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Short communication

Covalent functionalization of hybrid multi-walled carbon nanotube-graphene with polyethileneglycol for targeted delivery of Temozolomide

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Introduction

The limited bioavailability and intrinsic toxicity of drugs force continually pharmaceutical industry to design and develop novel drug carriers in order to improve physicochemical properties of the drugs and overcome the physiological and (bio)chemical barriers. Carbon nanostructures such as multi-walled carbon nanotube-graphene (MWCNT-G) hybrids, with their nanoscale structure and propensity to functional modification are useful for controlled drug delivery (Wu et al., 2013). However, the poor solubility and cellular toxicity restrict their use in this field. Their functionalization is required to improve their solubility and biocompatibility, alter their cellular interaction pathways and reduce their cytotoxic effects. The covalent attachment of polyethylene glycol (PEG) to their surface is one of the emerging techniques to increase their application. PEGylated carbon nanostructures display lower cytotoxicity and longer blood circulation half-life by which their *in vivo* opsonization is impeded and the uptake by reticuloendothelial system reduced (Ravelli et al., 2013). In addition, their high-surface-area-to volume-ratio facilitates drug encapsulation.

Having this in regard, the current research is focused on the suitability of covalently PEGylated MWCNTs-G as carriers of temozolomide (TMZ), imidazotetrazine second generation agent and first line alkylating and radiosenzitizing agent used for treatment of the most aggressive brain tumors such as glioblastoma multiforme and anaplastic astrocytoma.

Materials and methods

MWCNTs-G were purchased from Incubation Alliance, Inc., Japan and then activated to MWCNTs-G-COOH in the presence of 8 M HNO_3. MWCNT-G-COOH were functionalized using PEG6000 (av. Mw 5000-7000 g/mol, Merck Schuchardt, OHG, Germany) following the procedure of Abdel Salam and Burk (2012). For the drug encapsulation, solution of TMZ was added to the suspension of MWCNTs-G-PEG in acidified water (pH 2.5), ultrasonicated for 1h and stirred for 72h. Afterwards the MWCNTs-G-PEG-TMZ were isolated by ultra-centrifugation, rinsed with double-distilled water and dried at room temperature.

The characterization of the blank and TMZ loaded carbon nanostructures was performed using thermogravimetric analysis (TGA) (Pyris 1 TGA, PerkinElmer, Shelton, CT, USA), infrared spectroscopy (IR) using KBr pellets (PerkinElmer 2000 FT-IR; Waltham, MA, USA), UV-VIS spectroscopy (Perkin Elmer Lambda 16, USA) and scanning electron microscopy (SEM) (FEI Quanta 200, acceleration voltage 30 kV, EDS Oxford Inca Energy 350, UK). Encapsulation efficacy (EE) was determined as a difference between the total amount of TMZ in the initial solution and filtrate (UV/VIS, λ=328 nm, Perkin Elmer Lambda 16, USA). Size distribution and zeta potential were determined by NanoZS-100 (Malvern Instruments Ltd., UK) after dilution of blank and MWCNTs-G-PEG-TMZ in PBS 7.4 (0.0001M).

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Results and discussion

Biopharmaceutical characterization of blank and TMZ loaded MWCNTs-G-PEG

EE of MWCNTs-G-PEG-TMZ was 33 ± 4% (n=6), while the DC was 17% (n=6) out of 25% (theoretical value). The pristine MWCNTs-G had a zeta potential of −26 *mV*, which understandably increased to –46 *mV* when MWCNTs-G were activated to MWCNTs-G-COOH. The PEGylated MWCNTs-G showed less negative zeta potential (−30 *mV*) since the PEGylation converts the carboxylic acid groups into ester bonds. Nonsignificant change in zeta potential alteration was observed between the blank and TMZ loaded MWCNTs-G-PEG (−34 *mV*), which attributes to the encapsulation of TMZ and the formation of new carboxyl groups on the surface in the medium in which TMZ was loaded. The average particle size (d50) of MWCNTs-G-COOH, blank MWCNTs-G-PEG and loaded with TMZ was 133 nm, 186 nm and 218 nm, accordingly, confirming the dominant localization of TMZ into the hybrid structure (as SEM image shows). In all series, the PDI was not higher than 0.400, which indicates homogenous particles distribution.

Physicochemical characterization of blank and TMZ loaded MWCNTs-G-PEG

TGA was performed on MWCNTs-G-COOH, MWCNTs-G-PEG and MWCNTs-G-PEG-TMZ at a heating rate of 10 °C/min. The weight of MWCNTs-G-COOH decreased with increasing temperature, but the weight loss at 800 °C was insignificant (ca. 25%) compared to the one of PEGylated particles (ca. 80% at 800 °C) due to decomposition of PEG. For comparison, pure PEG weight decreased sharply with increasing temperature and reached 98% weight loss near 400 °C. For TMZ loaded MWCNTs-G-PEG the weight loss at 800 °C was 30%.

In the IR spectra of MWCNTs-G-COOH a characteristic peak for the carbonyl C=0 at 1730 cm^{-1} was observed, which was not present in the IR spectra of the PEGylated carbon nanostructures (MWCNTs-G-PEG). Also, in the IR spectra of MWCNTs-G-PEG, the peak at ca.3450 cm^{-1} (characteristic of an H bonded O-H stretch) became more pronounced due to the hydroxyl group present in the PEG. Also, a peak at 1100 cm^{-1} was present, corresponding to the C-O stretch of the ether group of PEG (the same peak appeared in the pure PEG). Two more strong peaks appeared between 2800 cm^{-1} - 3000 cm^{-1} due to the C-H stretching in the PEG chain. The same peaks were less intense compared to those in pure PEG, probably because of low amount of PEG in the functionalized carbon nanostructures i.e. small part of functionalized surface. In the IR spectra of MWCNTs-G-PEG-TMZ, the characteristic peaks of PEG were present, however, the ones of TMZ were not visible, which can be explained by relatively low DC. However, in the UV-VIS spectra of MWCNTs-G-PEG-TMZ (0.1 mg/mL in distilled water), two peaks at 255 nm and 328 nm were observed corresponding to the active hydrolytic metabolite 5-(3-methyl triazen-1-yl) imidazole-4-carboxamide (MTIC) of TMZ and the prodrug TMZ, respectively. These peaks do not appear in the blank MWCNTs-G-PEG.

The morphological study revealed that the outer diameter of the MWCNTs-G nanostructures significantly increased and their surface became non-uniform after they were covalently functionalized with PEG. From the images of TMZ loaded MWCNTs-G and MWCNTs-G-PEG one can reveal that the TMZ is dominantly localized inside the tubes, but also wrapped around the hybrid structure.

Conclusion

In this study, MWCNTs-G were functionalized with PEG and successfully loaded with TMZ. Functionalization was confirmed using different techniques. Biopharmaceutical properties TMZ loaded MWCNTs-G-PEG are suitable for effective drug delivery in brain tumor cells.

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