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Polyglycerol-coated nanodiamond as a macrophage-evading platform for selective drug delivery in cancer cells

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ABSTRACT

A successful targeted drug delivery device for cancer chemotherapy should ideally be able to avoid non-specific uptake by nonmalignant cells, particularly the scavenging monocyte-macrophage system as well as targeting efficacy to bring the drug preferentially into tumor cells. To this purpose, we developed a platform based on detonation nanodiamond (dND) with hyperbranched polyglycerol (PG) coating (dND-PG). dND-PG was first demonstrated to evade non-specific cell uptake, particularly by macrophages (U937). RGD targeting peptide was then conjugated to dND-PG through multistep organic transformations to yield dND-PG-RGD that still evaded macrophage uptake but was preferentially taken up by targeted A549 cancer cells (expressing RGD peptide receptors). dND-PG and dND-PG-RGD showed good aqueous solubility and cytocompatibitlity. Subsequently, the anticancer agent doxorubicin (DOX) was loaded through acid-labile hydrazone linkage to yield dND-PG-DOX and dND-PG-RGD-DOX. Their cellular uptake and cytotoxicity were compared against DOX in A549 cells and U937 macrophages. It was found that dND-PG-DOX uptake was substantially reduced, displaying little toxicity in either type of cells by virtue of PG coating, whereas dND-PG-RGD-DOX exerted selective toxicity to A549 cells over U937 macrophages that are otherwise highly sensitive to DOX. Finally, dND-PG was demonstrated to have little influence on U937 macrophage cell functions, except for a slight increase of TNF-α production in resting U937 macrophages. dND-PG is a promising drug carrier for realization of highly selective drug delivery in tumor cells through specific uptake mechanisms, with minimum uptake in and influence on macrophages.

1. Introduction

In drug treatment of cancerous diseases, systemically administered chemotherapeutic agents are distributed throughout the body acting indiscriminately on both cancer and normal tissues. This often leads to inadequate therapeutic efficacy due to insufficient drug availability in cancer tissues and significant toxicity in normal tissues. Thus, preferential accumulation of chemotherapeutic drugs in the malignant cells is highly desirable and carrier-based, highly targeted drug delivery systems (DDS) are instrumental in realization of this strategy. While the targeting moiety largely determines the specificity and efficacy of a DDS for cancer therapy, it is recognized that the success of a targeted DDS also depends on how well it avoids the non-specific uptake by nonmalignant cells and the scavenging system i.e. the mononuclear phagocyte system (MPS). The MPS consists of specialized phagocytic cells including monocytes and macrophages in the reticular connective tissue, Kupffer cells of the liver and tissue histiocytes [1]. MPS is a vital component of the immune system performing crucial immune functions i.e. antigen presentation, phagocytosis, and immunomodulation through production of cytokines and growth factors [1]. Recognition and elimination of exogenous particles that enters the body is a primary function of the MPS. Elimination by the MPS negatively affects the *in vivo* efficacy and circulation time of a particle-based DDS [2,3]. MPS can also be activated by an exogenous particulate matter to initiate immune responses [4,5]. Various targeted DDS based on nanoparticles have thus far been devised that focus on increasing accumulation of chemotherapeutic drugs in the malignant cells [6,7], but few have addressed the issue of scavenging by the MPS and their influence on the MPS.

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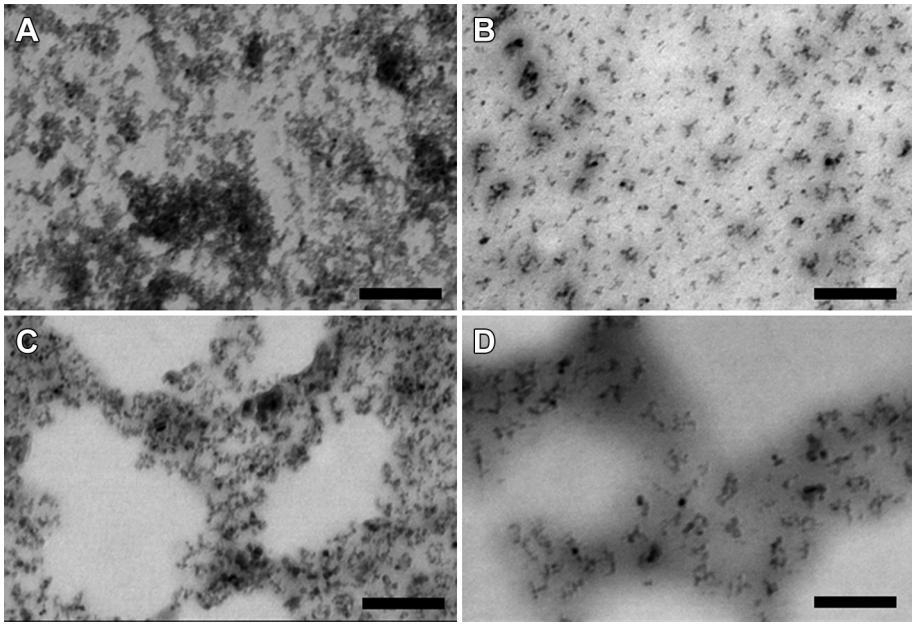

Fig. 1. STEM images of (A) dND, (B) dND-PG, (C) dND-PG-DOX and (D) dND-PG-RGD-DOX. All scale bars represent 100 nm.

Non-specific cellular uptake of particulate matter is largely dependent on the surface chemistry and surface charge of the particles [8,9]. Thus, drug carrier particles are often coated with an electrically neutral hydrophilic surface layer, a coating that shields against non-specific cellular uptake [9]. The most widely used coating material with this effect thus far is polyethylene glycol (PEG), a non-ionic hydrophilic polymer that prevents the adsorption of proteins and subsequent cellular uptake. Despite its wide usage, such significant drawbacks have been found of PEG coating as impeding endosomal escape [10] and inducing immune response [11]. A variety of alternative coating materials have been proposed, among which is hyperbranched polyglycerol (PG) [9,12]. Hyperbranched PG has recently attracted attention for biomedical application due to its biocompatibility, hydrophilic property and anti-fouling effect [13–16]. It was reported that PG polymers have long plasma half-lives [17] and PG coating can prolong liposome circulation [16,17]. In addition, PG is more amenable for further functionalization than PEG due to numerous surface hydroxyl groups [18,19].

Nanodiamond (ND) is a promising platform for biomedical applications such as imaging and drug/gene delivery due to its high specific surface area, tunable surface structures and

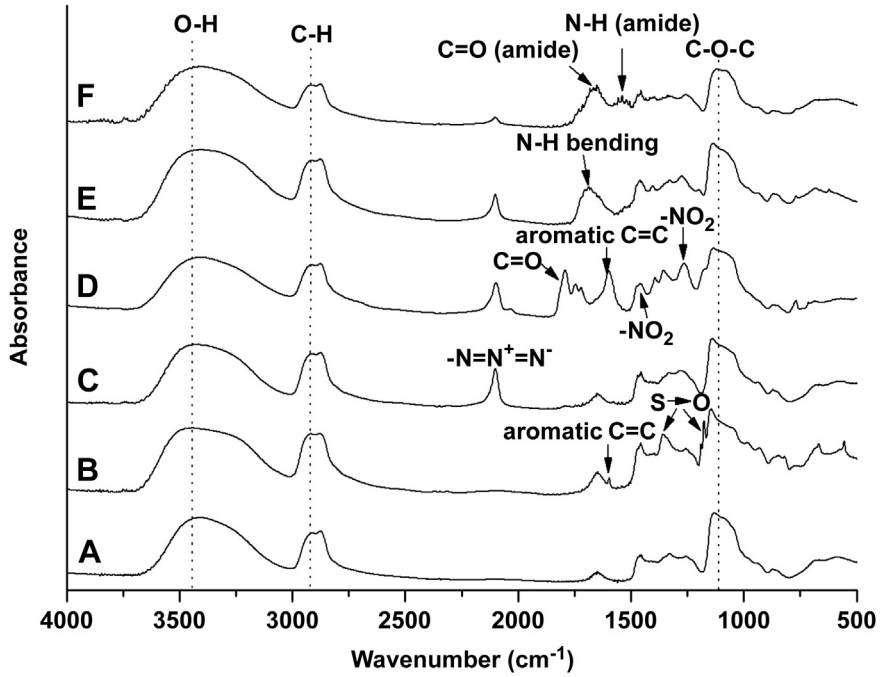

Fig. 2. FTIR spectra of (A) dND-PG, (B) dND-PG-OTs, (C) dND-PG-N_3, (D) dND-PG-N_3-PhNO_2, (E) dND-PG-N_3-$NHNH_2$ and (F) dND-PG-RGD-$NHNH_2$. Arrows indicate new absorption bands in each step.