

Mechanism of Cellular Interaction, Binding and Internalization of Functionalized Carbon Nanotubes

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ABSTRACT

The interaction between cells and carbon nanotubes (CNT) is a critical issue that will determine any future biological application of such structures. In this communication we will show that various types of functionalized-carbon nanotubes (*f*-CNT) exhibit a capacity to be uptaken by a wide range of cells (prokaryotic and eukaryotic) and can intracellularly traffick through different cellular barriers^[1, 2]. The mechanism by which *f*-CNT are able to cross cell membranes and deliver their cargo will also be discussed. Energy-independent mechanisms are explained based on the cylindrical shape and high aspect ratio of *f*-CNT that can allow their penetration through the plasma membrane, similar to a 'nanosyringe'^[3].

Keywords: carbon nanotubes, internalization mechanisms, nanosyringe, transmission electron microscopy

1 INTRODUCTION

The development of nanomaterials for biomedical and biotechnological applications is an area of research that holds great promise and intense interest. CNT are one of the types of nanomaterials thought to lead to novel types of nanomedicines^[4]. One of the key advantages that CNT offer is the possibility to translocate through plasma membranes and allow their utilization for the delivery of therapeutically active molecules in a manner that resembles the widely reported cell-penetrating peptides. Moreover, exploitation of their unique electrical, optical, thermal and spectroscopic properties in a biological context is hoped to yield great advances for diagnostic, imaging and therapeutic proposes. Here we present general principles behind the mechanism of CNT plasma membrane penetration and some of the different therapeutic modalities investigated in our laboratories based on these novel nanostructures.

2 CARBON NANOTUBES (CNT)

One of the most important parameters in *in vitro* and *in vivo* studies with CNT is the type of nanotubes used, which is determined by the process by which they are made biocompatible. Interactions with cells and tissues have to be performed using biocompatible CNT, achieved by either covalent or non-covalent surface functionalisation that results in water-dispersible CNT. A variety of different functionalisation strategies for CNT have been reported by different groups^[5]. In our laboratories, CNT were made compatible with physiological environments after functionalization by the 1,3-dipolar cycloaddition reaction^[6, 7].

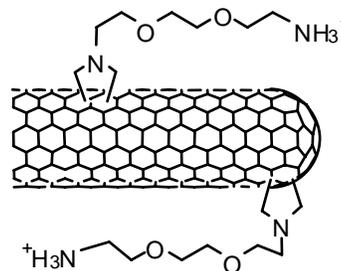


Figure 1. Molecular structure of CNT-NH₃⁺.

The preparation of ammonium functionalized-CNT (CNT-NH₃⁺, Figure 1) consists on the decarboxylation of ammonium salts obtained by the reaction of an α -amino acid with an aldehydes in the presence of CNT. The free amino function increases dramatically the solvent compatibility of both single- and multi-walled CNT (SWNT and MWNT, respectively) which are well dispersed in aqueous solvents and available for further derivatization. This has allowed their exploration as a platform to construct delivery systems for drugs, peptides and nucleic acids *in vitro*^[8] and *in vivo*^[9].

3 CNT-CELL INTERACTION

Once the issue of CNT compatibility with the aqueous biological milieu was adequately overcome, it was possible to explore their interaction with living cells. We have demonstrated that CNT are able to interact with and cross plasma membranes, and traffick within the cell cytoplasm, reaching the perinuclear region (Figure 2)^[2, 10]. Furthermore, the effect of functional group type at the surface of *f*-CNT was also investigated using techniques such as confocal microscopy, flow cytometry and protocols which inhibit energy-dependent internalization mechanisms (incubation at 4°C and addition of sodium azide or 2,4-dinitrophenol to cell culture media). Interestingly, the observations showing the ability of CNT to pierce or penetrate the plasma membrane to a large extent by a process independent of energy were confirmed, regardless of cell type or characteristics (e.g. surface charge) of the functional group attached onto the CNT^[1].

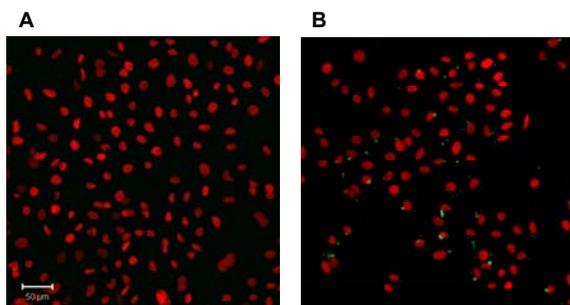


Figure 2: Confocal images of A459 cells incubated for 2 h at 37 °C (5% CO₂) in the absence (A) and presence (B) of SWNT-NH₃⁺ (green signal). Cellular nucleus counterstained in red with PI.

4 “NANONEEDLE” HYPOTHESIS

In Figure 3, a high resolution transmission electron microscopy (TEM) image of MWNT-NH₃⁺ is showing the initial interaction of the nanotubes with mammalian cells. We have observed that the nanotubes adopt a perpendicular orientation towards the plasma membrane of the cells through the process of cellular internalization^[11]. Recently, Lopez et al. have proposed that model nanotube structures can interact with lipid bilayers and cross them via a diffusion process directly through the biomembrane as illustrated in Figure 4^[3, 12]. The authors described the spontaneous diffusion of nanotubes functionalised with hydrophilic termini through lipid bilayer membranes using a molecular dynamics simulation study. These theoretical determinations have shown the implication of the charge interactions on the cell binding and internalization process

by positively-charged CNT. Our experimental data is in agreement with such theoretical simulations^[11].

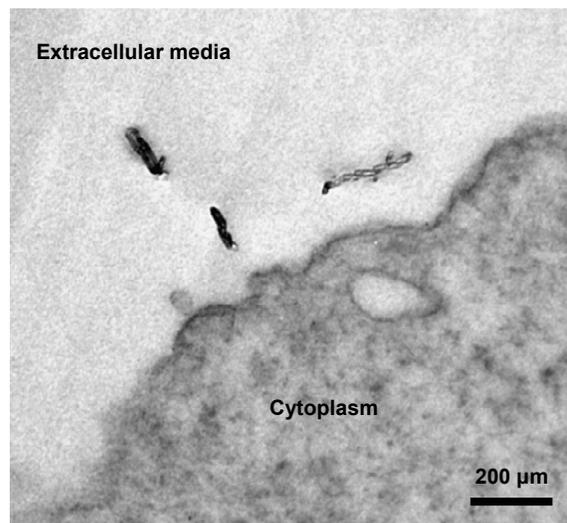


Figure 3: TEM image of A459 cells incubated with MWNT-NH₃⁺ for 1 h at 37 °C (5% CO₂).

Interestingly, spontaneous transmembrane penetration via flipping of membrane lipid molecules is, contrary to endocytosis, an energy-independent process, not dependent on receptor, coat or lipid raft interactions, therefore potentially relevant to all cell types. Furthermore, the hypothesis of *f*-CNT acting as ‘nanoneedles’ on plasma membrane, has recently been shown with CNT functionalized by other method than the covalent 1,3-dipolar cycloaddition reaction: a) non-covalently functionalized block copolymer-coated MWNT^[13], and b) oxidized, water-dispersible CNT^[14]. In summary, the work accumulating gradually by different groups is confirming that novel, very interesting mechanisms other than ‘classical’ endocytosis are contributing to the cellular internalization of CNT.

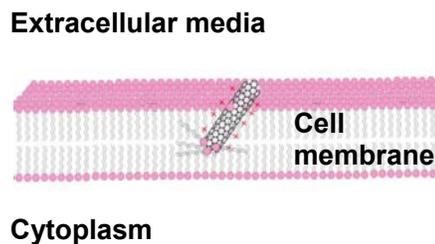


Figure 4. Schematic representation of “nanoneedle” mechanism for *f*-CNT cross cellular membranes.

5 CNT AS DELIVERY SYSTEMS

So far water-dispersible, individualized CNT have shown interesting properties that can be utilized in biomedical applications. Currently CNT are being considered and explored as novel carrier systems of therapeutics and diagnostics because: a) CNT can be internalized by a wide range of cell types; and b) their high surface area can potentially act as a template of cargo molecules such as peptides, proteins, nucleic acids and drugs. CNT have been described as delivery systems in, mainly proof-of-principle, studies for a variety of different biomedical applications^[8].

In our laboratories we have observed that *f*-CNT are able to facilitate the intracellular transport of plasmid DNA^[11, 15], peptides^[10] and drugs^[16, 17]. Therefore, water-dispersible CNT are been considered vectors for gene delivery^[11, 15], scaffolds for the cellular delivery and receptor presentation of immunologically active molecules with the ultimate aim to achieve novel tools for effective vaccination^[18, 19] and smart delivery systems for imaging and therapeutic proposes in cancer^[16].

6 CONCLUSIONS

Different types of CNT with adequate aqueous dispersibility have been shown to interact with various types of cells leading to cellular internalization of CNT. The ability of CNT to internalize different types of cells by different or combinations of mechanisms transform CNT in one of the most promising types of novel nanomaterials into a useful and clinically-relevant biotechnological and biomedical tool.

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