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Vesicle-Based Drug Delivery

Near-IR Remote Release from Assemblies of Liposomes and Nanoparticles**

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Nanotechnology offers novel insights and concepts for drug delivery.[1] The release of encapsulated materials remotely is desired in drug delivery for minimizing drug toxicity, controlling the properties of surfaces and interfaces,[2] and studying intracellular processes.[3] Light-stimulated remote release is of special interest because of the possibility for external control of the light intensity and modulation and because of its noninvasive character, which is desirable for bioapplications. Potential applications of such an approach are expected in photothermal therapy, in which the dynamic photothermal effect can be augmented by the delivery cargo. Release from polyelectrolyte microcapsules functionalized with metal nanoparticles (NPs), by burst opening and deformation, has been demonstrated previously (see the Supporting Information). On the other hand, liposomes have traditionally been used in drug delivery,[4,5] as well as for nonmedical applications involving reactions in confined volumes.[4,6,7] We have previously demonstrated temperature-triggered release of a liposome cargo from surface-supported vesicles embedded inside biocompatible polyelectrolyte multilayers.[8] In an effort to enlarge the scope of application of remote release and to extend it further to other surface-supported drug delivery vesicles, we have applied remote release to liposome–gold nanoparticles, referred to as assemblies or complexes (Lip-NP). The goals of this work were to show that Lip-NP assemblies could be prepared in a controlled manner in terms of size and nanoparticle state and then to use near-IR light to selectively release encapsulated dye from the assemblies.

Light-triggered release of liposome contents[9] has been demonstrated with visible light by using covalent lipid coupling with light-sensitive moieties[10] and the synthesis of metal NPs in the vesicles.[11,12] However, the efficient light-induced controlled release in space and time of liposome-encapsulated solutes without damage to environmental tissues and cells still remains challenging. Minimization of light absorption could be accomplished by choosing a wavelength in the "biologically friendly" window, that is, the near-IR part of the spectrum.[3] Therefore, in this work, we study light-sensitive Lip-NP complexes and tune the light absorption of the NPs into the near-IR spectral range by controllable NP aggregation. We utilize the complexation phenomenon between soft liposomes and hard metal NPs in order to demonstrate the feasibility of creating light-sensitive composite structures. Gold NPs serve as absorption centers for energy supplied by a laser beam.[13,14] The vesicles were composed from 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dipalmitoyl-3-trimethylammonium propane (chloride salt) (DPTAP), and cholesterol (CL). Near-IR irradiation induces local heating of the NPs, with the aim being to release the vesicle contents. The temperature rise is concentrated in the vicinity of the absorbing NPs, which results in highly efficient heat transfer with minimum contact to the encapsulated material. Heating leads to a subsequent phase transition (transition temperature of DPPC liposomes is reported to be in the range 40–50 °C)[15] or (and) disruption of the lipidic bilayer due to the creation/collapse of vapor bubbles.

Lip-NP assemblies were generated by mixing a solution of liposomes filled with the fluorescent marker 5(6)-carboxyfluorescein (CF) and gold NPs, either as single NPs (Figure 1a,b) or preaggregated by salt (Figure 1e,f). Control of the NP aggregation behavior allows the formation of Lip-NP complex structures by means of clustering through single NPs or NP aggregates and, thus, creates assemblies of types I and II, respectively. This complexation phenomenon is similar to that we have studied for DPPC-based liposomes and polylysine:[16,17] it is driven by electrostatics and results in progressive growth of vesicle assemblies to a definite size until charge compensation occurs. The NPs are not integrated into the lipidic bilayer due to their hydrophilic nature and large size.

In principle, the complexation between NPs and liposomes allows the preparation of complexes of various sizes from single well-stabilized vesicles to large aggregates (the aggregation profile is presented in the Supporting Information). The structure and dimensions of the Lip-NP assemblies are functions of the vesicle and NP characteristics, namely size and charge, as well as their mixing ratio upon complexation. These parameters, extracted from colloidal aggregation, allow one to tune the properties of the complex structures of liposomes and metal NPs. In general, the proposed approach is applicable to various NP-functionalized liposome structures, including the assemblies used as models in this work and single vesicles that could be covered by the NPs. Herein, the Lip-NP large assemblies were used for light-irradiation experiments for visualization.

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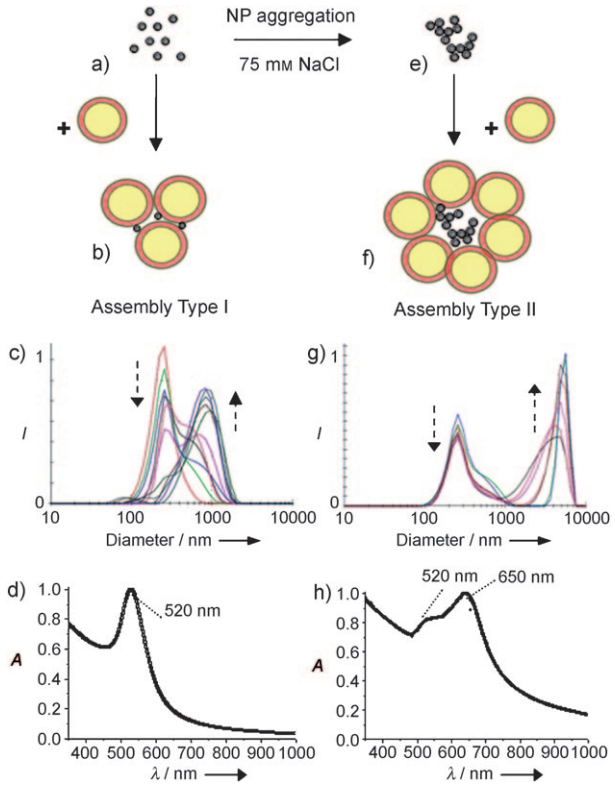

Figure 1. a, b, e, f) Lip-NP assembly formation. Liposomes are in orange and yellow; NPs are in gray. c, g) Time evolution of the assembly size distribution (volume weighted) for type I (c) and type II (g) assemblies. Recording of the normalized scattered intensity was every 5 min. The arrows show the direction of peak evolution. d, h) Normalized absorption spectra of the type I (d) and type II (h) assemblies.

The evolution of Lip-NP assemblies growth over time is presented in Figure 1 c, g. Assembly type I, forming at an equivalent Lip to NP ratio, are progressively grown due to diffusion of both components (Lip and NPs) from the bulk to the assembly surface, which gives stable assemblies with diameters near 800 nm (Figure 1 c). Accumulation of assemblies of a definite size is a known phenomenon in colloidal chemistry.[18] Assemblies of type II are composed not from single NPs but from NP aggregates of around 300 nm, which have a high cumulative electrostatic charge. The NP aggregates attract a larger number of liposomes to compensate for the charge excess; this gives bigger assemblies, which are roughly 5 μm in diameter. The latter is illustrated by a simultaneous decrease in the scattered light intensity corresponding to the NP aggregates and an enlargement of the peak for the Lip-NP assemblies (Figure 1 g). The assemblies are stable and the vesicles preserved their integrity for both kinds of Lip-NP structures; this was tested by a leakage test of the liposome contents. Transmission electron microscopy (TEM) images of the assemblies are presented in Figure 2, which shows single NPs and their aggregates in Lip-NP assemblies of types I and II, respectively.

Single NPs exhibit a surface plasmon resonance in the visible part of the spectrum (at ≈520 nm). Aggregates of

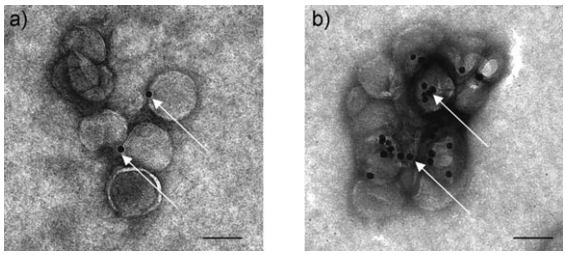

Figure 2. TEM images of the Lip-NP assemblies depicted in Figure 1: a) type I; b) type II. The scale bars represent 100 nm.

nanoparticles allow tuning of absorption and release due to the interaction of dipole moments on neighboring NPs.[19] A comparison of the absorption spectra of assemblies of types I and II (Figure 1 d and h, respectively) clearly shows the difference, with the characteristic redshift of NP absorption being exhibited in the latter case (Figure 1 h). This is consistent with the aggregation profile of gold nanoparticles.[20]

We have focused on release experiments with the type II assemblies, in which nanoparticle aggregation leads to higher near-IR absorption, for this feasibility study. Under identical illumination conditions, type I assemblies showed no release (see the Supporting Information) due to the lower absorption of the nonaggregated nanoparticles (at least an order of magnitude, that is, ≈0.02 at 830 nm versus ≈0.1 at 520 nm).[19]

In the remote-release experiments, the micron-sized aggregate of the type II Lip-NP was illuminated by a focused laser beam, which resulted in release of the encapsulated fluorescent dye, as evidenced by confocal laser scanning microscopy (CLSM) images (Figure 3). The assembly marked with a white dashed circle, which is larger in size, was chosen for the experiment. The fluorescence profile of the liposome assembly before release (blue trace in Figure 3 d taken across the assembly as marked by the blue line in Figure 3 a) shows a uniform filling of the liposome complex. After release took place, the fluorescence intensity of the illuminated part (under the orange dashed line in Figure 3 d) decreased to zero (the flat part of the red curve in Figure 3 d), whereas the fluorescence of the nonilluminated area did not change and remained at the same level as that before the exposure (the two peaks on the red curve in Figure 3 d). The targeted area and the profile of fluorescence illustrate precise control of the area of release. Also, the experiment shows a fast response to light irradiation, within five seconds of light exposure. It should be noted that we used biocompatible polymers and gold nanoparticles in this study.[21] The synthetic lipids were used as model lipids.

In conclusion, it has been shown that the Lip-NP complexes can be prepared in a controlled manner in terms of size (kinetics of complex formation) and nanoparticle state (single or aggregated) by using a complexation phenomenon driven by electrostatic interactions. The complexes release encapsulated dye upon stimulation with near-IR light due to local heating of the NPs. Closely located NPs induce near-IR absorption that allows efficient heat transfer directly to liposome lipidic bilayers upon near-IR laser illumination and results in release of encapsulated dye molecules. This study

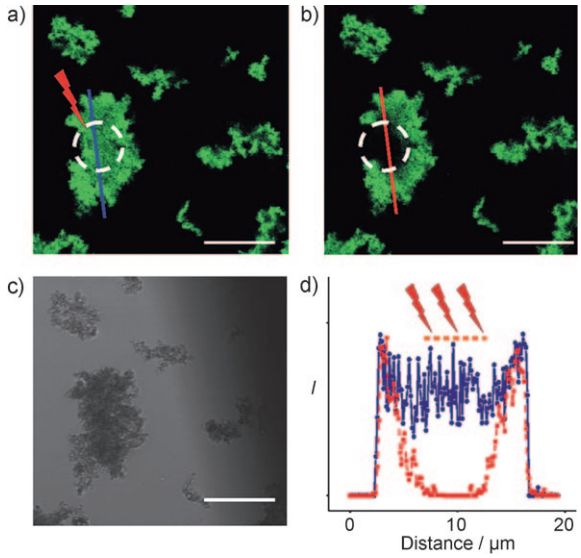

Figure 3. Remote release from Lip-NP assemblies (type II). CLSM images in fluorescence mode a) before and b) after light exposure. The illuminated area is marked by dashed circles. c) Transmission image of the same aggregate. The darker area on the right-hand side is the marked area used for identification. d) Profiles of fluorescence intensity before (blue trace corresponding to the blue line in (a)) and after (red trace corresponding to the red line in (b)) remote release. A projection of the illuminated area is marked by the dashed line. The scale bars in (a–c) represent 10 μm.

extends the remote-release methods to a variety of carriers that can be modified by light-sensitive NPs. Functionalized liposome–nanoparticle assemblies and liposomes can be used for transdermal applications in which an active compound is delivered through the skin, which is easily accessible by light. Due to quite deep IR-light tissue penetration,[7] the light-responsive liposome assemblies and liposomes could serve as active constituents of implanted devices.

Experimental Section

Liposomes filled with CF (Sigma–Aldrich, Germany) were prepared by mechanical extrusion with preliminary freeze–thaw cycling according to the previously described method[16] and were separated from free dye molecules by gel-permeation chromatography. The liposomes were composed from a DPPC/DPTAP/CL (60/30/10 w/w) mixture and have an average hydrodynamic diameter of 128 ± 3 nm, as calculated from dynamic light-scattering measurements. Lip-NP assemblies were made by adding liposome suspension to 20 nm hydrophilic gold NPs (Sigma–Aldrich, Germany) dispersed in either 7.5 mM or 75 mM NaCl, for type I and type II assemblies, respectively. A high salt concentration lets the NPs aggregate so the aggregates form complexes with the liposomes (type II assembly). Further details of liposome and Lip-NP preparation and characterization can be found in the Supporting Information. The integrity of the liposomes in Lip-NP assemblies was tested by fluorescence measurement in the supernatant of the centrifuged Lip-NP complexes.

Remote release experiments were conducted according to the previously described method by using a near-IR laser setup (see the Supporting Information) Confocal micrographs were taken with a Leica confocal scanning system mounted on a Leica Aristoplan apparatus and equipped with a 100× oil immersion objective (numerical aperture 1.4). The sample, confined between two microscope slides, was sealed (and marked for identification). Subsequently, an aggregate of the liposomes was found in the transmission mode on the confocal microscope (Leica, Germany). Once an appropriate transmission image was obtained, a single fluorescence confocal scan was taken, with care taken to assure that no photobleaching occurred. The sample was then placed in the laser setup for release experiments; the same aggregate was found in the transmission mode and illuminated for ≈5 s with an incident intensity of 50 mW (wavelength 830 nm; see the Supporting Information). The sample was then brought back to the confocal microscope and the same aggregate was recorded by using the same procedure as that used before release. Photobleaching experiments were also conducted (see the Supporting Information).

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