Near-Infrared Triggered Decomposition of Nanocapsules with High Tumor Accumulation and Stimuli Responsive Fast Elimination

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Abstract: A near-infrared (NIR) induced decomposable polymer nanocapsule is demonstrated. The nanocapsules are fabricated based on layer-by-layer co-assembly of azobenzene functionalized polymers and up/downconversion nanoparticles (U/DCNPs). When the nanocapsules are exposed to 980 nm light, ultraviolet/visible photons emitted by the U/DCNPs can trigger the photoisomerization of azobenzene groups in the framework. The nanocapsules could decompose from large-sized nanocapsule to small U/DCNPs. Owing to their optimized original size (ca. 180 nm), the nanocapsules can effectively avoid biological barriers, provide a long blood circulation (ca. 5 h, half-life time) and achieve four-fold tumor accumulation. It can fast eliminate from tumor within one hour and release the loaded drugs for chemotherapy after NIR-induced dissociation from initial 180 nm capsules to small 20 nm U/DCNPs.

Nanoparticles have emerged as promising carriers for drugs in cancer treatment owing to the enhanced permeability and retention (EPR) effect, increasing delivery efficiency, and suppressing side effects.[1–3] The design and development of sophisticated nanocarriers for targeted delivery of theranostic agents to solid tumors hold great promise for improving treatment efficacy and minimizing systemic toxicity. However, nanomedicine often suffers from multiple biological barriers, especially the low accumulation & delivery efficiency toward tumor because of clearance by organs during circulation in the blood stream.[4] It has been demonstrated that the particle size plays a vital role in dominating the delivery efficiency: particles with a diameter of 100–200 nm generally provide long-lasting blood circulation and less clearance of organs.[5–8] Thus, the conventional solution for size-dependent high accumulation in tumor is to optimize particle diameter to this range. However, large nanocarriers are difficult to be eliminated from the organs after their functionalities exhausted.[6,8] Therefore, it is critically desired to develop next-generation smart theranostic agents:[9–10] nanocarriers with a large initial size (100–200 nm) to cross biological barriers, provide prolonged blood circulation and achieve high tumor accumulation, which then could be responsively decomposed into small fragments to release loaded guests and be quickly metabolized.

Much effort has been made to develop stimuli-triggered delivery systems in response to a given stimulus, such as pH,[11–14] temperature,[15,16] and enzyme.[17] Among these approaches, light-triggered drug delivery has attracted much attention because it does not rely on changes in specific chemical properties of environment.[18–20] A variety of light-responsive systems have been designed based on ultraviolet (UV) or visible (Vis) light excitations with low tissue penetration and are biological harmful.[21] As a promising candidate, near-infrared (NIR) light would bring new opportunities owing to minimal absorbance and maximum penetration of tissues.[22–24] The polymer nanocapsules, in which polymers can act as a “cage” and encapsulate small functional nanoparticles or drugs. However, unlike condensed materials, polymers are more likely to deform and reshape.[25–27] Thus they are often chosen as building blocks of stimuli responsive systems.

Herein, taking the advantages of the core–shell structured NaGdF4:Yb,Tm@NaYF6:Nd@NaYF4 dual-mode up/ downconversion nanoparticles (U/DCNPs) and light-responsive polymer, we demonstrate a novel NIR light-responsive decomposable polymer nanocapsule (Figure 1). The 180 nm nanocapsules were constructed through a simple electrostatic interaction induced layer-by-layer assembly strategy on the surface of spherical SiO2 colloids (Supporting Information, Figure S1).[30,31] U/DCNPs with negative surface charges[32–34] were also incorporated in the polymer shells during the alternative assembly of polymers. The hollow-structured nanocapsules can be obtained after the SiO2 templates were selectively etched. To realize the NIR-responsive decomposition of the nanocapsules, the U/DCNPs were doped with Tm3+ to transmit...
NIR photons into UV/Vis photons, and UV/Vis sensitive azo functional group was also introduced into the polymer frameworks (Figure 1). The reversible photo-isomerization of the azo group in the polymer framework stimulated by UV/Vis upconversion luminescence creates continuous rotation–inversion movement, inducing the decomposing of initial 180 nm nanocapsules into scattered polymers and 20 nm U/DCNPs. The transportation, accumulation, and metabolization of the nanocarriers can be carefully monitored based on the NIR window (NIR-II) downconversion fluorescence of the core/multishell structured U/DCNPs. The transportation, accumulation, and metabolization of the nanocarriers can be carefully monitored based on the NIR window (NIR-II) downconversion fluorescence of the core/multishell structured U/DCNPs.

The core–multishell structured NaGdF$_4$:Yb,Tm@NaYF$_4$:Nd@NaYF$_4$ U/DCNPs were fabricated and employed to functionalize the polymer nanocapsules (Supporting Information, Figure S2). In this nanostructure, Tm$^{3+}$ doped nanoparticles were constructed as core for emission of UV/Vis upconversion luminescence under 980 nm excitation. The NaGdF$_4$:Nd layer can emit downconversion NIR-II fluorescence under 800 nm excitation for downconversion bio-imaging. Transmission electron microscopy (TEM) and the high-angle annular dark-field scanning TEM (HAADF-STEM) images (Figure 2A) of the as-prepared U/DCNPs show discernible contrast for the multi-layer structure with a uniform diameter of about 20 nm. High-resolution TEM images (Figure 2B) and the X-ray diffraction (XRD) patterns (Supporting Information, Figure S3) of the obtained U/DCNPs show that the nanoparticles are a highly crystalline hexagonal phase without any remarkable impurity.

Two types of polymers, poly(diallyldimethylammonium chloride) (PDADMAC) and poly[1-{4-(3-carboxy-4-hydroxyphenylazo)benzenesulfonamido}-1,2-ethanediyl] (PAZO), with positive and negative charges, respectively, were layer-by-layer assembled through electrostatic interaction onto the surface of colloidal SiO$_2$ nanoparticles. Negatively charged U/DCNPs were anchored into the composites in the same way. TEM images of the obtained nanocomposites clearly show uniform core–shell structured SiO$_2$@polymer@U/DCNP nanocomposites (Figure 2C). Uniform hollow nanocapsules with small U/DCNPs embedded in the shells were obtained after etching of the SiO$_2$ templates (Figure 2D,E). The polymer shells retained the spherical structure very well, and the size of the nanocapsules shrank from 200 to 180 nm. The nanocapsules possess NIR-to-UV/Vis upconversion optical properties under 980 nm excitation as well as downconversion NIR-to-NIR-II fluorescence at around 1060 nm excited by 808 nm light (Figure 2F). The result shows that the intensity of the upconversion luminescent emissions in the UV/blue region (300–500 nm) were significantly lowered after coating of the azo groups containing polymer. More than 85% of the photoenergy at about 310 and 365 nm was transferred to the polymer shells owing to the strong absorption in the UV region. About 30% of the photoenergy at circa 475 nm was absorbed by the polymer shells (Figure 2G).
The decomposability of the nanocapsules was carefully examined. TEM images of the nanocapsules after a certain period of NIR radiation clearly display the decomposition process: the nanocapsules are completely intact at the beginning (Figure 3 A); an obvious collapse is observed after 1.5 W cm\(^{-2}\) 980 nm irradiation for 15 min (Supporting Information, Figure S4). After 30 min, the polymer frameworks are totally dissociated and the embedded small U/DCNPs are released (Figure 3 B). With the increase of NIR irradiation duration, the maximal absorbance of the azo moiety at 350 nm decreases considerably, indicating that UV and visible light from U/DCNP inlays can cause the trans–cis photo-isomerization of azo molecules in the polymer frameworks (Figure 3 C). Dynamic light scattering (DLS) measurements show that the diameter of the nanocapsules shifts from about 180 to about 20 nm after 980 nm irradiation for 30 min, which is consistent with the sizes of the nanocapsules and U/DCNPs (Figure 3 D). The results clearly indicate that the isomerization of azo group eventually led to the disassembly of the polymer frameworks, realizing the decomposing procedure of the nanocapsules.[38–40]

The composition of the nanocapsules have strong effects on their decomposability. Without enough U/DNCPs to convert NIR into UV/Vis (Supporting Information, Figure S7), the decomposability of the nanocapsules would be seriously hindered (Supporting Information, Figure S8). On the other hand, the thickness of the polymer shells is also very important. When the number of layers of the assembly charged polymers is less than 8, for example 5, the polymers cannot support the U/DNCNPs, thus the hollow structure collapsed after etching of the SiO\(_2\) template (Supporting Information, Figure S9). When the number of the polymer layer is more than 8, the stability of the structure is significantly improved. However, if the thickness of the polymer shell increases alone without tuning the amount of the embedded U/DNCNPs, the structure would be too stable to be decomposed (Supporting Information, Figure S10). Thus, the ratio of nanoparticle/polymer should be carefully monitored to achieve satisfying decomposability. The size of the nanocapsules can also be well tuned by using colloidal SiO\(_2\) nanospheres with different diameters, and the decomposing ability can be retained very well (Supporting Information, Figures S11–S13). By alternately introducing of the polymers and inorganic nanoparticles, the hollow-structured nanocomposites with nanoparticles anchored to multiple layers can be obtained (Supporting Information, Figure S14).

An in vivo bio-distribution study was then conducted. The nanocapsules were very stable in a broad pH range and various biological media (Supporting Information, Figure S15), which laid a foundation for the long-term intracorporeal circulation. Nude mice bearing U87-MG solid tumors were intravenously injected at the caudal vein with the U/DCNP-functionalized nanocapsules (Figure 4 A). Considering the optimal particle size for a long blood circulation and high accumulation in tumor, the initial U/DCNP-functionalized polymer nanocapsules with the diameter of 180 nm were selected.[41,42] Bio-distribution and tumor accumulation were monitored by Nd\(^{3+}\) dominated down-conversion NIR-II fluorescence under 800 nm excitation (Figure 4 B). The results clearly reveal that the NIR-II fluorescence signal at the tumor sites for the 180 nm nanocapsule group is extremely bright in 6 h, compared with that of the free U/DCNPs under the same condition (to avoid the interaction from the signal of...
non-interested area, a screener with only the tumor area exposed was used). The NIR-II downconversion fluorescence signal can last over 48 h, which is much longer than that of bare U/DCNPs, suggesting the enhanced accumulation in tumor due to EPR effect with optimized size (Supporting Information, Figure S17). However, after applying 1.5 W cm\(^{-2}\) 980 nm radiation on the tumor region for only 5 min at 6 h post-injection, the fluorescence signal faded swiftly and totally vanished after merely 6 h. Such quick elimination demonstrates the fast internal clearance owing to the smaller size of the dissociated U/DCNPs. Compared with the free U/DCNPs, the large nanocapsules exhibit significantly higher accumulation rate at tumor sites owing to the longer blood retention and higher propensity of extravasation across the vasculature owing to the large size (Figure 4C,D; Supporting Information, Figure S18). There is a significant decrease in the concentration of Gd\(^{3+}\) for the nanocapsules injection group after exposing the tumor to the radiation of 1.5 W cm\(^{-2}\) 980 nm for 5 min (Figure 4C). It is attributed to the fast clearance of small 20 nm U/DCNPs after the decomposition of the initial large nanocapsules. On the basis of the above results, it can be concluded that the NIR-responsive decomposable nanocapsules can not only provide prolonged blood circulation, and thus achieve highly efficient accumulation in the tumor, but also decomposes into small-sized fragment nanoparticles for fast clearance.

Drugs loaded in the nanocapsules can be released during the decomposing process. DOX could reach a 50% release in 60 min (Figure 5A; Supporting Information, Figure S19) under the irradiation of 1.5 W cm\(^{-2}\) 980 nm light. In sharp contrast, less than 10% of DOX was leached out without the NIR irradiation-induced decomposing. According to the cell viability evaluation, DOX-loaded nanocapsules showed a significantly enhanced cytotoxicity to the cancer cells upon prolonged irradiation time, while control groups missing any of the three key elements do not result in any significant decrease in the cell viability (Figure 5B). Encouraged by the NIR light-responsive release of drugs, the DOX-loaded smart decomposable nanocapsules were used in the chemotherapy of U87-MG solid tumor. Owing to the high tumor accumulation efficiency and NIR triggered controllable drug release, a significantly improved therapeutic effect was observed (Figures 5C, D; Supporting Information, Figure S23). These results are consistent with that of the hematoxylin and eosin (H&E) staining analysis (Figure 5E; Supporting Information, Figure S24). No remarkable tissue damage or any other side effect was observed on heart, liver, spleen, lung, kidney, and weight fluctuations, indicating a good biocompatibility and negligible toxicity of this smart decomposable drug-delivery system.

In summary, NIR-responsive decomposable drug delivery nanocapsules with controllable sizes (180–500 nm) were fabricated through layer-by-layer self-assembly of U/DCNPs and light-responsive polymers. 980 nm NIR light can trigger the decomposition process of the U/DCNP–polymer nanocomposite, transforming the particle from large-sized nanocapsules to small-sized polymer fractions and U/DCNP nanoparticles. According to the results of the in vivo downconversion NIR-II monitoring of the circulation, accumulation, and metabolism, the nanocapsules with optimized initial large particle size (180 nm) can effectively avoid biological barriers, provide a long blood circulation (ca. 5 h half-lifetime) and achieve four-fold tumor accumulation. The NIR-induced dissociation from initial large capsules to small polymer fractions and 20 nm U/DCNPs allows a fast elimination from tumor within an hour and releases the loaded drugs for chemotherapy. The smart delivery and release capability of this NIR-activated decomposable nanocarriers lays solid foundations towards a novel platform for next-generation nano-therapeutics with both spatial and temporal external control.

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**Figure 5.** A) DOX releasing profiles from the decomposable nanocapsules with and without NIR radiation. B) Cell viability under different conditions. C) Tumor growth profiles and D) body weight of the mice under different conditions. E) H&E and TUNEL staining of U87-MG solid tumors after various treatments.
Conflict of interest

The authors declare no conflict of interest.

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