (Cha & Myung 2007). Results obtained using reduced nanoparticle concentrations (0.24–2400 $\times 10^{-9}$ g ml⁻¹) compared with other studies (Hussain *et al.* 2006a; Liu *et al.* 2009) suggest that concentration is an important parameter when assessing exposure to cells.

In a separate study, up to 30 $\mu\text{g ml}^{-1}$ single-walled CNTs (SWCNTs) significantly decreased the overall DNA content in chicken embryonic spinal cord or

Figure 7. Measurement of ROS generation in PC-12 cells by flow cytometry. The cells were cultured with nano-TiO₂ at concentrations of (a) 0 µg ml⁻¹, (b) 10 µg ml⁻¹, (c) 50 µg ml⁻¹ and (d) 100 µg ml⁻¹ for 24 h. ROS levels are dose dependent. The corresponding linear diagram of flow cytometry is shown (e). $n = 3$; mean $\pm$ SEM; *statistically significant difference compared with controls ($p < 0.05$); ** $p < 0.01$ (Liu *et al.* 2010).

dorsal root ganglia. This effect was more pronounced when cells were exposed to highly agglomerated SWCNTs than when they were exposed to better dispersed SWCNT bundles (Belyanskaya *et al.* 2009).

3.3. The patch clamp technique

The patch clamp technique is a laboratory technique in electrophysiology that allows the study of single

or multiple ion channels in cells. The technique can be applied to the study of excitable cells such as neurons, cardiomyocytes, muscle fibres and pancreatic beta cells.

This technique has been used in studying the effects of nanoparticles on ion channels (Zhao *et al.* 2009). This can be demonstrated using single rat hippocampal pyramidal neurons, isolated by enzymatic digestion and mechanical dispersion (according to the method of

Figure 8. (a) Schematic diagram of hippocampal CA1 pyramidal neurons in the brain. (b) Whole cell patch clamp recording in CA1 pyramidal neuron from 14–18 Wistar rats.

Zou *et al.* 2000). Sample preparation includes slicing the entire hippocampus and subiculum horizontally (400 μm in thickness) using a vibratome (VT1000M/E, Leica, Germany) and incubating with artificial CSF (ACSF). Hippocampal CA1 neurons were then visualized on a monitor connected to a low light-sensitive charge-coupled device camera (figure 8; Liu *et al.* 2009).

Whole-cell currents of pyramidal neurons were recorded using an EPC10 patch clamp amplifier (HEKA, Germany; figure 9). After the rupture of the membrane and the establishment of a whole cell voltage-clamp configuration, compensation (80%) for series resistance was routinely used. These data were low-pass filtered at 2.9 kHz, digitized at 10 kHz (four-pole Bessel filter) and used PULSE 8.74 software (HEKA, Germany) at the ambient temperature (21–23°C). The effect of metal nanoparticles on voltage-gated channels (hippocampal neurons) can be shown using ZnO nanoparticles (concentration of $10^{-4} \text{ g ml}^{-1}$; Zhao *et al.* 2009). Here, the transient outward potassium current (I_A) and delayed rectifier potassium current (I_K) increased considerably (figure 10). However, it is apparent that the ZnO solution/suspension did not shift the steady-state activation curve of I_A and I_K , and nor did it have a significant effect on the inactivation and the recovery from the inactivation of I_A . Peak amplitude and overshoot of the evoked single action potential was increased and the half-width was diminished in the presence of the $10^{-4} \text{ g ml}^{-1}$ ZnO solution (Zhao *et al.* 2009). Using different nanoparticles, such as CuO, other studies (Xu *et al.* 2009) have shown that CuO nanoparticles

Figure 9. Experimental set-up for recording the response of neuron cells in ion channel currents.

($5 \times 10^{-5} \text{ g ml}^{-1}$) have no effects on I_A , but inhibited I_K (figure 10). Furthermore, CuO nanoparticles did not shift the steady-state activation curve of I_K and I_A , but the inactivation curve of I_K was shifted negatively. The effects on the inactivation curve of I_A have no statistical significance (Xu *et al.* 2009).

More recent work (Xu *et al.* 2009) demonstrated that ZnO nanoparticles increase the peak amplitudes of the voltage-gated sodium current (I_{Na} ; figure 11), while the inactivation and the recovery from inactivation of the I_{Na} are promoted by ZnO. The data also show that the steady-state activation curve of the I_{Na} has not been shifted by ZnO nanoparticles. When the effects of Ag nanoparticles on the I_{Na} were examined with increasing concentrations (10^{-6} , 5×10^{-6} , $10^{-5} \text{ g ml}^{-1}$), the results revealed that only concentrations of $10^{-5} \text{ g ml}^{-1}$ reduced the amplitude of the I_{Na} (figure 11). Similar to ZnO, Ag particles produced a hyperpolarizing shift in the activation–voltage curve of I_{Na} . Ag nanoparticles delay the recovery of the I_{Na} from inactivation (Liu *et al.* 2009), but the ZnO accelerates the process (Zhao *et al.* 2009).

ZnO also increases the evoked single action potential and repetitive firing rate. Action potentials are a fundamental property of excitable cells in the mammalian CNS. ZnO enhances peak amplitude and overshoot and demonstrates decreased half-width of the evoked single action potential. Conversely, peak amplitude and overshoot of the evoked single action potential are decreased and half-width is increased in the presence of a $10^{-5} \text{ g ml}^{-1}$ Ag nanoparticle solution (Liu *et al.* 2009).

4. CONCLUSION

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 focussing on the interaction with hippocampal cell membranes, as carried out for CNS drug toxicity. The effects of nanomaterials

Figure 10. The original traces of (a)(i)(ii) I_A and (b)(i)(ii) I_K . The contrasting effects of ZnO and CuO nanoparticles on $I-V$ currents and peak amplitudes of I_A and I_K on hippocampal pyramidal neurons from Wistar rats (Xu *et al.* 2009; Zhao *et al.* 2009). (a,b) (i) Filled square, control; open circle, nano-ZnO; (a,b) (ii) open square, control; open circle, nano-CuO.

on ion channels within neurons may specifically relate to Na^+ [I_{Na} (A)] and K^+ [I_K (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.

5. FUTURE RESEARCH WORK AND POTENTIAL RESEARCH DIRECTIONS

Nanotoxicity research can be applied to a number of applications, such as determining composition levels in coatings for medical devices, medical-grade sheet moulding 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/nanotoxicity 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

Figure 11. The original traces of the I_{Na} . (a) The increased effects of the nano-ZnO (filled square, control; open circle, nano-ZnO) and (b) decreased effects of nano-Ag on $I-V$ currents and peak amplitudes of I_{Na} on hippocampal pyramidal neurons from Wistar rats (Liu *et al.* 2009; Zhao *et al.* 2009 filled square, control; open circle, nano-Ag).

models, such as ischaemia, 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 nanofunctions and features. Further research on nanotoxicity 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, Fe_2O_3 , Al_2O_3 , ZnO, SiO_2 , TiO_2 , CuO, Cu_2O , and WC, etc.) have been investigated in terms of the aetiology, pathology, physiology and epidemiology; however, no report has been obtained on CNS neurons owing to the complexity and high costs associated with assessments.

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