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Effects of charge and surface defects of multi-walled carbon nanotubes on the disruption of model cell membranes

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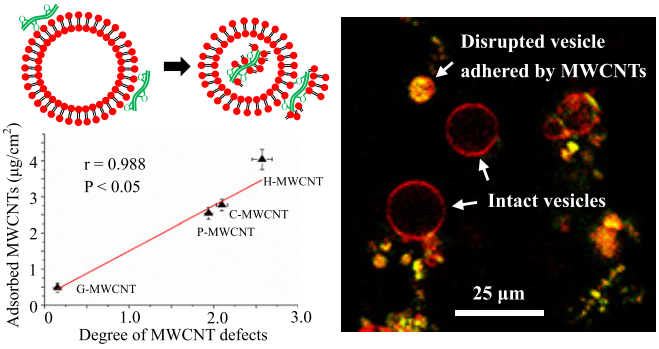
HIGHLIGHTS

- CNT-membrane interaction is electrostatically mediated.
- Lipids in the membrane can be extracted to CNTs.
- Defect sites on MWCNTs correlate with their interaction with membranes.

GRAPHICAL ABSTRACT



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ABSTRACT

The direct contact between multi-walled carbon nanotubes (MWCNTs) and cell membranes causes membrane disruption, potentially leading to cytotoxicity. However, the role of electrostatic forces and MWCNT properties is still open to debate. In this study, the influences of charge and MWCNT surface defects on membrane disruption were investigated by microscopy and a quartz crystal microbalance with dissipation monitoring (QCM-D). Positively/negatively charged giant unilamellar vesicles (GUVs) and supported lipid bilayers (SLBs) were made as model cell membranes. Negatively charged MWCNTs disrupted the GUVs containing positively charged lipids, which confirmed the electrostatically mediated interaction. However, the mass loss was detected from the negatively charged SLBs after MWCNT exposure, which suggests the extraction of phospholipids. The defect degree of MWCNTs correlated with their adhesion amount on the membranes. Both the oxygenated functional groups and unoxidized dangling carbon bonds were active sites for MWCNT-membrane interactions. The MWCNTs were observed to be engulfed inside the GUVs. The results clearly demonstrate that phospholipid extraction by MWCNTs could occur in electrostatically repulsive conditions, and MWCNT defects were active binding sites whether or not they were oxygenated. Our findings should be helpful in the design and safe applications of carbon nanomaterials.

1. Introduction

Carbon nanotubes (CNTs) possess remarkable electronic, mechanical and thermal properties (Milowska and Majewski 2013) and are

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therefore excellent candidates for many potential applications in capacitors, biosensors, drug carriers, medical imaging agents, cancer diagnosis, electroporation, and tissue engineering (Barinov et al. 2009; Mu et al. 2009a; Geng et al. 2014; Abdolahad et al. 2012, 2013; Cai et al. 2008; Tonelli et al. 2012). Although carbon-based nanomaterials are widely considered as safe materials with promising prospects in medical or clinical applications (Tonelli et al. 2012), there are still many reports on the cytotoxicity of CNTs (Pasquini et al. 2012; Zhang et al. 2012a; Shen et al. 2009) and their harmful effects on cellular physiological activities (Liu et al. 2012; Bhattacharya et al. 2013). One particular type of CNTs possibly poses a greater health risk. Greater toxicity of MWCNTs is typically observed when the CNTs were uncapped, needle-like, debundled, short, and/or dispersed in a solution (Kang et al. 2008; Akhavan et al. 2011; Palomäki et al. 2011). Therefore, the physiochemical and structural characteristics of CNTs (including the surface chemistry, functional group density, residual catalyst contamination, shape, length and diameter) can affect their toxicity through the direct contact of the cells and the CNTs (Kang et al. 2008; Akhavan et al. 2011; Palomäki et al. 2011) resulting in the rupture of the cell membrane and harmful inflammatory effects.

The cytotoxicity of CNTs typically starts from their adhesion to cell membranes and their consequent internalization into cells (Mu et al. 2009b; Chen and Bothun 2013). The surface charge of nanoparticles (NPs) has been proposed to play a leading role in their interaction with membranes (Xiao et al. 2012). Cationic NPs are well known to induce membrane leakage and cytotoxicity through binding to net negatively charged cell membranes (Leroueil et al. 2008; Verma and Stellacci 2010; Li and Malmstadt 2013). However, studies on the effects of negatively charged NPs on membranes have presented inconsistent results. Anionic carboxylic acid modified NPs show an absence of interaction with neutral/negatively/positively charged membranes (Laurencin et al. 2010) and have negligible effects on membrane depolarization (Arvizo et al. 2010). Conversely, negatively charged Au and TiO_2 NPs induce the leakage of negatively charged lipid bilayer vesicles (Montis et al. 2014; Moghadam et al. 2012). In another study working on carboxylic acid modified Au NPs and quantum dots, positively charged lipids in the membrane were necessary for negatively charged NPs to cause membrane disruption (Xiao et al. 2012). CNTs are typically negatively charged due to their oxygenated surface groups (—COOH, —OH, —C—O—C—, etc.) or protein binding in biological fluids (Mu et al. 2009b), which results in mechanistic uncertainty with respect to their interaction with cell membranes. Computer simulations suggest that CNTs lead to perturbations of membrane and affect cell structure (Parthasarathi et al. 2012; Baoukina et al. 2013). CNTs can attach to the lipid membrane in the presence of cations (Yi and Chen 2013a). Moreover, CNTs are suggested to insert into the membrane and form transmembrane channels revealed by the increased transmembrane current fluxes, which indicates membrane disruption (Corredor et al. 2013). A recent study demonstrated that CNT adhesion fluidizes bacterial membrane, which was revealed by changes in the polarization ratio possibly due to disruption and destabilization of the membrane structure (Zhu et al. 2014). However, it is still debated how the CNTs interact with lipid membranes in both electrostatic attractive or repulsive conditions. The role of charged lipid groups in CNT-membrane interactions has not been well addressed. A direct observation of CNT-induced membrane disruption and a quantification of CNT adhesion on membranes will greatly help understand the process.

Another unclear issue is the role of CNT surface defects in their binding to cell membranes. CNT defects include incomplete bonds, surface functionalities, sp^3 hybridized carbon atoms and ring shapes other than hexagon (Charlier 2002), which can form during the manufacture and the consequent chemical functionalization (Maciejewska et al. 2014). CNT surface defects are proposed to affect CNT cytotoxicity. The mechanisms are commonly attributed to the existence of functional groups. However, the CNT functionalization can either reduce (Sayes et al. 2006) or increase its cytotoxicity (Magrez et al. 2006; Zhang et al. 2011). The enhanced CNT hydrophilicity and opportunity to be internalized by cells have been suggested to increase the cytotoxicity (Magrez et al. 2006; Zhang et al. 2011), but the cellular uptake of CNTs also has been reported to be independent of functional groups (Kostarelos et al. 2007). In addition to functional groups, unoxidized dangling carbon bonds have been suggested to cause acute lung toxicity (Muller et al. 2008). Although defect-induced toxicities have been reported, the role of CNT defects in membrane damage is largely unknown. Specifically, no experiments have been conducted to explore the influence of CNT defects on membrane adhesion.

Considering the present knowledge limitations with respect to CNT-membrane interactions, this research aims to conduct systematic research to analyze the impacts of MWCNTs on both positively and negatively charged membrane, to evaluate the influence of MWCNT surface defects, and to better understand the process of membrane disruption. Giant unilamellar vesicles (GUVs) and supported lipid bilayers (SLBs) were used as model cell membranes. MWCNT adhesion on SLBs can be monitored quantitatively using a quartz crystal microbalance with dissipation monitoring (QCM-D) (Yi and Chen 2013a). MWCNT-induced GUV disruption process can be imaged in situ under microscopy (Li and Malmstadt 2013; Montis et al. 2014; Wei et al. 2015), which has not yet been applied to study CNT-membrane interactions. Moreover, model cell membranes exclude uncertainties in living cells and clarify the effects of CNT-membrane physiochemical interactions (Chen and Bothun 2013; Laurencin et al. 2010; Moghadam et al. 2012). Understanding the interactions between carbon nanomaterials and biological membranes is important for their future production and application.

2. Experimental section

2.1. Materials

Pristine MWCNTs (P-MWCNTs), graphitized MWCNTs (G-MWCNTs have the greatest graphite crystallization), hydroxylated MWCNTs (H-MWCNTs) and carboxylated MWCNTs (C-MWCNTs) were supplied by Chengdu Organic Chemistry Co. Ltd., Chinese Academy of Sciences. Bovine serum albumin (BSA) was purchased from Sigma-Aldrich. Fluorescent dye fluorescein isothiocyanate (FITC) was purchased from Xiya Reagent, China. The phospholipids 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-[phosphor-*rac*-(1-glycerol)] (sodium salt) (DOPG), 1,2-dipalmitoyl-3-trimethylammonium-propane (chloride salt) (16:0 TAP) and the fluorescent probe 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (RhB-PE) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). The lipid molecular structures are provided in the SI.

2.2. Characterizations of MWCNTs

The morphologies and length distributions of untreated and BSA-stabilized MWCNTs (BSA-MWCNTs) were imaged using transmission electron microscopy (TEM, JEM-1011, JEOL, Japan) at the electron emission of 100 kV. The MWCNT suspension was added to the copper grid, and the sample was dried using an infrared lamp. Surface oxygen and carbon atomic percentages were analyzed using X-ray photoelectron spectroscopy with an Al Kα X-ray source (XPS, ESCALAB 250, Thermo Fisher Scientific, USA). The electrophoretic mobility of the MWCNTs was measured in 0.1 *M* glucose at pH 6.5 on a Malvern Zetasizer (Nano ZS90, Malvern Instruments, UK). The degree of the MWCNT structural defects was assessed using a Raman spectrometer (LabRAM HR 800, HORIBA Jobin Yvon, France) (Osswald et al. 2007; Fenoglio et al. 2008). Samples of four different MWCNTs were placed on a microscope slide, and the Raman spectra were collected at the excitation wavelength of 633 nm. Specific surface areas (SSA) of the MWCNTs were calculated using the multi-point Brunauer-Emmett-Teller (BET) method from the N_2 adsorption isotherms at 77 K by use of a NOVA 2000e instrument (Quantachrome, USA). The metallic impurities in