Acta Biomaterialia 49 (2017) 36–44

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Review article

Carbon nanotubes as gene carriers: Focus on internalization pathways related to functionalization and properties

Cécile Caoduro [a], Eric Hervouet [b], Corine Girard-Thernier [c], Tijani Gharbi [a], Hatem Boulahdour [a], Régis Delage-Mourroux [b], Marc Pudlo [c,*]

[a] Nanomedicine, Imagery and Therapeutics Laboratory, EA4662, Université de Bourgogne Franche Comté, F-25000 Besançon, France
[b] Laboratoire de Biochimie, EA3922, Expression Génique et Pathologies du Système Nerveux Central, SFRIBCT FED 4234, Université de Bourgogne Franche-Comté, F-25000 Besançon, France
[c] Fonctions et Dysfonctions Epithéliales, EA4267, Université de Bourgogne Franche-Comté, F-25000 Besançon, France

ARTICLE INFO

Article history:
Received 14 April 2016
Received in revised form 15 September 2016
Accepted 3 November 2016
Available online 5 November 2016

Keywords:
Carbon nanotubes (CNTs)
Functionalization
DNA
Cellular uptake mechanism

ABSTRACT

Carbon nanotubes represent promising transporters for delivery of DNA and other biomolecules into living cells. Various methods of CNTs surface functionalization have been developed. These are essential to improve CNTs dispersibility and permit their interactions with biological structures that broaden their use in advanced biomedical applications.

The present review discusses the different single walled carbon nanotubes and multiwalled carbon nanotubes functionalization methods, leading to the formation of optimized and functionalized-CNT complexes with DNA. F-CNTs are recognized as efficient and promising gene carriers. Emphasis is then placed on the processes used by f-CNTs/DNA complexes to cross cell membranes. Energy independent pathways and uptake mechanisms dependent on energy, such as endocytosis or phagocytosis, are reported by many studies, and if these mechanisms seem contradictory at first sight, a detailed review of the literature illustrates that they are rather complementary. Preferential use of one or the other depends on the DNA and CNTs chemical nature and physical parameters, experimental procedures and cell types.

Statement of Significance

Efficient non-viral gene delivery is desirable, yet challenging. CNTs appear as a promising solution to penetrate into cells and successfully deliver DNA. Moreover, the field of use of CNTs as gene carrier is large and is currently growing. This critical review summarizes the development and evaluation of CNTs as intracellular gene delivery system and provides an overview of functionalized CNTs/DNA cellular uptake mechanisms, depending on several parameters of CNTs/DNA complexes.

Contents

* Corresponding author.
E-mail address: marc.pudlo@univ-fcomte.fr (M. Pudlo).

http://dx.doi.org/10.1016/j.actbio.2016.11.013
1742-7061/

1. Introduction

Carbon nanotubes (CNTs) are emerging nanomaterials with unique dimensions and properties. Specifically Single-walled CNTs (swCNTs) (**1**, Fig. 1) consist of a single sheet of graphene wrapped into a seamless cylinder with a diameter of close to one nanometer and length of fifty nanometers to several microns, whereas multi-walled CNTs (mwCNTs) (**2**, Fig. 1) consist of multiple rolled layers (concentric tubes) of graphene with diameters from one to one hundred nanometers and length of one to ten microns [1,2]. Chemical modifications, improving dispersibility [3–6] and biocompatibility [7] of CNTs, enable their use in a wide range of biomedical applications (for recent reviews on CNT biomedical applications see [8–12]). For example, CNTs have been used as DNA and protein biosensors [13,14], ion channel blockers [15] and for intracellular delivery of bioactive molecules [16,17]. Among these, CNT-based delivery systems (for recent reviews on CNT-based vectors see [18–20]) have been considered with the aim to deliver genetic materials with little immunogenicity. Typical length to diameter ratio and propensity to be modified allow CNTs to transport and release a wide variety of genetic materials in large amounts [16,21]. The use of functionalized CNTs (f-CNTs) (**3**–**7**, Fig. 1) to form CNTs/DNA complexes (**8**–**10**, Fig. 1) has been widely investigated and successful experiments of internalization provide proof of concept. Nevertheless, the extensive literature on the subject reveals a wide disparity in the mechanism of internalization. The mechanism of internalization is yet a key point for design and efficiency of transfection. The chemical and physical properties of CNTs/DNA complexes are greatly influenced by length and functional groups on distinctive CNTs' surfaces and by the different forms of DNA [22,23]. The present review begins with a brief report on the development of CNT-based gene delivery systems, placing emphasis on current studies and different uptake mechanisms proposed to explain penetration of such delivery systems into cells.

2. CNTs as DNA transporters

The side wall of pristine ("as prepared") CNTs may form π-stacking interactions with aromatic nucleotide bases and flexibility of nucleic acid would appear to be a crucial determinant to allow them to maximize interactions with CNTs [11]. Therefore single strand DNA (ssDNA) [6] and small interfering RNA (siRNA) [24] have been shown to bind very strongly to CNTs and the last one has been successfully used to deliver siRNA into cancer cells [24]. Thus, modifications of CNTs are needed to immobilize double strand DNA (dsDNA). In addition functionalization of CNTs improves dispersibility and allows complexation of all types of nucleic acids. Functionalization of CNTs has been widely reviewed (for reviews on the chemical modification of CNTs see [25,26]: and for a review on DNA functionalized CNTs in the field of DNA biosensor see [27]). In the field of CNT DNA vectors, functionalization follows a classical approach. The well-known oxidizing acid treatment is thus the usual way to cut and add carboxylic acid groups on CNTs (oxidized swCNT **3**, Fig. 1) [28]. Covalent binding

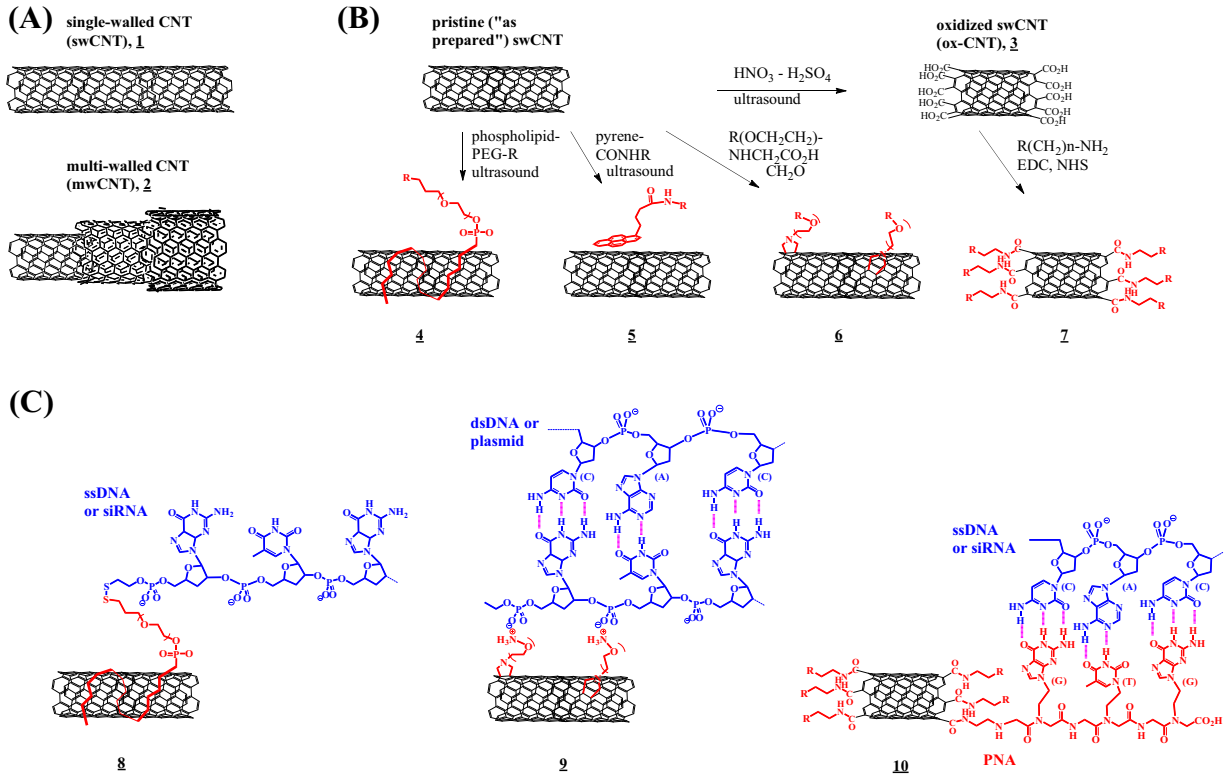

Fig. 1. (A) Single-walled carbon nanotubes (swCNTs) and multi-walled carbon nanotubes (mwCNTs); (B) Selected example of carbon nanotubes functionalization [phospholipid wrapping: [43,42,38,39,14]; (C) Selected example of CNT-based gene delivery system [43,38,39,14]. For clarity, functionalized CNTs and complexes are representing as single walled carbon nanotubes (swCNTs).