Near-infrared light–triggered platelet arsenal for combined photothermal-immunotherapy against cancer

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To address long-standing issues with tumor penetration and targeting among cancer therapeutics, we developed an anticancer platelet-based biomimetic formulation (N+R@PLTs), integrating photothermal nanoparticles (N) and immunostimulator (R) into platelets (PLTs). Exploiting the aggregative properties of platelets and high photothermal capacity, N+R@PLTs functioned as an arsenal by targeting defective tumor vascular endothelial cells, accumulating in a positive feedback aggregation cascade at sites of acute vascular damage induced by N-generated local hyperthermia, and subsequently secreting nanosized proplatelets (nPLTs) to transport active components to deep tumor tissue. The immunostimulator augmented the immunogenicity of antigens released from ablated tumors, inducing a stronger immunological response to attack residual, metastatic, and recurrent tumors. Following activation by low-power near-infrared light irradiation, the photothermal and immunological components synergistically provide exceptionally high therapeutic efficacy across nine murine models that mimicked a range of clinical requirements, and, most notably, a sophisticated model based on humanized mouse and patient-derived tumor xenograft.

INTRODUCTION

Long-standing issues with efficacy and adverse side effects that accompany traditional anticancer strategies, such as chemotherapy and radiotherapy, have led to an urgent call for exploration and development of new high-performance modalities. Recently, photothermal therapy (PTT) has attracted increasing attention for its particular advantages of minimal invasiveness, spatiotemporal precision, reproducible activity, negligible acquisition of drug resistance, and phototoxicity confined to the targeted regions (1–4). Considering the less biological toxicity (5) and superior tissue penetration depth (e.g., more than 1 mm upon 808-nm irradiation) (6), near-infrared light (NIR) is always used for PTT treatment. The photoenergy can be converted to heat by a photosensitizer (such as indocyanine green, Au nanorods, and copper sulfide) through vibration relaxation, surface plasmon resonance, or lattice structure (7, 8), and the resulting hyperthermia at the tumor site can efficiently kill tumor cells. To improve the accumulation of photosensitizers specifically at tumor sites, many types of nanoscaled vectors have been developed to use the enhanced permeability and retention effect of stochastic leakage from the abnormal openings and defects in tumor vessels (9–11). The further functionalization of targeting ligands has enabled the further endowment of these nanovectors with an active affinity to tumor cells with active affinity to tumor cells (12, 13), thereby offering the potential to optimize anticancer potency.

Although promising, nanovector-based PTT still faces a series of critical issues. For example, current methods for active targeting are highly reliant on successful identification of the receptors specifically expressed on tumor cells. Unfortunately, cancer heterogeneity, especially in the unstable and nonhomogeneous expression of these receptors during tumor development and/or among different tumors and patients, substantially compromises targeting efficiency (14, 15). Furthermore, intratumoral penetration by nanovectors is restricted by the compact extracellular matrix and its associated abnormally high interstitial pressure (16, 17). For this reason, complete ablation of large tumors is exceedingly difficult in most scenarios because of the presence of residual tumor mass at the untreated margins (18). In addition, the main site of action traditional monomodal PTT is confined to local tumors that are accessible to the triggering light but does not affect disseminated metastatic tumors that are beyond the range of action. Thus, to improve these approaches, it is crucial to develop new PTT-based agents that are reliably enriched at and penetrate tumor sites and that show increased therapeutic efficacy via additive or even synergistic effects (19–21).

Recently, the platelet (PLT) has been used as an effective anticancer delivery vector via several mechanisms, such as blood vessel endothelium adhering, surgery damage–induced aggregation, and nanosized vesicles secreted after activation (22–28). For example, the PLT was used to realize the target delivery of antibodies to tumor via vascular adhesion, thus inhibiting the tumor growth (25). Wang et al. (27) also reported that engineered PLTs decorated with anti-PDL1 could be activated and aggregated at surgery wound for preventing postsurgical tumor recurrence. In yet another example, the activated engineered PLTs could secrete checkpoint inhibitor–containing nanosized vesicles, which significantly enhanced antileukemia immune responses (28). These innate physiological features thus vote for the Advancement of Science. No claim to original U.S. Government Works. Distributed under a Creative Commons Attribution NonCommercial License 4.0 (CC BY-NC).
Inspired by these studies on the PLT-based drug delivery and synergistic mechanisms of PTT and immunotherapy, we here report the development of a biomimetic PLT arsenal for combined cancer therapy (fig. S1). For this construction, a block copolymer, naphthalene diimide–bithiophene derivative (NDI-BT), is designed as the photothermal material, and then, the photothermal nanoparticles (N) are synthesized and imported into PLTs together with the immunostimulator R837 hydrochloride (R) to construct the engineered PLTs (N+R@PLTs). After intravenous injection, N+R@PLTs function as circulating sentinels in the bloodstream and have a sensitive response to vascular damage. As the junctions between vascular endothelial cells in the vicinity of tumor tissue are always weakened by defects, a proportion of N+R@PLTs can act as spearheads to prime adhesion at these vascular endothelial cells, thus initially transporting the N+R cargos to the tumor vicinity through a natural route. After irradiation with NIR, local hyperthermia results in acute vascular damage, which subsequently induces an aggregation cascade to form an occlusion at the tumor vessel. In this respect, many more reinforced PLTs are potentially recruited in a feedback-dependent manner, enabling the further accumulation of N+R cargos to form an arsenal in situ. Subsequently, nanosized proplatelets (nPLTs) are further generated from the plasma membrane upon these activated PLTs, which relay the cargos into deep tumor tissue, expanding the area of attack (37, 38). Following tumor ablation induced by PTT, the immunogenicity of the released tumor-associated antigens induces the body’s immunologic response to attack residual, metastatic, and recurrent tumors. This effect is markedly improved with the assistance of the immunostimulator R. We systematically verified the abovementioned merits of PLTs and the synergy of N and R in vivo and demonstrated potent therapeutic effects in nine different murine models. Most notably, we also demonstrated the efficacy of the N+R@hPLT arsenal, using human PLTs (hPLTs), in a sophisticated model based on humanized mouse and patient-derived tumor xenograft (PDX). Together, these results showed great promise for utilization of this biomimetic PLT platform in high-performance and combined anticancer therapies.

RESULTS AND DISCUSSION

Synthesis of photothermal polymer nanoparticles

To construct the target biomimetic N+R@PLT platform, we first fabricated nanoparticles with high photothermal conversion efficiency (Fig. 1A). Specifically, a typical photothermal copolymer (NDI-BT) was synthesized by integrating thiophene and naphthalene diimide moieties into the polymer skeleton via the Suzuki reaction [number-average molecular weight (Mn) = 163473; fig. S2A]. Subsequently, hybrid nanoparticles were constructed with this photothermal copolymer residing in the hydrophobic core and with hydrophilic polyethylene glycol (PEG) segments of distearoyl phosphoethanolamine (DSPE)–PEG, an amphiphilic polymer, decorating the surfaces via a typical coprecipitation strategy. The resulting nanoparticles exhibited an well-defined spherical shape with an average hydrodynamic diameter of approximately 50 nm, with appreciable monodispersity and negative surface charge (Fig. 1B and fig. S2, B and C). In addition, the nanoparticles showed little cytotoxicity to normal cells, which ensured the safety of administering them in vivo (fig. S3, A and B).

The capability of these hybrid nanoparticles for capturing and converting NIR light to heat was then evaluated by acquiring the temperature alternation of the aqueous nanoparticle dispersion samples upon irradiation of an 808-nm laser at a low power (0.65 W/cm²). In a sample with a nanoparticles concentration of 100 μg/ml, 5-min exposure to the laser generated temperature up to 60°C (Fig. 1C), indicating that these nanoparticles had the capacity for superb photothermal conversion. Specifically, we determined that the photothermal conversion efficiency (η) of our nanoparticles was 69.2% (fig. S2D), considerably higher than η values of previously reported photothermal agents, such as Cu2-xSe (22.0%) (39), Cu2S (25.7%) (40), polypyrrole nanoparticles (45.0%) (41), dopamine-melanin nanoparticles (40.0%) (42), MoS2 nanosheets (24.4%) (43), carbon dots (58.2%) (44), and Cu2S–SiO2(OH)2 (48.3%) (45).

This exceptional capacity for photothermal conversion was directly attributable to its high efficiency for harvesting light and the low radiation-based energy loss of the photoactive polymer nanoparticles developed here. Specifically, the alternating donor-acceptor system with thiophene rings as the donors and naphthalene diimide as the acceptor and the appropriate donor system size together intrinsically imparted relevant excitation with predominant charge transfer (46), which enabled efficient light-harvesting capacity. In addition, the low radiation-based energy loss, related to the appropriate optical gap of the polymer component, and the aggregation-induced quenching in nanoparticles jointly contribute to the high efficiency of light-to-heat conversion (47). This photothermal performance of our nanoparticles (100 μg/ml) could be reliably maintained upon irradiation of a laser (0.65 W/cm²) alternatively switched on and off (Fig. 1D), thus indicating highly consistent thermostability and photostability. In addition, in an aqueous medium, these nanoparticles generated unequivocal photoacoustic signals with an intensity that was in perfect linearity with nanoparticle concentration (Fig. 1E), implying their potential for photoacoustic imaging guidance in vivo.

Construction and characterizations of N+R@PLTs

To functionalize the photothermal nanoparticles with additional PLT responsiveness and immunogenicity, the nanoparticles were then decorated with biotin, while CD42a on the PLT membrane was pretreated with avidin-labeled anti-CD42a antibody. We hypothesized that the highly specific interaction between biotin and avidin would trigger the binding of nanoparticles to anti-CD42a antibodies, promoting the internalization of the nanoparticles into PLTs via the CD42a molecules, which we verified by detection of the nanoparticles inside PLTs using cell-slice transmission electron microscopy (TEM) imaging (yellow arrows in Fig. 1F, compared with PLT shown in fig. S3C). The immunostimulator R837 hydrochloride uniformly dispersing in the medium was also imported during the internalization process, resulting in the simultaneous presence of N and R signals in the superresolution PLT images (Fig. 1G). Specifically, our calculations suggested that approximately 60 nanoparticles and 4.8 million R837 hydrochloride molecules were loaded into each PLT (fig. S2E). Such an internalization had almost no influence on the size and surface charge of PLTs and faithfully retained the photothermal and photoacoustic properties (fig. S2, C and F, and Fig. 1, H and I).

Regarding the PLT physiology, no discernible hemolysis was observed after incubating red blood cells with the engineered PLTs (fig. S3, D and E), indicating their good biocompatibility. In addition, the aggregation behavior of N+R@PLTs after activation was also evaluated by flow cytometry (FCM) (Fig. 1J) and fluorescence imaging strategies (fig. S3F). In a typical protocol, two parallel samples prepared from the same batch of PLT formulation were labeled
with two kinds of dyes and then used for aggregation analysis. We found that both N@PLT and N+R@PLT samples displayed an unequivocal increase in aggregation signal upon adenosine diphosphate treatment. This finding was highly consistent with the characteristic responsiveness of natural PLTs, indicating that PLTs retained their natural response function after loading with photothermal nanoparticles and immunostimulator molecules.

Further evidence for this responsiveness was provided by the TEM images (Fig. 1K, left), showing that resting-state N+R@PLTs were circular with no discernible pseudopodia, while active-state
N+R@PLTs became more dendritic and expansive, similar to natural PLTs. In addition, activation led to the generation of abundant nPLTs, which was also verified by TEM and scanning electron microscopy (SEM) (Fig. 1K, left inset, and fig. S3G) and by FCM data showing an increased signal for nanosized vesicles with high PLT marker CD62P expression (Fig. 1K, middle). Within nPLTs, NDI-BT nanoparticles (N) were observed (fig. S3H). Meanwhile, the characteristic signals for N and R were also identified in nPLTs (Fig. 1K, right), indicating that these two components could be entrapped within nPLTs for further transport.

**In vivo targeting and photothermal performance**

Having successfully constructed N+R@PLTs, we next investigated their fate and performance in vivo using mice xenografted with 4T1 triple-negative breast cancer tumors (Fig. 2A). Considering that heat diffusion resulting from the excessive temperature potentially confers collateral damage to healthy, adjacent tissues, we used a gentle PTT approach, i.e., a single NIR irradiation at a low power (0.65 W/cm²), for subsequent experiments. Following intravenous injection, N+R@PLTs showed a circulation time similar to that of natural PLTs, which was much longer than that of naked photothermal nanoparticles (fig. S3, I and J). This relatively long persistence period facilitated the function of N+R@PLTs as circulating sentinels in the bloodstream.

Owing to the adhesion receptors on PLTs (such as C-type lectin-like receptor 2, intercellular adhesion molecule 1, and glycoprotein) and “platelet margination” effect in the bloodstream (48–51), N+R@PLTs were endowed natural sensitivity to vascular damage in the vicinity of a tumor. As a result, a few N+R@PLTs spearheaded to tumor sites with a peak time of 1 hour (fig. S4, A and B). Upon local NIR
irradiation at 1 hour, we observed a gradual increase in signal intensity, and the peak time was extended to 8 hours (Fig. 2B and fig. S4B). Similar results were also observed via photoacoustic imaging that used the photoacoustic properties of these nanoparticles. For example, following a 10-min irradiation treatment, the photoacoustic signal at the tumor site increased 10-fold over the initial signal, suggesting that many more N+R@PLTs had accumulated in response to the acute vascular damage caused by local hyperthermia (Fig. 2C). For verification, we evaluated N+R@PLT fates by multiphoton confocal microscopy (Fig. 2D), which showed that, initially, the first detectable N+R@PLTs attached to the tumor vessel wall, attributable to their affinity for tumor vascular endothelial cells. After NIR irradiation, we observed an occlusion in the tumor vessel, suggesting substantial N+R@PLT aggregation.

Given that nPLTs secreted by the activation of PLTs could extravasate from tumor vessel and further penetrate into the depths of tumors (fig. S4C), the N and R cargos entrapped in these nanovesicles could thus be transported into the deep tumor tissue, which was demonstrated by the colocalization of N and R signals in immunohistochemical (IHC) tumor sections (Fig. 2E). This self-reinforcement and offspring generation produced a formidable arsenal around the tumor in addition to providing a mechanism to overcome the resistance to intratumoral infiltration that hinders traditional cell-mediated delivery systems. The expanded attack area thus induced potent hyperthermia throughout the solid tumor (Fig. 2F). Compared to the stable temperature in the natural PLT group, the temperature in the N (L) group showed a moderate increase (to 44°C) within 10 min because of the poor targeting performance (fig. S4, D and E). Notably, we observed that the temperature rapidly increased to 56°C in the N+R@PLTs (L) group (Fig. 2F), indicating the highest expression of heat shock protein (HSP) in tumor tissue (Fig. 2G).

Immunological response after photothermal treatment

Next, we evaluated the immunological response initiated by the PTT (Fig. 3A). To this end, we monitored the serum levels of typical cytokines [interleukin-6 (IL-6) and tumor necrosis factor–α (TNF-α)], their utility as indicators of the immunological response (Fig. 3B). As expected, treatment with N (L) had a minor effect on cytokine production because of the low accumulation of N at the tumor site and subsequent low photothermal effect. This effect was ameliorated in the N@PLTs (L) group because of the improved tumor accumulation via laser irradiation–induced PLT aggregation (fig. S4, D and E) and subsequent enhanced photothermal effect (Fig. 2F and fig. S3K). In the N+R@PLT group, the absence of laser irradiation excluded local hyperthermia effect and the moderate elevation of cytokine should only be attributed to the loaded R. With the synergism of these two aspects [N+R@PLTs (L)], the levels of both IL-6 and TNF-α further increased but remained within the normal range, indicating a safe and efficient immunological response.

Because serum cytokine levels peaked at day 3, this time point was chosen for detailed investigation in the tumor-draining lymph node (TLN), which lies immediately downstream of tumors and can be remodeled from immunosuppressive to immunostimulatory for anticaner immunotherapy. As shown in Fig. 3C, almost no sign of a tumor antigen (TA) was detected in the control group, and only a few signals were found in the N-based PTT [N (L)] group. In contrast, single-modality treatment with PTT [N@PLTs (L)] or immunostimulation (N+R@PLTs) could produce more TA signals scattered in dendritic cells (DCs). With the combination treatment [N+R@PLTs (L)], a large amount of exposed TA was transported to the TLN, increasing uptake by DCs, which was accompanied by the highest level of DC maturation (indicated by CD80 and CD86 in Fig. 3D). Correspondingly, using Ki67 staining, we detected abundant immune cell proliferation throughout the TLN (Fig. 3E). Further quantification by a carboxyfluorescein succinimidyl amino ester (CFSE) assay showed that ~84.9% of CD8+ T cells had proliferated in the N+R@PLTs (L) group, while the frequencies of proliferation in the other groups varied below 40% (Fig. 3F). As a result, many more CD8+ T cells from the immunostimulatory TLN infiltrated into the tumor in the N+R@PLTs (L) group (Fig. 3G), indicating a superior immunotherapeutic effect that complemented the PTT performance.

Therapeutic effects on various murine tumor models

In light of the strong performance by N+R@PLTs in these experiments, we next systematically evaluated the therapeutic efficacy of our engineered PLTs in different murine models to establish whether this treatment could accommodate a broad range of clinical anti-cancer requirements. In the first model, mice were inoculated subcutaneously in the left flank with 4T1 cells and received a single treatment with different formulations on day 7 (Fig. 4A). Similar to the rapid development of tumors in the phosphate-buffered saline (PBS) group, tumor development observed in the N (L) group indicated the limited efficacy of this treatment due to the weak photothermal effect. In contrast, the anticancer effect was moderately ameliorated after treatment with a single biomimetic therapeutic modality, as in either the N@PLTs (L) group with local hyperthermia or the N+R@PLT group with immunostimulation, facilitated by its characteristic PLT aggregation cascade (Fig. 4B).

In the N+R@PLTs (L) group, the synergy of these two modalities was evident by the halted tumor proliferation, leading to an almost complete inhibition of tumor development and 100% survival over the 100-day time course (Fig. 4, C and D). In addition to assessing primary tumors, we also evaluated metastases to the lungs and bones, which were the preferred sites for breast cancer metastasis in the clinic. While metastatic foci in the lungs and metastasis-induced erosion in the tibias were found to varying extents in most groups, these effects were not observed in the combined therapy N+R@PLTs (L) group (Fig. 4E and fig. S8). Notably, safety evaluation study showed that few abnormalities in the organs, serum, coagulation capability, temperature, and weight were observed in tumor-bearing mice or non–tumor-bearing mice (figs. S6 and S7), thus confirming the safety of our biomimetic PLT platform.

To further confirm the superiority of photothermal anticancer therapies featuring engineered PLTs, we included in the comparison a typical photothermal agent Au nanorod (A), which was coated with PLT membrane (A@PM) or loaded into whole PLTs (A+R@PLTs) following previously reported PLT-based formulations (fig. S8) (35). To provide an equivalent comparison, the same adjuvant R837 hydrochloride was added in the groups of A+R@PM, A+R@PLTs (L), and A+R@PLTs (L) to match the combination immunotherapy (fig. S8, A and B). We found that the therapeutic effects of A+R@PLTs (L) and A+R@PLTs (L) were both significantly lower than that of our N+R@PLTs (L) (fig. S8, C to E). Specifically, following a single irradiation of 0.65 W/cm², only one of six mice survived after 4 weeks of A+R@PLTs (L) treatment. Although the survival rate of the A+R@PLTs (L) group could be improved to 50% after 4 weeks, the performance was still lower than that of the N+R@PLTs (L) group (with 100% mice surviving for 100 days). The substantially greater success
Fig. 3. In vivo immune responses after different treatments. (A) Schematic illustration of the immune responses after PTT. (B) Serum cytokine levels of mice receiving different treatments. ns, not significant. (C) Representative fluorescence images of TLN sections. Blue, cell nuclei; green, DCs; red, TA. (D) FCM analysis for DC maturation in TLNs (gated on CD11c^+ DCs). (E) Representative IHC analysis for Ki67 expression in TLNs. Blue, cell nuclei; red, Ki67. (F) FCM analysis for CD8^+ T cell proliferation in TLNs. (G) Representative IHC analysis for the intratumoral infiltration of CD8^+ T cells. Blue, cell nuclei; green, CD8^+ T cells. Data in (B) represent means ± SD (n = 3). Statistical difference was tested using unpaired Student’s t test for the data at 72 hours in (B).
Fig. 4. N+R@PLT-based photothermal-immunological combined therapy for 4T1 primary tumors and hematogenous metastasis. (A) Schematic illustration of antitumor therapy for primary tumors. (B) Individual growth kinetics of primary tumors in different groups (n = 6). (C) Survival curves of the mice in (B). (D) Representative IHC analysis for Ki67 expression in tumors. (E) Representative photographs of spontaneously metastatic lung nodules (left) and representative computed tomography images of spontaneous tibia metastases (right). (F) Schematic illustration of advanced hematogenous metastasis model. (G) Representative bioluminescence images of tumors in different groups. (H) Quantitative analysis for bioluminescence signals of tumor and lung metastases. (I) Survival curves of the mice in (H). Data in (H) represent means ± SD (n = 6). The photographs of lungs in (E) were taken by Y.L. (Institute of Process Engineering, Chinese Academy of Sciences). Statistical difference was tested using the log-rank test for (C) and (I) and two-way ANOVA test for (H).
of the N+R@PLT-based PITT formulations [N+R@PLTs (L) group] could be attributed to the following improvements: First, the photothermal material ND1-BT nanoparticles displayed higher photothermal conversion efficiency (η ~ 69.2%), especially compared to that of Au nanorods (52.8%) (fig. S8, F to H). Therefore, a single NIR irradiation at a low power could efficiently induce local hyperthermia in the N+R@PLTs (L) group rather than the A+R@PM (L) group or A+R@PLTs (L) group (fig. S8I). What is more, the whole PLT formulation showed highly efficient targeting of tumor vessels for aggregation cascade upon natural PLT activation, which continuously accumulated, in a feedback-dependent manner, to sites of acute vascular damage caused by local hyperthermia. In addition, the nPLT “offspring” secreted by the PLT arsenal at the tumor site exhibited high capability for penetration of deep tissues compared to adherence-based strategies. In contrast, the coated membrane in the A+R@PM (L) group lacked these capacities of self-reinforcement and nPLT production, but only targeted tumor vessels via an adhering strategy (fig. S8J).

To mimic a more advanced metastatic process, we established a hematogenous metastasis model for further investigation. Typically, mice xenografted with 4T1 tumors received a single treatment with different PLT formulations on day 7, followed by another intravenous injection of 4T1 cells on day 8 (Fig. 4F). As these 4T1 cells were preengineered with luciferase expression (denoted as Luc-4T1), the development of the primary tumor and lung metastases could be simultaneously monitored by detection of bioluminescence (Fig. 4, G and H). Compared to the PBS control group, both the photothermal [N@PLTs (L)] and immunological (N+R@PLTs) therapy alone groups had a moderate effect on the suppression of signals corresponding with the primary tumor and lung metastases. In contrast, the combination therapy of N+R@PLTs (L) resulted in the disappearance of bioluminescence signals at both the primary and metastatic sites (fig. S9A), thus indicating the complete eradication of tumor cells throughout the body. Correspondingly, the N+R@PLTs (L) treatment group exhibited a 100% survival rate after 100 days, whereas all mice in the other treatment groups died within 4 to 7 weeks (Fig. 4I).

To further explore the potential for activation of photothermal-immunological synergism in physically distant tumors, we continued to use a dual-tumor model (Fig. 5A) wherein the tumor in only one flank received a single treatment with different PLT formulations. Historically, this dual-tumor model has posed a challenging obstacle for conventional photothermal strategies because these treatments generally require direct access to the source of NIR for effective treatment. We found that under conditions of a single NIR irradiation at low power, N@PLTs (L) treatment only induced a moderate inhibitory effect on tumor development at both the primary and distant tumor sites (Fig. 5B), as was similarly observed in N+R@PLT immunostimulation–only mice. Correspondingly, mice in these two groups died rapidly on approximately day 40. In contrast, both primary and distant tumors were completely inhibited in the N+R@PLTs (L) group, and all mice remained alive after day 100, indicating the necessity for synergism to achieve a potent therapeutic effect (Fig. 5C).

Given that the immune response is usually associated with a characteristic durable memory effect that is critical for a favorable cancer prognosis, we continued to evaluate the long-term anticancer immunity induced by our engineered PLT platform (Fig. 5D). As a control, challenging healthy mice with 4T1 cells led to induction of rapid tumor development and gradual death within the following 4 weeks (Fig. 5E). When mice were xenografted with 4T1 tumors and received a single treatment with N+R@PLTs (L), we observed a significant increase in effector memory T cells in the spleen even after 50 days (Fig. 5F). After establishing long-term immune memory, subsequent rechallenge with 4T1 tumor cells resulted in complete inhibition of tumor formation in the contralateral flank and 100% survival at 100 days (Fig. 5G).

As a common clinical problem, residual tumor cells missed by surgical excision frequently result in tumor recurrence (35, 52). To address this issue, we next investigated the applicability of the PLT platform for the prevention of postsurgical recurrence. To this end, we established a recurrence model by surgically resecting most of the primary tumor (Fig. 5H). Correspondingly, the signal intensities of residual Luc-4T1 cells were almost equal across treatment groups after the surgical operation. In agreement with previous experiments, the bioluminescence signal continued to spread in the PBS group during the following 2 weeks, resulting in a 0% survival rate due to recurrence of the malignant tumor (Fig. 5I). However, the N+R@PLTs (L) regimen, performed immediately after the incomplete surgical resection, resulted in complete disappearance of the residual bioluminescence signal within 1 week (fig. S9B). Correspondingly, all mice remained tumor-free, with a 100% survival rate over the course of a long-term (100 days) observation period. In addition to 4T1 breast cancer, we also verified this potent anti-recurrence effect in models for CT26 colorectal carcinoma, B16 melanoma, Lewis lung carcinoma (LLC), and hepatoma-22 (H22) liver cancer, thereby verifying the universality of anticancer efficacy for the PLT-based biomimetic platform (Fig. 5, J to M).

**Construction of hPLT-based formulation and verification of efficacy in a sophisticated humanized PDX model**

To further confirm the clinical applicability of our PLT platform, we constructed an hPLT platform (N+R@hPLTs) and evaluated its therapeutic effect in a sophisticated humanized PDX model (Fig. 6A). Initially, CD45⁺ hematopoietic stem cells isolated from human umbilical cord blood were transferred into NOD.Cg-Prkd⁻cd11b⁻H2rg⁻imLyIvSt⁻ Vst (NPG) mice for reconstruction of the human immune system (fig. S10A). Concurrently, a primary tumor sample was resected from a patient with breast cancer. After engraftment for three passages, the tumor sample was subcutaneously transplanted into the axilla of the humanized mice to establish the humanized PDX model. The construction of human N+R@hPLTs followed the same method as mouse N+R@PLTs except that mouse PLTs were replaced by hPLTs. Imaging by structured illumination microscopy (SIM) demonstrated the successful construction of N+R@hPLTs (Fig. 6B), and results of FCM analysis confirmed the aggregation behavior of N+R@hPLTs after activation (Fig. 6C).

Subsequently, we used N+R@hPLTs to treat the humanized PDX mice and found that the fluorescence signal of the PDX tumor gradually increased upon local NIR irradiation, consistent with our observations of mouse N+R@PLT administration (Fig. 6D and fig. S10B). We also observed thrombi in the PDX tumor after laser irradiation, while the blood vessel lumen was clean in the control group (Fig. 6E), which directly demonstrated the aggregation cascade behavior of N+R@hPLTs after laser irradiation. Therefore, the self-reinforcing behavior of N+R@hPLTs led to comparable photothermal efficacy in humanized PDX tumors as that found for N+R@PLTs in the 4T1 tumor model (Fig. 6F and fig. S10C). We further evaluated the immunological response activated by N+R@hPLTs (L). Compared with the PBS group, the combined therapy induced a significant
increase in serum levels of IL-6 and TNF-α cytokines (Fig. 6G), as well as substantially greater levels of tumor-infiltrated CD8⁺ T cells (Fig. 6H), indicating a strong immune response caused by the light-induced cascade responses. Because of the comparatively superior performance in both photothermal effect and immunotherapeutic effect, we found that 100% of the humanized PDX mice remained alive and tumor-free, with few abnormalities in serum, following N+R@hPLT administration (Fig. 6, I and J, and fig. S10D).

In summary, we have developed a new type of biomimetic delivery system by loading photothermal nanoparticles and the immunostimulator R837 hydrochloride into PLTs. This design incorporates recent nano/biotechnology to overcome long-standing obstacles in ferrying therapeutics to target sites and bypass in vivo complexities. The nature of PLTs enables them to function as an arsenal by first adhering to defective tumor vascular endothelial cells and then by subsequent aggregation cascade, accumulating in...
Fig. 6. Characterization of N+R@hPLTs and corresponding antitumor effects in a sophisticated model based on humanized mouse and PDX. (A) Schematic illustration of experimental plan used to evaluate antitumor activity in vivo using the sophisticated model. (B) SIM image of N+R@hPLTs. Red, hPLTs; green, nanoparticles (N); blue, adjuvant R837 hydrochloride (R). (C) FCM analysis for the aggregation behavior of hPLTs and N+R@hPLTs in vitro. (D) In vivo animal fluorescence images of N+R@hPLTs in tumors with or without laser irradiation for different periods. (E) H&E staining of tumor sections from humanized PDX mice that received laser irradiation or not. Dashed red lines indicate thrombi in vessels. (F) Representative in vivo IR thermal images of humanized PDX mice under laser irradiation after intravenous injection with hPLT-based formulations. (G) Serum cytokine levels in humanized PDX mice 3 days after administration of N+R@hPLTs or PBS buffer. (H) Representative IHC analysis for intratumoral infiltration of human CD8+ T cells. Green, CD8+ T cells. (I) Individual tumor growth kinetics of PDX tumors after treatment with N+R@hPLTs or PBS buffer. (J) Survival curves of the humanized PDX mice in (I). Data in (G) represent means ± SD (n = 3). Statistical difference was tested using unpaired Student’s t test for (G) and log-rank test for (J).
a feedback-dependent manner at the site of acute vascular damage caused by NIR-induced local hyperthermia. Ultimately, nPLTs are secreted to transport N and R cargos into deep tumor tissue. Thus, photothermal-immunological synergism is achieved with even a single, low-power treatment of NIR irradiation. As a result, we observed safe and high-performance therapeutic efficacy across nine different murine models that matched a broad range of clinical requirements, including long-term survival, and efficacy against secondary and metastatic tumors. Moreover, administration of this PLT platform in a sophisticated model based on humanized mouse and PDX showed significant clinical therapeutic efficacy for our PLT-based biomimetic formulation. Together, these results strongly suggest that our PLT-based platform offers a promising combination therapy modality for potentially wide clinical adoption in the treatment of a wide range of difficult and otherwise intractable cancers.

**MATERIALS AND METHODS**

**Materials**

Phycoerythrin (PE)/allophycocyanin (APC)–conjugated anti-mouse CD9, PE/APC-conjugated anti-human CD31, PE-conjugated antihuman CD3, eFluor 450/PE–conjugated anti-mouse CD8a, APC-conjugated anti-mouse CD80, APC/Cy7-conjugated anti-mouse CD86, and peridinin chlorophyll protein (PerCP) Cy5.5–conjugated anti-mouse CD62L antibodies were purchased from eBioscience (Beijing, China). Adjuvant R837 hydrochloride (R) solution was obtained from Gibco (Beijing, China). Adjutant R837 hydrochloride, HAuCl4, AgNO3, NaBH4, resveratrol, and cetyl trimethylammonium bromide (CTAB) were purchased from Sigma-Aldrich (Shanghai, China). PBS, Hepes solution, and enzyme-linked immunosorbent assay (ELISA) kit were purchased from Solarbio (Beijing, China). Other chemicals were purchased from J&K (Beijing, China) and used without purification.

**Cell lines**

4T1 murine breast tumor cells, Luc-4T1 murine breast tumor cells, B16 murine melanoma LLC cells, H22 cells, and murine CT26. WT colon tumor cells were purchased from Peking Union Medical College Hospital (Beijing, China). 4T1, Luc-4T1, B16, H22, and CT26.WT cells were cultured in RPMI 1640 medium, while LLC cells were cultured in DMEM. All the media were supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C with 5% CO2, and all the cells were passaged at approximately 80% confluency.

**Animals**

Four-week-old Balb/c mice and C57BL/6 mice (14 to 18 g, female) were obtained from Vital River Laboratories (Beijing, China). Six-week-old female NPG mice were purchased from Shanghai Model Organisms Center Inc. The animal protocol was approved by the Institutional Animal Care and Use Committees at the Institute of Process Engineering, Chinese Academy of Sciences.

**PLT extraction (53)**

Mouse whole blood was harvested by retro-orbital puncture and collected in heparinized mouse blood collection tubes. The whole blood was diluted with 2× volume of Hapes medium. The diluted blood was centrifuged for 10 min at 100g, and the collected PLT-rich plasma (PRP) was diluted further in Hapes medium. PRP was centrifuged for 5 min at 2400g, and the white precipitate was pure PLTs.

**Synthesis of the photothermal polymer**

The photothermal polymer NDI-BT was synthesized via the Suzuki reaction. 4,9-DibromoMo-2,7-bis(2-oclyldecyl)benzo[1Mn][3,8]phenanthroline-1,3,6,8(2H,7H)-tetrone and 2,2-bithiophene-5,5-diboronic acid bis(pinacol) ester were polymerized under anhydrous conditions in a N2 atmosphere. The NDI-BT polymer was characterized by gel permeation chromatography. The Mn value of NDI-BT was 163,473.

**Synthesis of photothermal nanoparticles (N)**

Photothermal nanoparticles were prepared by the coprecipitation method as described previously (6, 54). In total, 1 mg of NDI-BT polymer and 2 mg of surfactant mixture (DSPE-PEG2000:DSPE-PEG2000-biotin = 9:1) were dissolved in 100 μl of tetrahydrofuran (THF). The solution was rapidly injected into 1 ml of ultrapure water under ultrasonic conditions. Then, the THF was removed by N2 flow.

**Construction and characterization of PLT biomimetic N+R@PLTs**

PLTs (1 × 108) were resuspended in 1 ml of PBS, incubated with a 1:100 dilution of a biotinylated anti-CD42a monoclonal antibody for 30 min, and then washed with PBS. Afterward, PLTs were incubated with avidin solution (2 mg/ml) for another 30 min and then washed again. Last, PLTs were incubated with 100 μg of photothermal nanoparticles (which had been linked to biotin) in 1 ml of PBS for another 30 min and then washed to obtain N@PLTs. During the incubation, an adjuvant imiquimod hydrochloride (R) solution was added into the medium in a final concentration of 80 μM to obtain N+R@PLTs. The final loading efficiencies of N and R were about 4.4 and 10.2%, respectively. The concentration of N+R@PLTs in photothermal effect study in vitro was 1 × 1010 N+R@PLTs/ml (~43.8 μg/ml or ~44.5 μM for N). For animal experiments, the doses of PLTs, N, and R were 1 × 1010 cells per mouse, ~4.4 μg per mouse, and 2.0 μg per mouse, respectively. In SIM image, R was replaced by a blue-emitting dye, coumarin derivative. The size and surface potential of N, PLTs, and N+R@PLTs were detected by dynamic light scattering (Malvern ZEN 3600 Zetasizer, UK).

**In vitro photothermal effect of N+R@PLTs**

PLTs, N@PLTs, and N+R@PLTs were resuspended in 1 ml of PBS at 1 × 1010 PLTs/ml, and N was suspended in 1 ml of PBS at 43.8 μg/ml (44.5 μM) in centrifuge tubes. Then, these tubes were irradiated upon 0.65 W/cm2 NIR laser for 10 min, and the thermal images were taken at 0, 1, 3, 5, and 10 min.

**Characterization of the aggregation ability of N+R@PLTs**

The aggregation ability of N+R@PLTs was measured by FCM and fluorescence microscopy (53). For FCM, PLTs in each group were divided into two portions, and either portion was modified with anti-CD9 APC- or PE-conjugated antibodies. The mixture of the two portions in each group was measured by FCM to obtain the aggregation rate of resting-state PLTs. Afterward, PLTs were activated by adenosine diphosphate and then evaluated to measure the aggregation behavior of the active-state PLTs. FCM analysis was performed using a BD Aria III flow cytometer and analyzed using FlowJo 7.6.
For fluorescence microscopy, confocal laser scanning microscopy (CLSM; UltraVIEW VoX, PerkinElmer, USA) was used. Samples were pretreated similar to how they were prepared for FCM. The statistics of aggregation were analyzed in the same field of vision.

Morphological characterization of the N+R@PLT activation
The N+R@PLT activation was confirmed by SEM and TEM to observe the nPLT secretion and contents. N+R@PLTs were activated by incubation in PBS containing thrombin (2 U/ml) at 37°C for 30 min. After activation, N+R@PLT suspensions were centrifuged at 800g for 10 min. The supernatants were collected for imaging by a TEM (JEOL, Japan) to observe nPLTs. Meanwhile, both the resting-state and active-state N+R@PLTs were resuspended in 4% (w/v) glutaraldehyde solution and incubated at 37°C for 30 min. After washing three times with PBS, N+R@PLTs were incubated in 20, 50, 80, and 100% (v/v) alcohol in sequence for 5 min in each concentration. Last, N+R@PLTs were imaged with a SEM (JEOL, Japan).

Hemolytic study of different formulations
The blood of Balb/c mice was harvested by retro-orbital puncture and collected in heparinized mouse blood collection tubes. Then, 2 ml of the whole blood was washed by mixing with 4 ml of PBS, followed by centrifugation at 10,000g for 10 min. The washing procedure was repeated until the supernatant was clear. The obtained cells were resuspended in 20 ml of PBS. Then, 0.2 ml of cells was mixed with 0.8 ml of different formulations (water, PBS, PLTs, N@PLTs, and N+R@PLTs). The water was added as positive control, while the PBS was added as negative control. After 4-hour incubation, these mixtures were centrifuged at 10,000g for 10 min followed by photographing and detecting the optical density (OD) of supernatant at 577 nm using Tecxan (Infinite M200). The hemolytic percent was calculated as follows

\[
\text{Hemolytic percent} = \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Negative}}}{\text{OD}_{\text{Positive}} - \text{OD}_{\text{Negative}}} \times 100\%
\]

The microphotograph was imaged by CLSM (Leica). The photographs in fig. S3 were taken by Y.L. (Institute of Process Engineering, Chinese Academy of Sciences).

Synthesis of Au nanorods (55)
The reagents were added into 3.9 ml of H2O in the following order: 5 ml of CTAB, 500 μl of HAuCl4, 95 μl of AgNO3, and 500 μl of resveratrol. Then, 15 μl of NaBH4 was added after the solution was mixed. Last, the whole solution was slowly heated to 65°C and maintained for 3 hours to obtain Au nanorods.

Construction of A+R@PM
The mouse PLTs were incubated in 0.25× PBS, followed by centrifugation at 9000 rpm for 10 min to obtain the PLT membrane. Meanwhile, the synthesized Au nanorods were incubated with R837 hydrochloride for 1 hour at room temperature to obtain A+R. Last, the PLT membrane and A+R were coexcluded through a porous membrane to form A+R@PM. The concentration of A was 8.8 μg/ml (44.5 μM) in photothermal effect in vitro, which was equal to the concentration of N in the same study. The dose of A was 0.9 μg per mouse (~4.5 nmol per mouse) in animal experiment.

Construction of A+R@PLTs
The construction of A+R@PLTs followed the methods reported previously (35). Briefly, 1 × 109 PLTs were mixed with 1 ml of PBS containing 50 μg of Au nanorods. Then, the mixture was treated in an electroporation process under normal conditions for a few seconds. The final A+R@PLTs contained about ~4.5 × 10−9 nmol Au per PLT, which was equal to the concentration of N in N+R@PLTs. The concentration of A+R@PLTs in photothermal effect study in vitro was 1 × 1010 A+R@PLTs/ml (8.8 μg/ml, 44.5 μM for A). For animal experiments, the dose of PLTs and A was 1 × 109 cells per mouse and 0.88 μg per mouse (~4.5 nmol per mouse), respectively.

Characterization of N+R@PLT activation behavior
The activation behavior of PLTs was analyzed by TEM and FCM. After activation, PLTs were centrifuged, and the supernatant was analyzed by FCM. The nanoplatelets were labeled with an anti-CD62P Cy5.5 antibody.

Fluorescence imaging of frozen sections of nPLTs
For nPLT penetration, Balb/c mice were subcutaneously injected with 1 × 106 4T1 cells per mouse in the right flank. When the tumors reached 200 mm3, these 4T1 tumor–bearing mice were injected with 1 × 107 N+R@PLTs and then randomly divided into two groups: One group was irradiated at the tumor site with NIR laser (0.65 W/cm2, 5 min) at 1 hour after N+R@PLT injection, while the other group was not. Twelve hours later, the tumors of both groups were collected and frozen in optimum cutting temperature (OCT) tissue compound (Sakura, Tokyo, Japan) on dry ice and then sectioned into 10-μm slices. The obtained tumor slices were incubated with CD31 and CD62P antibodies, followed by orderly staining with fluorescence secondary antibody and 4′,6-diamidino-2-phenylindole (DAPI) solution (0.1 μg/ml). Last, the slices were imaged with an automatic multispectral imaging system (PerkinElmer).

For R, N, and nPLT colocalization, Balb/c mice were treated in the same way as mentioned above except that the tumor-bearing mice were injected with N+R@PLTs in which R and N were labeled as mentioned in SIM experiment. These mice were randomly divided into two groups after injection: One group was irradiated with 808-nm NIR laser at the tumor site at 1 hour after injection, while the other group was not. Twelve hours later, the tumors of both groups were collected and frozen in OCT tissue compound on dry ice and then sectioned into 10-μm slices. The obtained tumor slices were incubated with CD31 and CD62P antibodies, followed by orderly staining with fluorescence secondary antibody and DAPI solution (0.1 μg/ml). Last, the slides were imaged with an automatic multispectral imaging system (PerkinElmer).

In vivo aggregation of N+R@PLTs in tumor tissue
The aggregation behavior of PLTs in vivo was tested with an animal imaging system [labeled with 1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine iodide (DiR), tested by Kodak, FX Pro], a real-time multispectral optoacoustic tomographic imaging system (MOST inVision 128, iThera, Germany), and a multiphoton laser confocal scanning fluorescence microscope (labeled with a two-photon dye, tested by FV1200MP-E-M, Olympus, Japan). After an injection (1 × 109 PLTs per mouse) and 808-nm laser irradiation, mice were imaged at 0, 5, and 10 min.
In vivo photothermal effect of N+R@PLTs in 4T1 tumor model

Balb/c mice were inoculated subcutaneously with $5 \times 10^5$ 4T1 cells in the left flank and then randomly divided into three groups: PLTs (L), N (L), and N+R@PLTs (L). On day 7, the mice in these groups were intravenously injected with $1 \times 10^9$ PLTs, 4.4 μg of N, and $1 \times 10^9$ N+R@PLTs. One hour later, these mice were irradiated at the tumor site with an 808-nm laser (0.65 W/cm$^2$). The thermal images were taken at 0, 1, 2, 4, 6, 8, and 10 min. Twenty-four hours after laser irradiation, the tumors of each group were individually collected, frozen in OCT tissue compound (Sakura, Tokyo, Japan) on dry ice, and then sectioned into 10-μm slices. Then, the slices were incubated with anti-HSP antibody at 4°C overnight followed by staining with fluorescence-labeled secondary antibody at room temperature for 1 hour. After staining with a DAPI solution (0.1 μg/ml), the slices were imaged with an automatic multispectral imaging system (PerkinElmer).

Tumor-associated antigen uptake by DCs

Balb/c mice were inoculated subcutaneously with $5 \times 10^5$ Luc-4T1 cells in the left flank and then randomly divided into five groups. On day 7, the mice in these groups were intravenously injected with PBS, N, or N+R@PLTs. In groups N (L), N@PLTs (L), and N+R@PLTs (L), the mice were anesthetized at 1 hour after injection, and the tumors were illuminated with an 808-nm laser (0.65 W/cm$^2$, 5 min). After 72 hours, the inguinal lymph nodes of each mouse were individually collected, frozen in OCT tissue compound (Sakura, Tokyo, Japan) on dry ice, and then sectioned into 10-μm slices. These slices were incubated with an anti-CD11c antibody and anti-luciferase antibody at 4°C overnight and then incubated with corresponding fluorescent secondary antibodies at room temperature for 1 hour. Last, after staining with a DAPI solution, the slices were imaged with an automatic multispectral imaging system (PerkinElmer).

In vivo DC maturation

Balb/c mice were inoculated subcutaneously with $5 \times 10^5$ 4T1 cells in the left flank and then randomly divided into five groups. On day 7, the mice in these groups were intravenously injected with 100 μl of PBS, 4.4 μg of N, or $1 \times 10^9$ N+R@PLTs. For groups N (L), N@PLTs (L), and N+R@PLTs (L), the mice were anesthetized at 1 hour after injection, and the tumors were illuminated with an 808-nm laser (0.65 W/cm$^2$, 5 min). After 72 hours, the inguinal lymph nodes of each mouse were individually collected and triturated into single-cell suspensions. Then, the cells were stained with FITC-conjugated anti-mouse CD11c, PE-conjugated anti-mouse CD40, APC-conjugated anti-mouse CD80, and APC/Cy7-conjugated anti-mouse CD86 antibodies. After washing, the cells were dispersed in 500 μl of PBS and analyzed by FCM.

In vivo CD8$^+$ T cells proliferation

For fluorescence imaging of frozen sections, mice were given the same treatments as those in the DC maturation study, and lymph node sections were prepared as described above. Then, the sections were labeled with an anti-Ki67 antibody followed by a fluorophore-labeled secondary antibody and then analyzed with an automatic multispectral imaging system (PerkinElmer).

For CFSE-based FCM analysis, mice were given the same treatments as those in the DC maturation study, and then, T cells from the spleens and lymph nodes of the mice in each group were labeled with CFSE. Then, CFSE-labeled T cells were intravenously injected into the mice that had been treated in the same way as the mice from which T cells were derived. Three days later, the inguinal lymph nodes and spleen of each mouse were collected and triturated into single-cell suspensions. Then, after labeling with an APC/Cy7-conjugated anti-mouse CD3 antibody and a PE-conjugated anti-mouse CD8a antibody and washing, the cells were dispersed in 500 μl of PBS and analyzed by FCM.

Intratumoral CD8$^+$ T cell infiltration study

Mice were given the same treatments as those in the DC maturation study. After 72 hours, the inguinal lymph nodes of each mouse were individually collected, frozen in OCT tissue compound (Sakura, Tokyo, Japan) on dry ice, and then sectioned into 10-μm slices. These slices were incubated with an anti-CD8 antibody at 4°C overnight and then incubated with fluorescent secondary antibodies at room temperature for 1 hour. Last, after staining with a DAPI solution, the slices were imaged with an automatic multispectral imaging system (PerkinElmer).

Antitumor studies in 4T1 tumor models

Balb/c mice were inoculated subcutaneously with $5 \times 10^5$ 4T1 cells in the left flank on day 0. Then, they were randomly divided into five groups. On day 7, these mice were given the same treatments as those described above in the DC maturation study. Then, tumor size, body weight, and body temperature were measured every 2 days, and the experimental end point was defined as either death or a tumor size greater than 1000 mm$^3$. Then, the lungs and hind legs of each mouse were collected at the experimental end point or on day 30 to study metastases originating from the primary tumor. Tibia metastases were imaged by computed tomography. The photographs in Fig. 4 were taken by Y.L. (Institute of Process Engineering, Chinese Academy of Sciences).

Anti-metastasis study with a Luc-4T1 tumor model

Balb/c mice were inoculated subcutaneously with $5 \times 10^5$ Luc-4T1 cells in the left flank and then randomly divided into four or two groups. On day 7, the mice were given the same treatments as those in the antigen uptake study described above. One day later, each mouse was intravenously injected with $3 \times 10^5$ Luc-4T1 cells, and lung metastases were imaged with IVIS Spectrum (PerkinElmer, USA) after an intraperitoneal injection of d-luciferin sodium salt at a dose of 3 mg per mouse at different time points. To facilitate imaging, each mouse was shaved before imaging.

Antitumor study with a distant-tumor model (dual-tumor model)

Balb/c mice were inoculated subcutaneously with $5 \times 10^5$ 4T1 cells in the left flank and then randomly divided into four groups. Seven days later, these mice were inoculated subcutaneously with $5 \times 10^5$ 4T1 cells in the right flank. On day 10, they were given the same treatments as those in groups PBS, N@PLTs (L), N+R@PLTs, and N+R@PLTs (L) in the DC maturation study described above. Then, the sizes of the left and right tumors were measured every 2 days, and the experimental end point was defined as either death or a tumor size greater than 1000 mm$^3$.

Long-term immune memory effects of N+R@PLTs

Balb/c mice were randomly assigned into two groups, and one group was inoculated subcutaneously with $5 \times 10^5$ 4T1 cells in the left flank, while the other was not. Seven days later, the mice with
tumors were given the same treatments as those in group v in the DC maturation study described above, and the other mice remained untreated. Fifty days later, the spleens and lymph nodes of some of the mice in each group were collected and triturated into single-cell suspensions. After labeling with an APC/Cy7-conjugated anti-mouse CD3 antibody, a PE-conjugated anti-mouse CD8a antibody, a PerCP Cy5.5–conjugated anti-mouse CD62L antibody, and an eFluor 450-conjugated anti-mouse CD44 antibody and washing, the cells were dispersed in 500 µL of PBS and analyzed by FCM. The other mice were inoculated subcutaneously with 1 × 10^5 4T1 cells in the right flank. Then, tumor size was measured every 2 days, and the experimental end point was defined as either death or a tumor size greater than 1000 mm³.

**Inhibitory effect on postoperative recurrence**

To establish a 4T1 model, Balb/c mice were subcutaneously inoculated with 5 × 10^5 Luc-4T1 cells in the left flank and then randomly assigned into two groups. On day 14 (the tumors reached ~500 mm³), the tumors were partially resected. On the same day, after surgery, the mice were given the same treatments as those in groups PBS and N+R@hPLTs (L) in the DC maturation study described above. The primary tumors and recurrent tumors were imaged with IVIS Spectrum (PerkinElmer, USA) after intraperitoneal injection of d-luciferin sodium salt at a dose of 3 mg per mouse. To facilitate imaging, each mouse was shaved before imaging. Recurrent tumor sizes and survival were measured every 2 days, and the experimental end point was defined as either death or a tumor size greater than 1000 mm³. For B16, CT26, LLC, and H22 tumor models, C57BL/6 mice (B16 cells) or Balb/c mice (CT26, LLC, and H22 cells) were inoculated subcutaneously with 5 × 10^5 corresponding tumor cells in the left flank and then randomly assigned into two groups. On day 14 (the tumors reached ~500 mm³), the tumors were partially resected. On the same day, after surgery, the mice were given the same treatments as those in groups i and v in the DC maturation study described above. Primary and recurrent tumor sizes were measured every 2 days, and the experimental end point was defined as either death or a tumor size greater than 1000 mm³.

**Antitumor study in humanized PDX model**

The PDX tumor samples were subcutaneously transplanted into the axilla of the humanized mice to establish the humanized PDX model, and then, these mice were randomly divided into two groups: PBS group and N+R@hPLTs (L) group. Two weeks later, when the tumors were visible (~250 mm³), the mice of these groups were intravenously injected with PBS and N+R@hPLTs, respectively. One hour later, some of the mice in the N+R@hPLTs (L) group were anesthetized and their tumors were illuminated with an 808-nm laser (0.65 W/cm², 5 min), while the other mice in this group were left with no administration and considered to be the control group in the in vivo aggregation study. Three days later, serum samples of these two groups were extracted to study the cytokine levels. Last, tumor sizes and survival percent were measured every 2 days, and the experimental end point was defined as either death or a tumor size greater than 1500 mm³. The construction and characterization of N+R@PLTs, in vivo aggregation study, in vivo photothermal study, and serum biochemical marker detection were administered in the same way as we used in the corresponding studies for N+R@PLTs except that the mouse PLTs were replaced by hPLTs, which were obtained from healthy donors. Hematoxylin and eosin (H&E) staining of tumor sections for the study of in vivo aggregation was administered in the same way as the H&E staining in other studies of this manuscript. The study was approved by Shanghai Tongren Hospital Medical Ethics Committee in accordance with the 1964 Declaration of Helsinki and 1982 International Ethical Guidelines for Human Biomedical Research (2019-032-01 and 2020-059-01).

**Safety estimation**

Tissue sections of the heart, liver, spleen, lungs, and kidneys were stained with H&E and analyzed by light microscopy for postmortem histopathological analysis. The serum levels of urea nitrogen (BUN), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) were analyzed spectrophotometrically using an automated analyzer (Hitachi-917, Hitachi Ltd., Tokyo, Japan). All tissues and sera were sampled at the end points of the corresponding experiments. The coagulation four indices including prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), and fibrinogen (FIB) were detected by Beijing CIC Medical Laboratory (Beijing, China).

**Statistical analysis**

All data are presented as means ± SD. Statistical analysis was performed with Prism 6.0 software (GraphPad Software) by an unpaired Student’s t test, log-rank test, and one-way or two-way analysis of variance (ANOVA).

**Supplementary materials**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/13/eabd7614/DC1

**References and Notes**

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