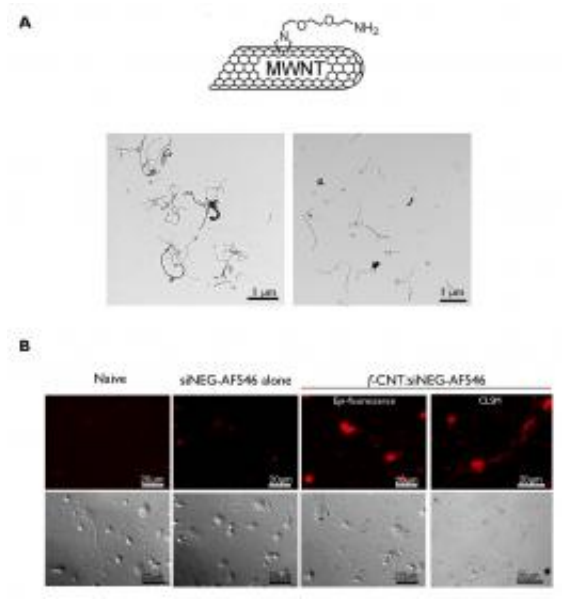
Talk softly but carry a tiny stick: Stroke prevention and recovery with nanotube-delivered siRNA

July 5 2011, by Stuart Mason Dambrot



Neuronal uptake of f-CNT in vitro. (A) f-CNT chemical structure and Transmission Electron Microscope images. (B) Epifluorescence and confocal laser scanning microscopy images of primary neuronal culture isolated from mouse brain motor cortex. (c) Image courtesy of PNAS, doi: 10.1073/pnas.1100930108

(Medical Xpress) -- Of the world's leading causes of death, stroke ranks second – and occurring 8 out of 10 times is ischemic stroke: reduced blood supply to the brain creates a shortage of oxygen, glucose and other

nutrients and an increase in metabolic waste, leading to neuronal damage that results in physiological impairment or death. At the molecular level, the genetic activation of the nucleic acid protein Caspase-3 – a member of the cysteine-aspartic acid protease (caspase) family – is a major factor in loss of neuronal tissue and associated apoptosis (programmed cell death). Post-stroke treatments known to be effective at reducing or reversing damage involve preventing Caspase-3 activation, either by genetic or pharmacological intervention. Recently, however, a group of European researchers combined these modalities by using functionalized carbon nanotubes (*f*-CNT) – nanotubes made soluble by attaching certain molecules to their sidewalls – to deliver siRNA (silencing RNA) to ischemically-impacted neuronal tissue *in vivo*.

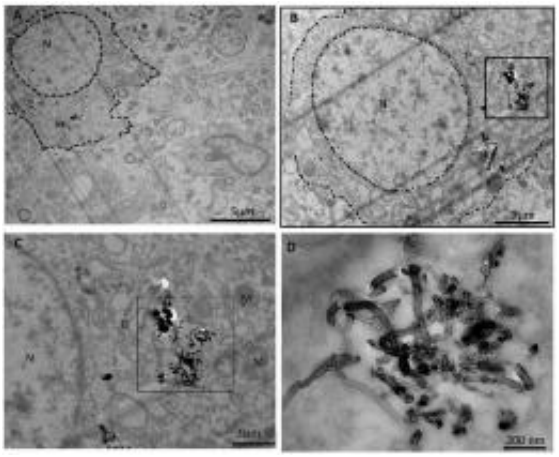
Led by Prof. Tommaso Pizzorusso at the Consiglio Nazionale delle Ricerche Neuroscience Institute and the University of Florence Department of Psychology, and Prof. Kostas Kostarelos at the Nanomedicine Laboratory of the University of London – and including Khuloud Al-Jamal, Lisa Gherardini, Giuseppe Bardi, Antonio Nunes, Chang Guo, Cyrill Bussy, M. Antonia Herrero, Alberto Bianco, and Maurizio Prato – the group encountered several neural and nanotechnological challenges in using biocompatible f-CNT to deliver Caspase-3 siRNA (siCas 3) intraneuronally into the rodent motor cortex. According to Kostarelos, the main challenge in delivering nucleic acids to neural tissue in general (not just using nanotubes) is ensuring that adequate amounts enter the cytoplasmic or nuclear target sites for siRNA and plasmid DNA, respectively. He notes that “neuronal tissue has proven exceptionally challenging to manipulate genetically because of inefficient means to transfer exogenous genetic information without using viruses.”

Specifically, says Pizzorusso, the team first had to reduce toxicity by opting to direct *f*-CNT injection at the lesion sties. “Systemic treatment requires large amounts of both vector and drug, which increases

toxicity," explains Pizzorusso. "Therefore, we had to setup a local lesion and injection protocol – so we needed nanotubes capable of staying in solution for microinjections, and able to bind and eventually release nucleic acids like siRNA." This also meant that they had to avoid injecting clumps of material. "Then we needed to set up a lesion procedure with relatively low variability of the lesion outcome. Each animal can respond differently to the lesion so we had to be as standardized as possible."

The team developed a number of innovative techniques to address these challenges, and is looking at ways of making their findings directly applicable to the human motor cortex.

"The key innovation used in this study is based on the previous discovery that chemically functionalized carbon nanotubes are capable of efficient, direct translocation into the cytoplasm of cells," Kostarelos stresses, "and in that way transport siRNA intracellularly." Pizzorusso points out that "CNT toxicity can vary as a function of various parameters, such as size and coating. While previous tests had been made to find the best usable vector, we will have to determine the vector's exact processing in the brain." Furthermore, he adds, even though local microinjections in patients are clinically feasible and practiced, the technique is still invasive and of course any long-term side-effects in the brain must still be studied. "Vectors that will be capable of easily reaching the target tissue – for example, to reach the brain the vector must cross the blood-brain barrier – will make such interventions really widespread." At the moment, lack of such capabilities by any vector system prevents their translation in human patients except in specialized neurosurgery clinics.



Internalization of f-CNT by neuronal tissue in vivo. (A) Transmission Electron Microscopy of brain cortical parenchyma at 48 hours after f-CNT administration showing internalization of nanotubes into brain cells. (B-D) Cells are identified by their morphological characteristics; neurons are marked by a dotted line and zoomed in with internalized f-CNT marked in a square. G, M, and N stands for Golgi apparatus, mitochondria, and nucleus respectively. (c) Image courtesy of PNAS, doi: 10.1073/pnas.1100930108

Moreover, Pizzorusso continues, “an open issue facing the team is that an injection given hours after the stroke was less effective than the injection simultaneous to the stroke. We should clarify whether this is an issue related with the amount of siRNA delivered – in which case we could change the dosage or improve the nanovector – or whether at greater post-stroke delays different biological processes have to be targeted. For instance, in addition to counteract cell death it would be important to promote brain plasticity in order to facilitate functional recovery at later times after the stroke, possibly in combination with rehabilitative physical therapy.”

In terms of applications, Kostarelos sees the most promising near-term application being a means to identify predominant genes implicated in neurological disorders by performing functional gene knockdown.

“Further ahead,” he adds, “is the therapeutic application of such nanoparticle-siRNA constructs for treatment of various brain diseases.” Pizzorusso holds the even more expansive view that “all fields of medicine in which a certain volume of cells can be directly accessed by a treatment are potentially suitable with our approach.”

Might the future hold the technology’s deployment in completely in vivo applications, such as autonomous nanorobotic siRNA delivery? “Potentially, yes,” opines Pizzorusso, but he again cautions that “brain treatments would require vectors able to cross the blood-brain barrier with low systemic toxicity before thinking about devices for automatic systemic delivery.”

“Our technology is suitable to many in vivo neurological applications,” envisions Kostarelos. ”Most of the experiments described in our paper are conducted in vivo and all subjects survived the procedures.” While he states that the idea of autonomous nanorobotic siRNA delivery is exciting as a possibility, he soberly acknowledges that “it’s too early to credibly assert whether it will ever be realistic.”

More information: Functional motor recovery from brain ischemic insult by carbon nanotube-mediated siRNA silencing, *PNAS* July 5, 2011 vol. **108** no. 27 10952-10957, Published online before print June 20, 2011, doi:10.1073/pnas.1100930108