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Chemical modification of multiwalled carbon nanotube with a bifunctional caged ligand for radioactive labelling

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Abstract

Carboxyl-functionalized multiwalled carbon nanotubes (MWCNTs) have been successfully radiolabelled with cobalt-57 (^{57}Co) ($T^{1/2} = 270$ days) via the attachment of the bifunctional caged ligand MeAMN$_3$S$_3$sar. In this study MeAMN$_3$S$_3$sar has been synthesized and coupled to MWCNTs to form the conjugate MWCNT–MeAMN$_3$S$_3$sar. Synthesis was confirmed with nuclear magnetic resonance. X-ray photoelectron spectroscopy (XPS) confirmed the conjugation. Non-radioactive labelling of this conjugate was completed with Cu(II) ions to confirm the stability of the MeAMN$_3$S$_3$sar after coupling with the MWCNTs. The complexation of the Cu(II) was also confirmed with XPS. Transmission electron microscopy was used to demonstrate that the coupling reaction had a negligible effect on the size and shape of the MWCNTs. Radiolabelling of the MWCNT–MeAMN$_3$S$_3$sar conjugate and pristine (untreated) MWCNTs (non-specific) with the gamma-emitting radioactive isotope ^{57}Co were compared. The radiolabelling efficiency of the MWCNT–MeAMN$_3$S$_3$sar conjugate was significantly higher (95% vs. 0.1%) ($P \leqslant 0.001$) than for the unconjugated pristine MWCNTs. This will allow for the potential tracking of nanoparticle movement in vitro and in vivo.

Keywords: Multi-walled carbon nanotube; Radioactive labelling; Bifunctional caged ligand; Nanoparticle tracking

1. Introduction

In recent years there have been increased efforts to determine how artificially introduced nanoparticles interact with human tissues and systems in vivo, with an emphasis on tracking their movement. Although nanoparticles are used in a number of consumer products, including photocatalysis [1], cancer therapy [2–6] and composites [7–20], not enough is understood about their biological interactions and toxicity. Such biological responses may be dependent on the surface chemistry of a nanoparticle, in addition to their size, shape and chemical composition. The ability to predict the relationship between physicochemical parameters and biological systems has proven to be a very complicated and challenging assignment to date. To further the understanding of nanoparticle interactions with biological systems, it is important to develop highly sensitive, reliable and robust methodologies to label such nanoparticle systems.

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Radioisotopic (active) labelling is one such method offering unparalleled detection sensitivity and compatibility with nanoparticle synthesis methodologies. The attachment of radioactive metal ions to multiwalled carbon nanotubes (MWCNTs) could potentially allow for labelling and tracking. One type of functionalized carbon nanotube (CNT) previously explored in biomedical applications is based on the covalent surface modification developed by Kostarelos et al. [21]. These CNTs have been studied for various applications, including imaging using various radionuclides (^{111}In, ^{86}Y) [22–24]. Another type of functionalized CNT that has been explored in vivo is based on the chemical modification of carboxylic acid groups after strong acid treatment. These CNTs have also been studied using tracing radionuclides (^{125}I, ^{14}C) [25,26]. However, in order to track CNT movement within a polymer matrix it is essential that in designing these labelling techniques, it can be demonstrated that the label is stable and that the native physical characteristics of the nanoparticles are not altered. Furthermore, these labelling techniques may then be used to monitor nanoparticle transport and interactions in typical, but realistic environments. The attachment of a radioactive metal ion to nanoparticles can be achieved by either direct labelling (where a radio-emitting element is inserted into the MWCNT structure) [27], or with the use of a bifunctional ligand. Direct labelling is difficult to control and may lead to unplanned changes to the geometry and behaviour of the nanoparticles. A more suitable technique (in theory) for attachment of radioactive metal ions to MWCNTs is via a bifunctional ligand. Such ligands offer a highly favourable three-dimensional, cage-like structure capable of the stable encapsulation of metal ions. In addition, the ligands are also able to readily bind to the target nanoparticles through a favourable functional group [27,28]. For example, the bifunctional caged ligand $MeAMN_3S_3sar$ has an aromatic amine (—NH_2) functional group attached, which would allow for coupling with nanoparticles that have a carboxyl (—COOH) group on the surface (Fig. 1). To achieve a high conjugation efficiency of the bifunctional ligand to a MWCNT, the ligand has to be free from any co-products formed during the reaction [29].

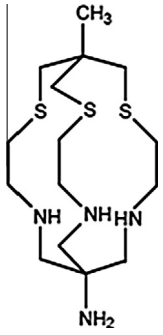

Fig. 1. Schematic diagram of the $MeAMN_3S_3sar$ bifunctional cage ligand [27].

The potential to radioactively label and track MWCNTs is of particular interest. Previously, we have demonstrated that the incorporation of MWCNTs of varied chemical functionality to polymethyl methacrylate (PMMA)-based bone cements can significantly augment the mechanical and thermal properties of the resultant composite cement [30–32]. Although our previous work has shown that the MWCNT–PMMA bone cements did not invoke a cytotoxic or negative response when in contact with osteoblast-like cells [33], it is still crucial to understand the potential distribution of these MWCNTs within the human body. Therefore, the aims of this study were twofold: (i) to radioactively label MWCNTs with cobalt-57 (^{57}Co) isotopes via coupling the MWCNTs with a bifunctional caged ligand ($MeAMN_3S_3sar$); and (ii) to assess the radioactive retention of the radioactively labelled MWCNTs.

2. Experimental procedures

2.1. Materials preparation

2.1.1. MWCNTs

The MWCNTs used in this study were carboxyl-functionalized (MWCNT-COOH) (4 wt.% COOH concentration) MWCNTs (Nanocyl SA, Belgium). These MWCNTs were all grown using chemical vapour deposition and had average diameters of 9.5 nm and average lengths of $\leqslant$1 μm [34].

2.1.2. Synthesis of the bifunctional caged ligand MeAMN3S3sar

The Co(III) complex $[Co(MeNH_3N_3S_3Sar)]Cl_4{\cdot}2H_2O$, and the free ligand $MeAMN_3S_3sar$ were prepared as described previously [27–29]. The desired bifunctional caged ligand $MeAMN_3S_3sar$ was isolated as a white solid. ^{13}C nuclear magnetic resonance (NMR) (D_2O, 1,4-dioxane): $\delta = 34.67, 40.39, 49.23, 54.91$ (—CH_2—), 42.64, 56.91 (C_q), 28.34 ppm (CH_3); mass spectroscopy (MS) (electron impact (EI), theoretical): *m/z*: 364.18; found: 365.23 $[MeAMN_3S_3sar]^+$; elemental analysis calculated (%) for $C_{15}H_{32}N_4S_3{\cdot}0.5H_2O$: C48.22, H8.90, N14.99, S25.75%; found: C48.23, H8.97, N14.82, S25.95.

2.1.3. Coupling MWCNTs with the bifunctional caged ligand MeAMN3S3sar

The COOH functional groups on the surface of the MWCNTs were coupled with the NH_2 functional group on the caged ligand to form a stable covalent bond. This was completed by an esterfication reaction using the coupling agents N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC) and N-hydroxysuccinimide (NHS) (all chemicals supplied by Sigma–Aldrich, UK unless otherwise stated) to form a stable active ester bond (Fig. 2).

The methodology described is a modified version of the technique used by Jiang et al. [35] for coupling proteins to MWCNT-COOH. This was achieved by suspending 20 mg MWCNTs in 60 ml 0.1 M MES-buffered solution (pH 6.2) via sonication at an amplitude of 10 mA for 4 min. This