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Review article

Functionalised carbon nanotubes: From intracellular uptake and cell-related toxicity to systemic brain delivery

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ABSTRACT

Carbon nanotubes (CNTs) have long been regarded as promising carriers in biomedicine. Due to their high surface area and unique needle-like structure, CNTs are uniquely equipped to carry therapeutic molecules across biological membranes and, therefore, have been widely researched for use in theranostic applications. The attractive properties of the CNTs entice also their use in the brain environment. Cutting edge brain-specific therapies, capable of circumventing the physical and biochemical blockage of the blood-brain barrier, could be a precious tool to tackle brain disorders. With an increasing number of applications and expanding production, the effects of direct and indirect exposure to CNTs on cellular and molecular levels and more globally the general health, must be carefully assessed and limited.

In this chapter, we review the most recent trends on the development and application of CNT-based nanotechnologies, with a particular focus on the carrier properties, cell internalisation and processing, and mechanisms involved in cell toxicity. Novel approaches for CNT-based systemic therapeutic brain delivery following intravenous administration are also reviewed. Moreover, we highlight fundamental questions that should be addressed in future research involving CNTs, aiming at achieving its safe introduction into the clinics.

Contents

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1. Introduction

Carbon nanotubes (CNTs) are defined as cylindrical nanomaterials composed of a continuous, unbroken hexagonal mesh of carbon atoms. The first observation of CNTs by electron microscopy, credited to Iijima in 1991, opened a plethora of applications for this material [1]. This included not only high-strength composites, energy storage, and field emission device, but also the use of CNTs for biomedical applications [2]. In particular, CNT ability to cross efficiently cell membranes and carry a large amount of molecules has encouraged the design of nanotube-based delivery systems [3,4].

The concept of drug delivery was probably introduced by Paul Ehrlich, in 1897, when he theoreticized the use of "zauberkugeln" (in English "magic bullets") intending to improve the efficacy of available therapeutics [5]. Long after this statement, delivery of therapeutic and imaging agents into specific organs or tissues has remained a promising approach to modulate the pharmacokinetics and bioavailability of therapeutics, and provide controlled release kinetics at a target site. Numerous materials with sizes between 10 and 1000 nm have been investigated, including liposomes, dendrimers, nanoemulsions, nanoparticles, quantum dots and CNTs. With their needle-like shape, CNTs display singular physico-chemical properties. Their large surface area, ranging from 50 to 1315 m^2/g, allows the conjugation with extensive amount of therapeutic and imaging molecules [6–8]. Moreover, the high CNT length-to-diameter ratio enables them to efficiently penetrate biological membranes and accumulate into intracellular compartments [9]. Consequently, attachment of molecules to CNTs helps overcoming several administration problems, including insolubility, poor biodistribution and inability of therapeutic or diagnostic molecules to cross cellular barriers [3].

Despite their undeniable potential, concerns have emerged regarding the toxicity of CNTs, as various reports showed that pristine nanotubes could induce biological damage [10]. Excessive nanotube length, the presence of impurities from the synthesis process and the introduction of carboxylic groups at the CNT surface could trigger unattended and detrimental cellular responses [11]. Such parameters must therefore be thoroughly controlled and characterised to design safe and biocompatible nanotubes applicable as delivery systems. The post-synthesis surface modification of nanotubes with hydrophilic molecules, named functionalisation, has been reported as an efficient approach to enhance their water dispersibility and reduce their toxicity [12,13]. This can be performed by covalently attaching moieties at the surface of CNTs or by non-covalent interactions between nanotube surface and hydrophobic/aromatic regions of amphiphilic molecules [14].

To tailor nanotube function, therapeutic molecules or imaging probes can be added to functionalised CNT (*f*-CNT) side-walls [4]. By taking advantage of their inner cavity, *f*-CNTs can also be filled to keep the surface available for further modifications [15]. Contrast agents can be combined to nanotubes to generate CNT-based hybrids with clinical imaging capabilities [16]. If such hybrids display desirable targeting capabilities, they become versatile imaging tools for diagnostic applications [17,18]. CNT hybrids can also help tracking administrated nanocarriers to assess in real-time their spatial distribution and therefore measure their biodistribution profile [19]. The major medical imaging techniques, namely ultrasound, nuclear and magnetic resonance imaging (MRI), display limitation in terms of sensitivity or image resolution. To improve this, the combination of synergistic imaging modalities in a single carrier, such as CNTs, could be particularly valuable [20]. Beyond the promising properties of CNT-based hybrids for multi-imaging capabilities, their dimensions need to be optimised in order to control their intrinsic imaging properties, improve their accumulation in target cells and enhance their biocompatibility profile. This dimension refinement is essential to demonstrate the potential of CNT-based hybrids and confirm their safety before conducting clinical studies.

A wide range of studies have also reported on the development of carbon nanotubes for brain delivery, with results showing that adequate functionalisation is essential to produce biocompatible CNTs capable of local or systemic delivery of therapeutics to brain cells [21].

In this review, a description of the physico-chemical properties and surface modification of CNTs needed for delivery will be presented. Moreover, the interaction between CNTs and mammalian cells will then be described, followed by a summary of their toxicity. Finally, we will look into the most recent advances involving CNT-mediated systemic brain delivery and *in situ* CNT biodegradation.

2. Physico-chemical properties and surface modification of CNTs for biomedical applications

2.1. Synthesis, classification and properties

Carbon nanotubes can be generated by electric arc discharge and laser ablation using vaporisation of graphite target [22,23]. Alternatively, they are synthesised by chemical vapour deposition which rely on the passage of carbon-containing vapours in a furnace containing metal catalysts [24]. CNTs can be classified as single-walled (SWNT) or multi-walled (MWNT) nanotubes, in accordance with the number of layers that compose a single nanotube (Fig. 1).

SWNT and MWNT exhibit a diameter of 0.4–2 nm and 10–100 nm, respectively [26]. Both types are utilised as delivery systems and display large aspect ratios with lengths ranging from 50 nm to several microns. The length and diameter can be tuned by controlling the production conditions, but the design of CNT-based delivery systems requires further post-synthesis shortening procedures to increase their biocompatibility and bioavailability [10,19]. A reduction in the CNT length to diameter ratio can be achieved by strong acid treatment, ultrasonication, steam-purification and mechanical methods [27–29].

The unique physicochemical properties of CNTs, namely high surface area and length-to-diameter ratio, optimal electrical conductivity, and thermo-chemical stability, make them particularly attractive for biomedical applications [30]. However, pristine CNTs must be functionalised to improve their hydrophilicity and biocompatibility.