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Functional long circulating single walled carbon nanotubes for fluorescent/photoacoustic imaging-guided enhanced phototherapy

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ABSTRACT

Nanotherapeutics have been investigated for years, but only modest survival benefits were observed clinic. This is partially attributed to the short and rapid elimination of nanodrug after intravenous administration. In this study, a long circulation single wall carbon nanotube (SWCNT) complex was successfully fabricated through a new SWCNT dispersion agent, evans blue (EB). The complex was endowed with fluorescent imaging and photodynamic therapy ability by self-assembly loading an albumin coupled fluorescent photosensitizer, Chlorin e6 (Ce6) *via* the high affinity between EB and albumin. The yielding multifunctional albumin/Ce6 loaded EB/carbon nanotube-based delivery system, named ACEC, is capable of providing fluorescent and photoacoustic imaging of tumors for optimizing therapeutic time window. Synergistic photodynamic therapy (PDT) and photothermal therapy (PTT) were carried out as guided by imaging results at 24 h post-injection and achieved an efficient tumor ablation effect. Compared to PDT or PTT alone, the combined phototherapy managed to damage tumor and diminish tumor without recurrence. Overall, our study presents a SWCNT based theranostic system with great promising in dual modalities imaging guided PTT/PDT combined treatment of tumor. The applications of EB on SWCNT functionalization can be easily extended to the other nanomaterials for improving their *in vivo* stability and circulation time.

1. Introduction

Conventional cancer therapy drug delivery systems are limited by nonspecific targeting, poor drug water solubility, and rapid drug clearance [1,2]. Nano-based carriers are attractive vehicles for drug delivery to circumvent these limitations because they offer lasting steady-state serum concentrations, allowing passive drug accumulation around leaky tumor vessels due to enhanced permeation and retention (EPR), which also minimizes off-target tissue toxicity [3–6]. Unfortunately, reports suggest that after intravenous injection, many nanoparticles are rapidly cleared from circulation after binding to serum proteins *via* opsonization [7,8]. Moreover, nanoparticles can be engulfed by circulating macrophages or destroyed in the liver or spleen *via* the mononuclear phagocytic system (MPS) or the reticuloendothelial system (RES) [8–10], which significantly reduces drug delivery efficacy. Thus, better approaches for delivering nanodrugs with lasting *in vivo* circulation are needed [11–13]. Currently, poly-(ethylene glycol) (PEG), a flexible and hydrophilic molecule, is used to prolong nanocarrier circulation [14,15]; however, recent studies indicate that a PEGylated nanomedicine system can induce anti-PEG IgM antibodies after an initial injection, significantly enhancing clearance of the subsequent nanoparticle administration as rapidly as non-optimized nanoparticles [16–18]. Thus, challenges remain for developing a safe and lasting drug delivery system.

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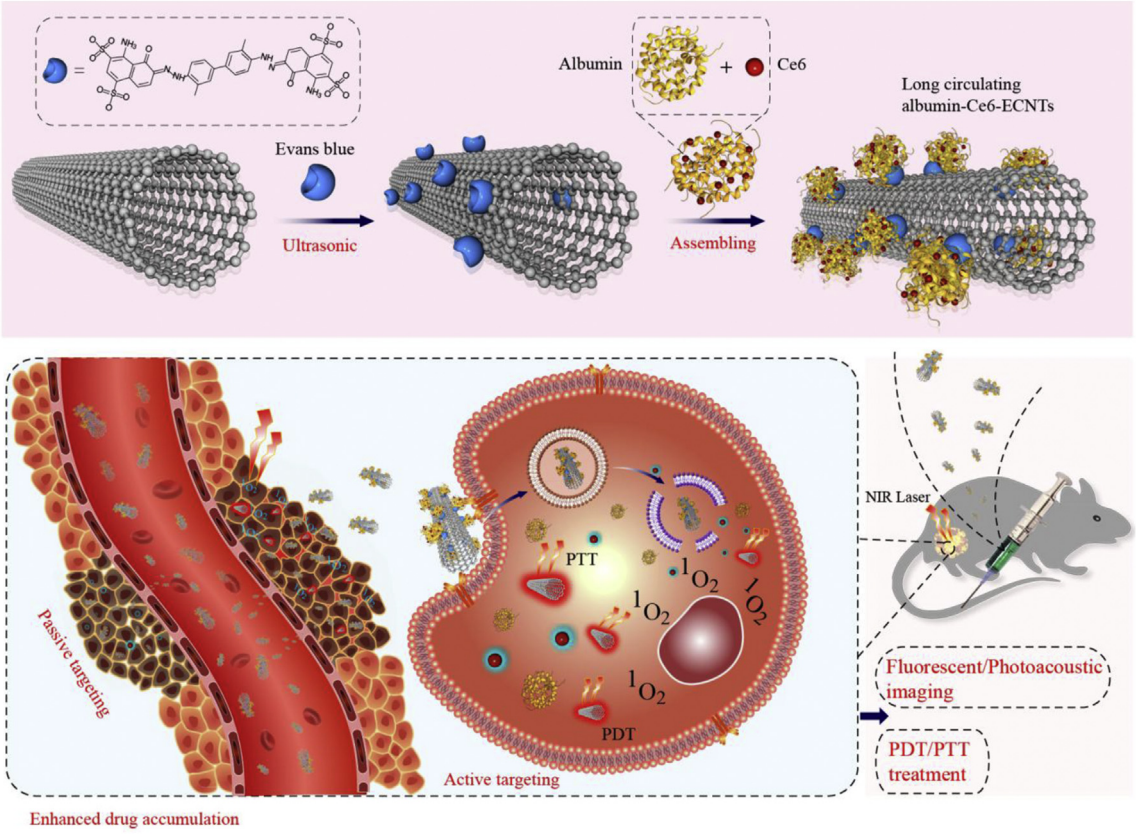
In recent years, various types of near-infrared light-responsive nanomaterials, such as gold nanoparticles, carbon materials and upconversion nanomaterials, have drawn tremendous attentions for phototherapy [19–21]. Among these particles, carbon nanotubes especially single walled carbon nanotubes (SWCNTs), offered more advantages over other nanoparticles including large and uniquely shape, excellent photo-thermal concerting efficacy, high cargo loading, excellent cell penetration ability and strong raman signals [22–25]. It is believed that SWCNT will be a promising cancer theranostic agent with appropriate surface modification to improve the biocompatibility and tumor targetability, because unmodified SWCNTs have highly hydrophobic surfaces and are not aqueous soluble. Modifying these hydrophilic surfaces can improve water solubility and improve SWCNTs performance *via* linking multiple active molecules such as peptides, genes, proteins, and drugs [26–33]. Evans blue (EB), a strong hydrophilic and non-toxic biological vessel stain, that can be used to coat SWCNTs *via* non-covalent adsorption and increased water solubility has been reported [34]. Importantly, EB modification of SWCNTs does not break their π network and preserves their physical properties, suggesting promise for drug loading and imaging applications [34]. In addition, EB can form stable complex with the serum albumin which is presently an FDA-approved biocompatible drug delivery carrier that can target cancer cells with the albumin receptor gp60 and SPARC [35]. It is known that albumin can prolong nanomaterial circulation [35,36], so we hypothesized that SWCNT/EB complexed to albumin could circulate longer than unmodified SWCNTs and drug accumulation would occur at target sites, for example tumors.

In this study, a novel long-circulating SWCNT-based therapeutic system was established to deliver Chlorin e6 (Ce6), a widely used photosensitizer with strong near fluorescence, for combined synergistic photodynamic therapy (PDT) and photothermal therapy (PTT). Specifically, Ce6 was encapsulated with albumin to increase its stability and hydrophilicity. Subsequence, the formed albumin/Ce6 complex was loaded onto the surface of EB-modified SWCNTs by exploiting the high affinity between albumin and EB. In this system, the strong optical absorbance of SWCNTs and Ce6 fluorescence allows photoacoustic (PA) and fluorescent (FL) imaging of the complex in tumors to guide PDT/PTT therapy (Scheme 1), which may improve tumor treatment efficacy without obvious side effects. Compared to single therapeutic treatment, combined PTT and PDT therapy can significantly improve treatment outcomes without recurrence. Overall, this new biocompatible delivery system allows complementary imaging modalities, a long circulation time and improved tumor accumulation for synergistic tumor PDT/PTT treatment. This simple, effective and long-circulating multifunctional SWCNTs delivery platform is ideal for targeted molecular image-guided therapy.

2. Materials and methods

Materials: Albumin, Evans blue (EB), DAPI, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 3,6-Di(*O*-acetyl)-4,5-bis[*N*,*N*-bis(carboxymethyl)aminomethyl] fluorescein, tetra-acetoxy-methyl ester (Calcein-AM) and 3,8-diamino-5-[3-(trimethylammonio)propyl]-6-phenyl phenanthridine (PI) were purchased from Sigma-Aldrich (St. Louis, MO). Ce6 was acquired from Life Science (Shanghai, China). DMEM cell culture medium, fetal bovine serum (FBS) and penicillin-streptomycin were acquired from Hyclone (Pittsburgh, PA). SWCNT (carbon (carbon as SWCNT) > 80%, diameter: 0.7–1.4 nm) was purchased from Sigma-Aldrich (St. Louis, MO).



Scheme 1. Preparation and application of albumin/Ce6 fabricated EB/carbon nanotube-based delivery system (ACEC).