Synthesis and Self-assembly of PEGylated Rosette Nanotubes

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Introduction

Nanostructured materials can accumulate in tumor tissues by escaping from leaky tumor blood vessels through enhanced permeability and retention effect (PER). This phenomenon makes nanomaterials for drug delivery advantageous in cancer chemotherapy.[1] In this work novel types of One-dimensional (1D) nanostructures, rosette nanotubes (RNTs), are examined, as they are especially effective for drug delivery due to the increased drug loading capacity that results from their large surface area.[2] Traditional 1D nanostructures, such as carbon nanotubes and peptide nanotubes, have limited biocompatibility, resulting in undesirable side effects when used as drug delivery vehicles.[3] Rosette nanotubes (RNTs), self-assembled from synthetic hybrid DNA guanine-cytosine (G∧C) motifs, are a novel member of the nanotubular structure family and have been established as a drug delivery vehicle with low cytotoxicity. RNTs have high stability as a result of the extensive Watson-Crick hydrogen bonds (H-bonds) network and the inter-rosette π-π stacking interactions. One of another great advantages of RNTs is the ability to functionalize their surface with a wide range of bioactive molecules, such as amino acids and peptides for biomedical applications [4]. Previous studies revealed that amino acid- and peptide-functionalized RNTs could affect cell viability at high doses, an effect attributed to high surface charge density on the RNTs.[5] Here we report on the design, synthesis and characterization of PEGylated RNTs in which polyethylene glycol (PEG) chains are displayed on the surface of the tubular structure (Figure 1). We hypothesize that the functionalization of RNTs with PEG will enhance the biocompatibility of 1D nanostructures for cancer drug delivery.

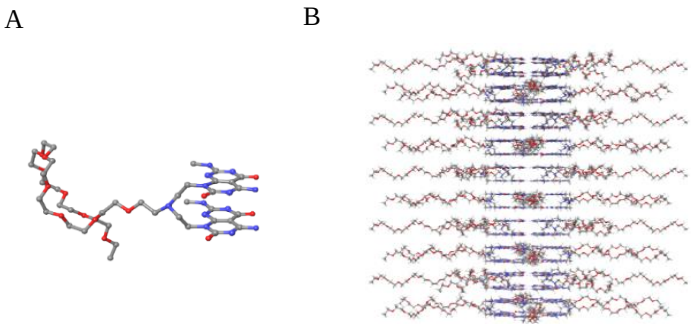

Figure 1. (A) Ball-and-stick model of PEGylated G∧C module. (B) Simulated model of self-assembled PEGylated RNTs.

Materials and Methods

The PEGylated G∧C motif was synthesized from the barbituric acid precursor in a series of 15 organic chemistry reaction steps. Nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) were used to characterize the synthesized molecule and verify the purity of the compounds. The self-assembly process was investigated at different aging times (from 1 hour to 3 months), temperatures (from 20°C to 100°C), and pH levels (from pH=3 to pH=11). Attainment of a tubular rosette nanostructure was confirmed with scanning electron microscopy (SEM), transmission electron microscopy (TEM) and UV-Vis spectroscopy.

Results and Discussion

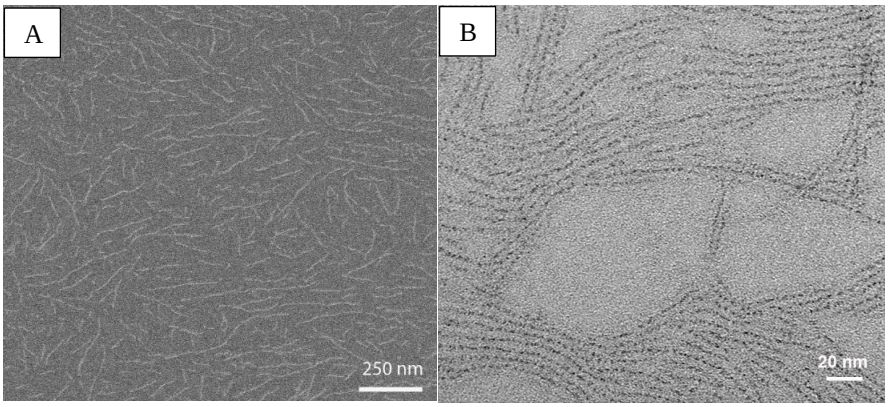

Figure 2. SEM (A) and TEM (B) images of PEGylated RNTs.

All synthesis steps result in high yields (>65%) with the desired structures. In the self-assembly studies, the PEG-functionalized motif is able to form RNTs in Dulbecco's phosphate buffered saline (DPBS) solutions and distilled water at a concentration of 1mg/mL. The formed self-assembled tubular structures have an outer diameter of about 6.5 nm. (Figure 2). SEM images reveal that tubular networks form after aging for 1 hour, and no continuous growing is observed for the following 4 days. We also found the PEGylated RNTs are stable for at least 3 months when stored at 4°C. In addition, the assembled nanotubes maintain their structure in temperatures ranging from 20°C to 100°C, but the length of the nanotubes decreases concomitantly with increasing temperature. This might result from the increased movement of PEGylated G∧C molecules at higher temperature. For the pH dependent studies, the PEGylated RNTs are observed at pH 7 to pH 11. In intracellular cellular conditions, the Waston-Crick H-bonds between the G∧C motifs are disassembled to off-load any encapsulated drugs. This suggests that PEGylated RNTs can be potentially utilized as drug delivery vehicles.

Conclusions

PEGylated RNTs are successfully synthesized and fully characterized with NMR, MS, SEM and TEM. On the basis of results from self-assembly studies, the tubular structures are stable at a wide range of temperatures and pH levels. Herein, we envision that numerous biomedical applications will be adapted in PEGylated RNTs.

Acknowledgements

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