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

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INTRODUCTION

Carbon nanostructures such as multi-walled carbon nanotube-graphene (MWCNTs-G) hybrids, with their nanoscale structure and propensity to functional modification are useful for controlled drug delivery in CNS (Wu et al., 2013). Their functionalization with polyethylene glycol (PEG) improves their solubility and biocompatibility, alters their cellular interaction pathways, reduces their cytotoxic effects, uptake by reticuloendothelial system and prolongs the blood circulation time (Ravelli et al., 2013). Having this in regard, the current research is focused on the suitability of covalently PEGylated MWCNTs-G as carriers of Temozolomide (TMZ), first line alkylating and radiosenzitizing agent used for treatment 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 8M 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 72 h. The characterization of the blank and TMZ loaded carbon nanostructures was performed using thermogravimetric analysis (Pyris 1 TGA, PerkinElmer, Shelton CT, USA), infrared spectroscopy using KBr pellets (PerkinElmer 2000 FT-IR; Waltham, MA, USA), UV-VIS spectroscopy (Perkin Elmer Lambda 16, USA), scanning electron microscopy (FEI Quanta 200, acceleration voltage 30 kV, EDS Oxford Inca Energy 350, UK), size distribution and zeta potential meters (NanoZS-100, Malvern Instruments Ltd., UK).

RESULTS AND DISCUSSION

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

Encapsulation efficacy (EE), drug content (DC), particle size distribution and zeta potential of covalently PEGylated carbon nanostructures are presented in Table 1. The pristine MWCNTs-G had a negative zeta potential, which negative value additionally increased when MWCNTs-G were activated to MWCNTs-G-COOH. The PEGylated MWCNTs-G showed less negative zeta potential since the PEGylation converts the carboxylic acid groups into ester bonds. Non-significant difference in zeta potential between the blank and TMZ loaded MWCNTs-G-PEG attributes to the formation of new carboxyl groups on the surface in the medium in which TMZ was loaded. In all series, the PDI was not higher than 0.400, which indicates homogenous particles distribution.

Table 1. EE, DC , PDI and zeta potential of covalently PEGylated carbon nanostructures

Series	EE/DC (theor. 25%) (± SD, n=6) %	d50 /PDI (nm)	Zeta potential (n=6) mV
MWCNTs-G	/	1043 nm/0.389	-26 *mV*
MWCNTs-G-COOH	/	133 nm/0.365	-46 *mV*
MWCNTs-G-PEG6000	/	186 nm/0.395	-30 *mV*
MWCNTs-G-PEG6000-TMZ	33±7.46 17±3.60	218 nm/0.379	-34 *mV*

The morphological study revealed that the outer diameter of the MWCNTs-G nanostructures 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.

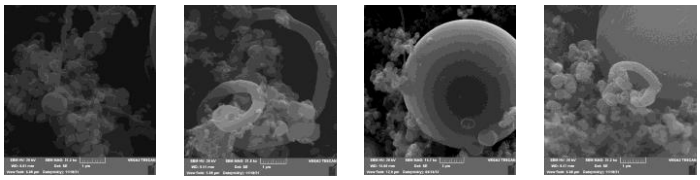

Figure 1. SEM images of MWCNT-G-COOH, MWCNT-G-TMZ, MWCNT-G-PEG6000 and MWCNT-G-PEG6000-TMZ

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

The weight loss of MWCNTs-G-COOH 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 MWCNTs-G-PEG-TMZ the weight loss at 800 °C was 30%.

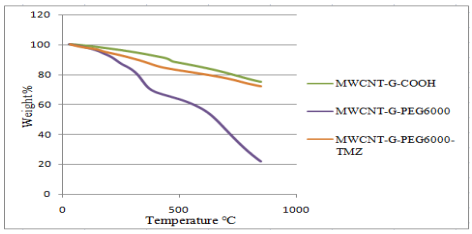

Figure 2. TGA plot of MWCNT-G-COOH, MWCNT-G-PEG6000 and MWCNT-G-PEG6000-TMZ

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 H bonded O-H stretch) became more pronounced due to the hydroxyl group present in the PEG. A peak at 1100 cm^{-1} was also 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.

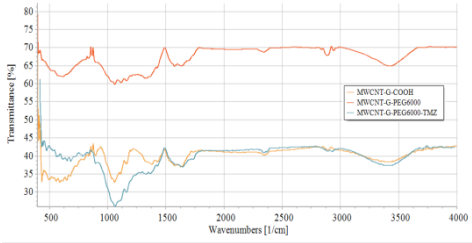

Figure 3. IR spectra of MWCNT-G-COOH, MWCNT-G-PEG6000 and MWCNT-G-PEG6000-TMZ

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 metabolite5-(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.

CONCLUSION

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.

REFERENCES

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