NEURAL REGENERATION RESEARCH
July 2015, Volume 10, Issue 7
www.nrronline.org

PERSPECTIVE

Metallic nanoparticles for peripheral nerve regeneration: is it a feasible approach?

The primary function of the peripheral nerves is to transmit signals from the spinal cord to the rest of the body, or to convey sensory information from the rest of the body to the spinal cord. In case of injury or a health disorder, this pathway can be partially or totally disrupted, resulting in pain, loss of sensation, reduced muscular strength, poor coordination, or complete paralysis. Even if peripheral nerves can spontaneously regenerate from injury, in the case of a complete nerve transection, a clinical operation must be performed in order to reconnect the portions of the injured axons. Although current clinical strategies include autografts, allografts and nerve guides, the maximum regeneration distance is limited to 25 mm. Researchers are currently focused on finding new methods and materials to improve this nerve regeneration distance in the case of gap injuries. The main methods currently investigated include: i) the use of scaffolds with different mechanical and chemical properties, ii) the use of specific molecules able to promote the growth process (*e.g.*, neurotrophins, extracellular matrix molecules, cell adhesion molecules or growth factors), and iii) the incorporation of support cells into the nerve conduits (Bell and Haycock, 2012).

Recently, researchers have explored the use of nanoparticles (NPs) for neural regeneration applications. NPs offer several benefits in terms of small size, physical properties that diverge from the bulk, surface functionalization and chemical stability. Due to their small size, nanotechnology devices can penetrate the blood-brain barrier and can be taken up within cells. This mechanism is particularly useful for drug delivery systems, as NPs can be engineered to target specific sites in the brain (Garcia-Garcia et al., 2005). Moreover, NPs have been shown to promote the outgrowth of neuronal cells. For example, Ciofani et al. (2010) discovered that the piezoelectric properties of boron nitride nanotubes can be used to increase the neurite length of PC12 cells *in vitro*. They observed a 30% increase in axonal outgrowth after exposing the boron nitride nanotubes to ultrasound. In other work, Kim et al. (2011) reported enhanced neuronal differentiation after endocytosis of iron oxide NPs in PC12 cells exposed to nerve growth factor. Most importantly, the neurite length at day 1 was almost three times higher than the outgrowth of cells treated with growth factor alone. Chitosan-gold NPs were also successfully grafted onto poly(D,L-lactide) nerve conduits, showing the feasibility of regeneration (in terms of degree of myelination, number of blood vessels and area of regenerated nerve) in an *in vivo* transection model of the rat sciatic nerve (Ni et al., 2010).

Several types of nanoparticles have already been synthesized and characterized, but amongst these, metallic NPs are particularly attractive because of their distinctive optical properties. Indeed, when metal NPs are illuminated by an external light field, they generate a resonant coherent oscillation called the localized surface plasmon resonance. The peak wavelength of the optical absorption mainly depends on the NP shape and aspect ratio. Gold nanorods (Au NRs) are of particular interest for biophotonics applications, because their localized surface plasmon resonance wavelength matches the therapeutic window of biological tissues (*i.e.*, the near infrared region of the spectrum, from 750–1,400 nm) (Paviolo et al., 2013). Collisional relaxation rapidly transfers the plasmon energy to the metal lattice as heat, after which it is conducted to the surrounding environment. Heat generated by laser exposure of plasmonic NPs has already been successfully applied in other nerve-related applications, such as the depolarization of primary auditory neurons (Yong et al., 2014) or to modulate neural activity (Yoo et al., 2014). Yong et al. (2014) demonstrated that near infrared irradiation of Au NRs can stimulate electrical activity in spiral ganglion primary neurons. For cells cultured with silica-coated Au NRs, they reported a linear increase of laser-induced current with pulse duration, until a threshold where action potentials were triggered. Similarly, Yoo et al. (2014) showed photothermal inhibition of neural activity in primary hippocampal neurons, pointing out that this nanotechnology approach might represent a valuable option in the treatment of brain disorders that currently rely on chemical or electrical suppression (*e.g.*, epilepsy, Parkinson's disease).

In our laboratory, we discovered that the heat released after plasmon excitation can also be used to stimulate cell outgrowth in NG108-15 cells (Paviolo et al., 2013). We used Au NRs with different coatings (bare, polystyrene sulfonate- and silica-coated) to evaluate cell behavior in terms of cytotoxicity, cell proliferation, NR uptake, and cell differentiation. NRs were internalized in the neuronal cells from day one of incubation, with their location predominantly in the cell cytoplasm. The plasmon peak was then excited using a range of optical pulse lengths and powers. The laser irradiation was observed to have a stimulatory effect on cell differentiation, but had no significant effect on cell viability. Indeed, significant increases were observed in the neurite length 3 days after laser exposure. The greatest outgrowth was observed after irradiating the endocytosed NRs with the highest laser dose (7.5 W/cm^2), obtaining an average increase in neurite length of almost 36% compared to the non-irradiated sample. This behavior was not specific to the NR surface chemistry; however, the response was correlated with optical absorption by the endocytosed Au NRs (Paviolo et al., 2013). A separate study on neurite outgrowth has also reported the use of near infrared light to guide axonal growth. In that case, the growth behavior was linked to localized heating of the cells and the activation of the transient receptor potential vanilloid channels (Ebbesen and Bruus, 2012).

Following our discovery that increased neurite outgrowth is correlated with laser exposure of Au NRs, we demonstrated that intracellular calcium transients (Ca^{2+}) can be induced after near infrared optical exposure of NG108-15 cells cultured with Au NRs (Paviolo et al., 2014) (**Figure 1**). Calcium ions are an integral part of common signaling pathways and have been extensively studied for their role in cell responses such as mitosis, apoptosis, muscle contraction, and neurite extension (Ebbesen and Bruus, 2012). In response to a stimulus, Ca^{2+} increases, oscillates and decreases, leading to the activation, modulation or termination of a specific cell function. Ca^{2+} ions can enter the cell cytoplasm in two different ways: through specific channels located on the plasma membrane, or after being released from internal cytoplasmic organelles, such as the mitochondria, endoplasmic reticulum, sarcoplasmic reticulum or the Golgi apparatus. Ebbesen and Bruus (2012) showed that cytoplasmic Ca^{2+} concentrations play a key role in the regulation of neurite growth. The neuronal growth cone uses surface receptors to mediate the growth of neurites, regulating both the process of neurite extension and motility. They observed that localized increases in cytoplasmic Ca^{2+} can result in both the protrusion of filopodia and neuronal growth cone turning, and that neurons can grow by following a Ca^{2+} gradient.

We activated intracellular Ca^{2+} transients by irradiating endocytosed bare- and polystyrene sulfonate-coated-Au NRs with a 780 nm laser diode modulated with a binary signal with variable frequencies and pulse lengths. The Ca^{2+} variations were mapped as a function of time by monitoring the fluorescence intensity of the calcium indicator Fluo 4-AM on single cells. We observed Ca^{2+} spikes with different amplitudes, with a 48% probability of NR laser-induced cell activation. The highest signal amplitudes were found for a radiant exposure of 0.33 J/cm^2, achieving an increase in fluorescence of 32 ± 2% from the baseline level. The observed laser-induced Ca^{2+} release appears to be associated with the transient heating arising from excitation of the localized surface plasmon resonance in the NRs. These thermal transients could potentially serve to: i) open some voltage-controlled channels by changing the cell membrane capacitance, ii) activate temperature sensitive transient receptor potential vanilloid channels, and/or iii) deplete the calcium storage of specific intracellular organelles (*e.g.*, mitochondria). Reduced amplitudes were observed at higher optical irradiation levels, confirming the potential for photothermal inhibition of neural activity observed in primary hippocampal brain tissue cultured with Au NRs (Yoo et al., 2014). Since it is known that cytoplasmic

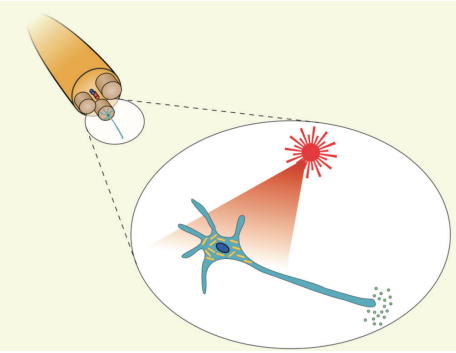

Figure 1 Schematic representation of laser-induced activation of cells incubated with Au nanoparticles.

Ca^{2+} concentrations also regulate the process of neurite extension and motility, we speculated that this pathway was also activated during the process of neurite increase observed after exposing the Au NRs at their plasmon wavelength.

Although the interest in Au NPs for neuronal and therapeutic applications is expanding, *in vivo* studies are still constrained by a lack of knowledge about the consequences of nanomaterials on intracellular pathways and inflammatory responses. It is known that a high concentration of metal nanoparticles in living organisms can cause cell oxidative stress and reactive oxygen species production, leading to other serious cellular dysfunctions, such as inflammation, cell damage, DNA damage, cancer or apoptosis. Reactive oxygen species and oxidative stress are mainly generated within cells as a byproduct of other reactions or as part of a molecular synthesis breakdown. If produced in excess, they may become cytotoxic by altering protein activity and/or interacting with lipids and nucleic acids. Physiological levels of reactive oxygen species are also critical for maintaining a dynamically active cytoskeleton and for controlling neurite extension in neuronal cells. Recently, Söderstjerna et al. (2014) recorded a significantly higher number of apoptotic and oxidatively stressed cells after exposing Au NPs in a primary tissue model of the mouse retina. Their images of nuclear uptake raise questions of whether NPs can cause DNA damage and what is the long-term impact of NPs on gene expression. In terms of electrical activity, it was also observed that intracellular Au NPs can increase neuronal excitability and aggravate seizure activation in hippocampal primary tissue, suggesting that intracellular NPs might alter neuronal functions and cause hyper-excitability in pathological conditions (Jung et al., 2014).

Moreover, Kim et al. (2011) postulated that NPs might be able to alter the interaction of neuronal cells with their substrate, by up-regulating cell adhesion molecules associated with the extracellular matrix. In a more comprehensive study looking at the biodistribution of Au nanoshells *in vivo*, it was determined that NPs mainly accumulate in the liver and spleen of mice over extended periods of time. These destinations are consistent with clearance of the particles from the blood stream. However, histopathology studies performed on animals one year after the infusion showed no signs of toxic effects in these organs (Gad et al., 2012). This lack of significant toxicity raises the possibility that convective flow conditions *in vivo* might favor the long-term biocompatibility of nanomaterials.

All of the evidence reported here suggests that the use of nanotechnology to treat neuronal disorders has great potential for future applications in neural prostheses and cell therapies. There are currently several proposed clinical applications of NPs for drug delivery, including tissue regeneration, cochlear and retinal implants, cartilage and joint repair, skin regeneration, antimicrobial therapy, correction of metabolic disorders, and targeted drug delivery to treat specific diseased sites (including applications to the central nervous system) (McMillan et al., 2011). However, there is a need to clarify the long term effects of NPs and the intracellular pathways that are activated. Different studies have shown that NPs might be capable of activating specific signaling pathways that regulate neuronal cell differentiation, but the long-term impact on gene expression still has to be clarified. Moreover, since ion channels in the plasma membrane of neurons determine the overall bioelectrical properties of the cells and contribute to the generation and propagation of action potentials, careful studies should also be undertaken to investigate the influence of NPs on single ion channels and ionic currents. For neural regeneration applications, the literature suggests that there is great potential to combine the stimulatory effects of Au NRs with other known methods to promote peripheral nerve outgrowth, such as aligned electrospun polycaprolactone fiber scaffolds (Ni et al., 2010; Bell and Haycock, 2012). However, further studies on neuronal cell inflammation and gene expression after optical exposure of the nanomaterials will be required. There is also potential to combine biodegradable materials in the nanoparticle preparation to allow sustained drug release (*e.g.*, growth factors) at the targeted sites. *In vitro* formation of neuronal circuits to study neuronal network communication can also be targeted as a future application of nanoparticle assisted neural regeneration.

Chiara Paviolo, Paul R. Stoddart *

Centre for Micro-Photonics (Paviolo C), Industrial Research Institute Swinburne (Stoddart PR), Faculty of Science, Engineering and Technology, Swinburne University of Technology, Hawthorn, Victoria, Australia

***Correspondence to:** *Paul R. Stoddart, Ph.D., pstoddart@swin.edu.au.*
***Accepted:** *2015-05-23*
***doi:**10.4103/1673-5374.160083* ***http**://www.nrronline.org/*
Paviolo C, Stoddart PR (2015) Metallic nanoparticles for peripheral nerve regeneration: is it a feasible approach? Neural Regen Res 10(7):1065-1066.

References

Bell JH, Haycock JW (2012) Next generation nerve guides: materials, fabrication, growth factors, and cell delivery. Tissue Eng B 18:116-128.
Ciofani G, Danti S, D'Alessandro D, Ricotti L, Moscato S, Bertoni G, Falqui A, Berrettini S, Petrini M, Mattoli V, Menciassi A (2010) Enhancement of neurite outgrowth in neuronal-like cells following boron nitride nanotube-mediated stimulation. ACS Nano 4:6267-6277.
Ebbesen CL, Bruus H (2012) Analysis of laser-induced heating in optical neuronal guidance. J Neurosci Meth 209:168-177.
Gad SC, Sharp KL, Montgomery C, Payne JD, Goodrich GP (2012) Evaluation of the toxicity of intravenous delivery of auroshell particles (gold-silica nanoshells). Int J Toxicol 31:584-594.
Garcia-Garcia E, Andrieux K, Gil S, Couvreur P (2005) Colloidal carriers and blood-brain barrier (BBB) translocation: a way to deliver drugs to the brain? Int J Pharm 298:274-292.
Jung S, Bang M, Kim BS, Lee S, Kotov NA, Kim B, Jeon D (2014) Intracellular gold nanoparticles increase neuronal excitability and aggravate seizure activity in the mouse brain. PLoS One 9:e91360.
Kim JA, Lee N, Kim BH, Rhee WJ, Yoon S, Hyeon T, Park TH (2011) Enhancement of neurite outgrowth in PC12 cells by iron oxide nanoparticles. Biomaterials 32:2871-2877.
McMillan J, Batrakova E, Gendelman HE (2011) Cell delivery of therapeutic nanoparticles. Prog Mol Biol Transl Sci 104:563-601.
Ni HC, Lin ZY, Hsu SH, Chiu IM (2010) The use of air plasma in surface modification of peripheral nerve conduits. Acta Biomater 6:2066-2076.
Paviolo C, Haycock JW, Cadusch PJ, McArthur SL, Stoddart PR (2014) Laser exposure of gold nanorods can induce intracellular calcium transients. J Biophotonics 7:761-765.
Paviolo C, Haycock JW, Yong J, Yu A, Stoddart PR, McArthur SL (2013) Laser exposure of gold nanorods can increase neuronal cell outgrowth. Biotechnol Bioeng 110:2277-2291.
Söderstjerna E, Bauer P, Cedervall T, Abdshill H, Johansson F, Johansson UE (2014) Silver and gold nanoparticles exposure to in vitro cultured retina – Studies on nanoparticle internalization, apoptosis, oxidative stress, glial- and microglial activity. PLoS One 9:e105359.
Yong J, Needham K, Brown WGA, Nayagam BA, McArthur SL, Yu A, Stoddart PR (2014) Gold-nanorod-assisted near-infrared stimulation of primary auditory neurons. Adv Healthcare Mater 3:1862-1868.
Yoo S, Hong S, Choi Y, Park J, Nam Y (2014) Photothermal inhibition of neural activity with near-infrared-sensitive nanotransducers. ACS Nano 8:8040-8049.