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Carbon Nanotubes for Gene Delivery

Edward Kim
First-Year Student (B.Sc. Nanoscience)
College of Physical and Engineering Science, University of Guelph, CANADA

Since their discovery in 1991 by Sumio Iijima, carbon nanotubes (CNTs) have been generating great interest in various fields of research (Reich, Thomsen & Maultzsch, 2004). A particular area of interest for CNT applications is in the field of cell medicine, because of the CNT's ability to permeate plasma membranes and interact with organelles within the cell (Lacerda, Raffa, Prato, Bianco & Kostarelos, 2007). Additionally, CNTs have been shown to bind readily to other molecules (Lacerda *et al.*, 2007), suggesting their possible use as a molecule-delivery mechanism. This paper aims to give a brief overview of the basic properties of CNTs, while also discussing their use as a vector for gene delivery in human cells for therapeutic purposes, and analyzing potential problems that researchers may face in the future.

Structurally, CNTs appear as hollow tubes of carbon atoms, which can be rolled as either a single layer or in multiple layers (Dresselhaus, 2009). These rolls form either single-walled carbon nanotubes (SWCNTs) or multi-walled carbon nanotubes (MWCNTs). A CNT is defined by a vector in the graphene plane, which specifies how the CNT should be rolled, its circumference, and whether or not the CNT has any chirality (Rotkin & Subramoney, 2005). The length of the CNT is not defined by this vector, however, since the pattern of the CNT's unit cell may be repeated indefinitely. Currently, CNTs can be manufactured at a maximum axial length of only a few dozens of millimetres, though they can easily be produced in bulk powders or suspensions (Beckman, 2007). Most CNT research is presently focused at the microscopic scale.

CNTs are primarily of interest to medical scientists and doctors as a result of their unique physical and chemical attributes: CNTs are incredibly strong, flexible and can be formed with a myriad of different diameters; their variable length, chirality and circumference allow them to exhibit specific chemical or physical properties as desired (Rotkin & Subramoney, 2005). They can be manufactured to be either conducting or semi-conducting, and their walls can accept covalent bonds to form functional groups (Rotkin & Subramoney, 2005). Though CNTs exhibit a number of novel properties, it is the ability to bind different molecules to the surface of CNTs that is the most important contributor to why they are invaluable as a potential vector for gene delivery.

An example of the CNT's promise is its possible use in the treatment of a disease that could potentially benefit from gene delivery treatment. The basic premise of gene delivery is to insert a gene into a cell and activate it through transcription and translation, thereby producing a protein as the end result (Gene Delivery, 2010). A prime candidate for CNT-based gene delivery treatment is cystic fibrosis, which is a disease caused by a mutated gene (Karp, 2010).

Though gene delivery shows great potential as a method of treating certain diseases, it could benefit from the use of CNT vectors to solve a major difficulty, intracellular transport. DNA degrades rapidly outside the nucleus due to enzymatic action in the cytoplasm, and as a result, it is necessary to associate it with a vector to assist the gene in its delivery to the nucleus (Lacerda *et al.*, 2007). Historically, viral vectors have been used extensively in gene delivery research. However, while viral vectors are effective due to their natural ability to invade cells, they can often trigger an unwanted immune response (Ke & Qiao, 2007). Since CNTs can be bound to various molecules to augment cell interactions, they present a novel solution to the problem of finding a suitable gene delivery vector (Lacerda *et al.*, 2007).

CNTs can easily be bonded to DNA molecules, allowing the CNTs to assist in transport across the plasma membrane and throughout the cell (Lacerda *et al.*, 2007). The combination of attaching DNA and additional molecules to CNTs has proven to be incredibly effective in gene delivery research. Experiments have shown, through the use of marker proteins produced by plasmid DNA (pDNA), that it is possible to attach specific genes to a CNT and have the CNT transport the gene into the nucleus. There, it is transcribed, producing mRNA which is then translated (Lacerda *et al.*, 2007). Unlike many other substances, entering the cell through the endocytotic pathway by vesicular encapsulation, CNTs are able to bypass this pathway and enter directly into the cell through the plasma membrane (Panessa-Warren, Maye, Warren & Crosson, 2009). This method of CNT-based

pDNA delivery has been employed to great success. Researchers have shown that conjugates of CNTs and pDNA can enter mammalian cells and achieve gene expression in 80-100% of the target cell population (Lacerda *et al*., 2007). The high success rate of this CNT-pDNA treatment suggests that CNTs may very well become a viable gene delivery vector in the near future.

One of the prominent challenges in using CNTs in any biological context is the fact that CNTs are extremely hydrophobic. The CNTs' hydrophobicity arises from the fact that they are made purely of carbon atoms, meaning that there is no difference in electronegativity in a CNT's molecular bonds. Thus, CNTs are non-polar whereas water is polar, and so they are insoluble. In multicellular organisms such as humans, cells are generally present in an aqueous environment, both in terms of extracellular fluid and the cytoplasm itself (Karp, 2010). It is necessary to modify the CNTs to make them water-soluble, otherwise they will not be able to easily associate with the aqueous interior of cells. This result would prove to be problematic when attempting to transport DNA through the cytoplasm and into the nucleus for transcription. A prime example is cationic functionalized CNTs being used to bind to DNA and transport it through the cell and into the nucleus (Liang *et al*., 2008). Since the functional groups used for increasing the solubility of CNTs are charged, they cause the CNT to become polar. The CNT's solubility in the cell is increased as a result.

The toxicity of carbon nanotubes does remain relatively unknown. Whenever medical procedures involve the introduction of foreign materials into cells, cytotoxicity is a factor that must be considered. Previous studies have shown that the aggregation of CNTs inside a human lung cell can cause cytoskeletal projections to emerge from the cell, indicating cell irritation (Panessa-Warren *et al*., 2009). These results suggest that using improper dosages of CNTs could result in serious harm to the patient. It is imperative, therefore, that research on the toxicity of CNTs be conducted in parallel with research on its potential applications for therapeutic purposes.

CNTs present a glimpse of a hopeful, yet challenging, future for gene therapy. Gene delivery has gone through many stages of experimentation, but CNTs are rapidly proving to be an effective vector for gene delivery into cells. Though there will certainly be difficulties in developing a standardized, safe approach for gene delivery though CNT vectors, the flexible and adaptive nature of CNTs suggest that it is only a matter of time until a solution is discovered.

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