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Highly biocompatible carbon nanocapsules derived from plastic waste for advanced cancer therapy

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ABSTRACT

Carbon nanotubes (CNT) are increasingly being investigated for their use in biomedical applications and nanomedicine. In order to be used as nano-carriers for delivering anti-cancer drugs (drug delivery), smart free standing carbon nanotubes (CNTs) with hydrophilic core were prepared from recycled plastic bags. These CNTs were loaded with doxorubicin (Dox) and its external surface was chemically functionalized with a biodegradable polymer (chitosan) by anchoring its polymeric chains to functional groups on the external surface of CNTs. The obtained Chitosan-coated CNTs (Ch-CNCs) nanocomposites were then tested for their localized and slow drug eluting property using the cellular vicinity of MDA-MB-231 TXSA, human breast cancer cell line. The preliminary results are very promising and confirm a 500 fold enhanced death rate in case of cells treated with Ch-CNCs compared to the pro-drug alone. This work shows that it is possible to develop a highly biocompatible carbon nanocapsules (CNCs) derived from plastic bags and further used them as cargo transporters to study an intra-tumoral delivery of an anti-neoplastic drug is possible.

1. Introduction

Cancer is the leading cause of death worldwide and has enticed researchers worldwide to look for better and advanced treatments as that to chemo and radiation therapy. Lack of specificity and high toxicity has been the major drawback of the general chemotherapeutic procedure. The ideal cancer drug delivery systems combine targeted delivery with controlled release to deliver and release in a selective fashion to the target cells [1–4]. Such systems not only improve the efficacy of the drug, but also reduce the toxic side effects of the drug. In recent years, a wide range of different nanoscale drug carriers have been developed and explored [5,6]. Notably, single wall carbon nanotubes (SWCNTs) have emerged as strong potential candidate due to their advantages as a high cargo loading, intrinsic stability and structural flexibility, over the more widely studied metal nanoparticle systems, which could prolong the circulation time and enhance the bioavailability of the therapeutic agent [7–16]. Moreover, SWCNTs have been shown to enter mammalian cells and thus investigated as potential delivery vehicles for intracellular transport of nucleic acids, proteins and drug molecules. Furthermore, SWCNTs have been functionalized with antibodies as targeting agents providing a high efficiency for nanotube internalization into cells. On the other hand, SWCNTs have also disadvantages relative to MWCNTs such as their higher tendency to aggregate into bundles that may be related to cytotoxicity if not controlled [17–23]. However, many groups have

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reported that the agglomeration of SWCNT in the air-ways is the primary cause of morbidity and granuloma formation. Drug molecules such as doxorubicin (DOX) can be loaded onto CNTs via hydrophobic and π - π stacking interactions and the release rate can be controllable by using nanotubes with different diameters [24,25]. However, inherent properties of SWCNTs such as poor aqueous solubility and tendency to form bundles limited their use for pharmacological applications. To overcome these drawbacks, a number of synthetic and natural polymers have been used to encase SWCNTs via non-covalent interactions to improve their compatibility with water and physiological environments. On the other hand, beside polymer attachment, also covalent functionalization is widely used to avoid the cytotoxic effect of CNTs [26–31]. Indeed; surface engineering of CNTs by covalent and noncovalent modifications enables site-specification drug delivery and targeting. The non-covalent functionalization is based on Van Der Waals, hydrophobic or the ability of the extended π-system of the carbon nanotube sidewall to bind guest molecules via π-π-stacking interactions. Its advantage depend on the preservation of the properties of the CNTs, while its disadvantage concerns weak forces between wrapped/coupled molecules that may lower the load transfer in the composite. The covalent functionalization can be performed at the sidewall site of CNTs or at the defect sites usually located at the tip. New properties can be added by this means of functionalization. Instead, electronic properties of SWCNTs are perturbed by covalent functionalization and double bonds are irreversibly lost. This may affect conductive property, preventing further CNT applications. Despite excellent progress in using SWCNTs as drug carriers, more research is needed to further optimize their ability to selectively accumulate in diseased tissues and release the pay load in a controlled manner. Notice that, most of the aforementioned studies using CNTs for drug delivery application are based on single walled CNTs and CNTs prepared using catalyst based CVD methods. This fabrication suffers from the difficulty in controlling the thickness of the CNTs wall and thus the nanotube diameter, shape morphology and importantly presence of metal catalyst particles from conventional CNTs (i.e. SWCNTs and catalyst based CNTs) restrict their use as drug delivery systems. Due to the aforementioned reasons, the toxicity of these CNTs is still a highly debatable issue. In addition, all the above studies target systemic route of therapeutic administration with no reports on the use of CNTs for local drug administration. In recent studies based on template synthesis of nanotubes for drug delivery, the focus has been on uniformity of these carriers so as to easily internalize into a cell and facilitate an apt calculation for the authenticity of the method. In this work, we have performed an innovative CVD approach to synthesize carbon nanotubes by using nanoporous anodic alumina membranes as templates and commercially available plastic bags as a carbon source without need to use any metal catalysts or solvent. This can prevent and reduce many synthesis disadvantages (use of poisonous chemical metal compounds and the expensive chemicals and production of commercial products as separation/filtration membranes) which make it a potential nanotechnological recycling approach, which could contribute directly to the conservation of our natural ecosystems in the near future. However, the principal aim of this work is to investigate and exploit the role of CNTs as an optimal drug carrier in the field of localized drug delivery. The localized and sustained delivery of an antineoplastic drug, doxorubicin (Dox), has been evaluated in this *in vitro* study. These chitosan-coated CNTs in their pristine form (i.e. without drug inside) were also tested on both, human breast cancer and human foreskin fibroblast cell lines in order to investigate the toxicity of these carbon based materials where hence concluding them to be an ideal intra-cellular drug transporter for breast cancer treatment.

2. Experimental section

2.1. Synthesis of carbon nanotubes (CNTs) or chitosan-coated CNTs

CNTs were prepared as following.

2.1.1. Nanoporous anodic alumina (NAA) template fabrication

NAATs were prepared using a two-step anodization with a constant voltage of 40 V for 20 h in 0.3M oxalic acid at 5 °C temperature as described in Altalhi et *al.* [32] Briefly, high purity Al chips (i.e. 1.5 cm in diameter) were cleaned under sonication in ethanol (EtOH) and double distilled water and subsequently electropolished in a mixture of perchloric acid ($HClO_4$) and EtOH 1:4 (v:v) at 20 V and 5 °C for 3min. Then, the 1st anodization step was carried out in an aqueous solution of oxalic acid ($H_2C_2O_4$) 0.3M at 40 V and at 6 °C for 20 h. Then, the resulting alumina (Al_2O_3) layer was selectively dissolved by wet chemical etching in a mixture of phosphoric acid (H_3PO_4) 0.4M and chromic acid (H_2CrO_7) 0.2M at 70 °C for 3 h. Next, the 2nd anodization step was performed under the same anodization conditions as the 1st one for 17 h. Once the anodization process finished, the remaining aluminium substrate was removed in a saturated solution of hydrochloric acid and cupric chloride ($HCl/CuCl_2$). Subsequently, a pore opening process was performed by wet chemical etching in an aqueous solution of H_3PO_4 5 wt% at 35 °C under current control conditions [33].

2.1.2. Synthesis of chitosan-coated CNTs

Carbon nanotubes (CNTs) were fabricated as reported by AlTalhi et al. [32] using template and catalyst free CVD synthesis inside nanoporous anodic alumina membranes (NAAMs). The fabrication process was carried out using a CVD system consisting of a two-stage furnace equipped with a cylindrical quartz tube with dimensions (43 and 1000 mm in diameter and length, respectively) and temperature and gas flow controllers. Particularly, shopping plastic bags were collected from a local grocery that are typically produced from linear low-density polyethylene (LLDPE) and used them as a carbon source to produce CNTs. After collection, these plastic bags were washed with a liquid soap and double distilled water, dried under nitrogen stream, cut into small squares of 1 cm^2 and kept in a container with inert atmosphere to prevent them from contaminations before the fabrication process. Then, these small pieces of plastic bag were placed in a ceramic crucible, which was introduced into the pyrolysis zone of the CVD reactor. To ensure the absence of oxygen during the CNTs synthesis, Argon inert (Ar) gas was flowed at 1 dm^3 min^{-1}. In the deposition zone of the CVD reactor, prepared NAAMs were placed where the carbon deposition took place. We performed the fabrication process at 850 °C and 30 min.

After CNTs synthesis, the CVD reactor was cooled down to room temperature. The resulting CNTs–NAAMs were saved in a container under inert conditions. An additional annealing process was used in some CNTs–NAAMs samples by heating under Ar atmosphere at 900 °C for 3 h. The obtained CNTs were followed by being taken out and ultrasonicated in HF solution in order to dissolve the alumina membrane. This was followed by centrifugation (15000 rpm) and sequential washing with deionized water. The centrifuged pellet hence obtained was then oxidized under air plasma by exposure for 30s and then re-dispersed in phosphate buffer, pH-8.5. The nanotubes remained dispersed for months.

2.2. Structural characterization

Prepared NAAMs, CNTs and CNTs-NAAMs are characterised by a scanning electron microscope (FEG-SEM FEI Quanta 450) equipped with energy dispersive X-ray spectroscopy (EDXS). The standard