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IR780-loaded zwitterionic polymeric nanoparticles with acidity-induced agglomeration for enhanced tumor retention

I.-Lin Lu[a,b,1], Te-I Liu[a,1], Hsiao-Chieh Lin[a], Siou-Han Chang[a], Chun-Liang Lo[c], Wen-Hsuan Chiang[d,*], Hsin-Cheng Chiu[a,*]

[a] Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Hsinchu City 30013, Taiwan, ROC
[b] Department of Surgery, Hsinchu Mackay Memorial Hospital, Hsinchu City 30071, Taiwan, ROC
[c] Department of Biomedical Engineering, National Yang-Ming University, Taipei City 112, Taiwan, ROC
[d] Department of Chemical Engineering, National Chung Hsing University, Taichung City 402, Taiwan, ROC

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ABSTRACT

The tumor-targeted delivery of near infrared (NIR)-triggered photothermal therapy (PTT) by varied nanocarriers has captured substantial attention. In order to boost the antitumor efficacy of PTT by promoting cellular uptake and tumor retention of PTT-carrying nanoparticles, the surface charge-changeable polymeric nanoparticles comprising poly(lactic-co-glycolic acid) (PLGA) cores covered with zwitterionic diblock copolymers, methoxypoly(ethylene glycol)-b-poly(methacrylic acid-co-histamine methacryamide), mPEG-b-P(MAA-co-HMA), were developed as vehicles of IR780. Due to the acid-triggered protonation of imidazole groups, the resulting positively charged HMA residues tend to form electrostatic complexes with negatively charged MAA residues, thus facilitating the embedding of outer PEG segments into the neutral and gel-like surfaces and thus the formation of inter-particle aggregation. Taking advantage of the weak acidity of tumor extracellular matrix, the IR780-loaded nanoparticles undergoing acidity-elicited dramatic structural transformation showed appreciably enhanced cellular uptake by TRAMP-C1 cells and prolonged tumor retention time.

1. Introduction

In the past few years, the tumor-targeted delivery of near infrared (NIR)-triggered photothermal therapy (PTT) by varied nanocarriers has captured substantial attention owing to its minimal invasion, high tissue penetration, low phototoxicity and exact local treatment [1–4]. In order to boost the antitumor efficacy of PTT, it is pivotal to enhance tumor accumulation of PTT-carrying nanoparticles by promoting their uptake by cancer cells. To this end, in virtue of the weak acidity (pH_e 6.0–6.8) of tumor extracellular matrix [5,6], various functionalized nanoparticles capable of altering size and/or surface charges in response to external pH_e have been recently developed as carriers of photothermal agents [7–11]. For instance, the gold nanorod-coated mesoporous silica loaded with indocyanine green, a photosensitizer, was equipped with pH-responsive surfaces composed of β-cyclodextrin/RLA/2,3-dimethylmaleic anhydride (DMA)-modified chitosan oligosaccharide-block-poly (ethylene glycol) polymer (CS(DMA)-b-PEG) [11]. Through the tumor acidity-triggered detachment of charge-switchable CS(DMA)-b-PEG and exposure of RLA peptide, the internalization of nanoformulations by MCF-7 cells was considerably enhanced, thus augmenting antitumor effect of photodynamic and photothermal therapy. Moreover, Ji's group utilized hemoglobin as a pH_e-responsive vehicle for tumor-targeted transport of IR780, a theranostic agent for cancer imaging and PTT. The IR780-carrying hemoglobin exhibited not only the enhanced uptake by KB cells in response to pH reduction from 7.4 to 6.5 but also the prolonged tumor retention by the acidity-induced hemoglobin aggregation [7]. Many attempts have also been made to develop pH-responsive polymeric therapeutic delivery systems capable of undergoing charge conversion in response to pH change with the nanoparticles entering the tumor acidic microenvironment. The uptake of the nanoparticles by cancer cells can thus be greatly enhanced with the acidity-induced generation of positive charges on nanoparticle surfaces and the electrostatic attraction between cationic nanoparticles and cancer cell membranes [9,12].

In this study, for improved tumor retention of IR780, the tailored-made pH_e-sensitive zwitterionic diblock copolymers, methoxypoly (ethylene glycol)-b-poly(methacrylic acid-co-histamine

* Corresponding authors.
E-mail addresses: whchiang@dragon.nchu.edu.tw (W.-H. Chiang), hscchiu@mx.nthu.edu.tw (H.-C. Chiu).
[1] The authors contributed equally to this work.

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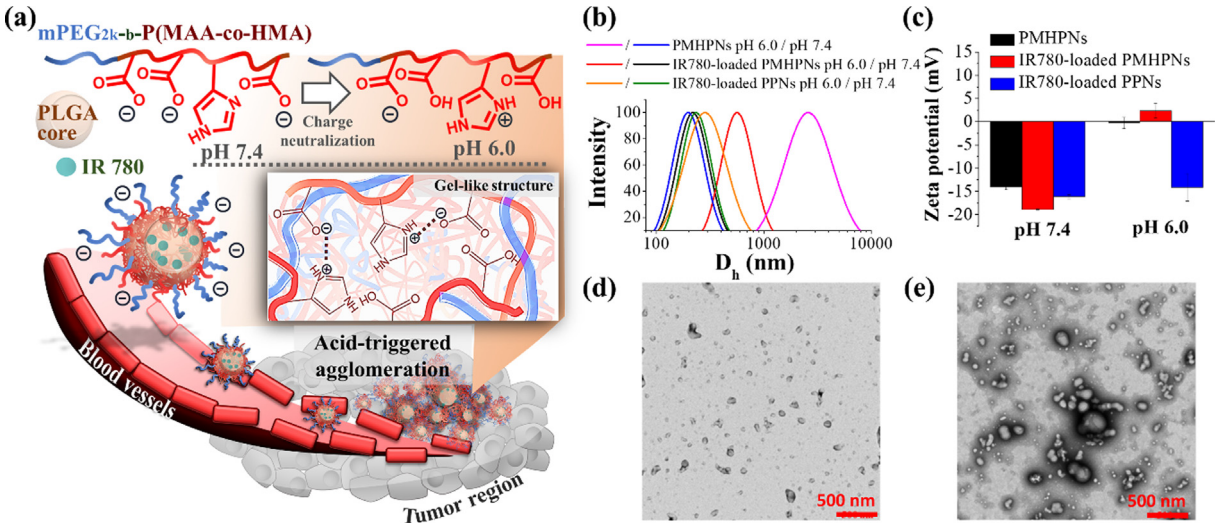

Fig. 1. (a) Illustration of the enhanced tumor retention of IR780-loaded PMHPNs by virtue of acid-triggered surface charge neutralization and agglomeration. (b) DLS particle size distribution profiles and (c) zeta potential values of various nanoparticles in aqueous solutions at different pH. Error bars represent mean ± s.d ($n \geq 5$). TEM images of IR780-carrying PMHPNs at pH 7.4 (d) and 6.0 (e) (Scale bars are 500 nm).

methacryamide) (mPEG-b-P(MAA-co-HMA) were coated on the surfaces of poly(lactic-co-glycolic acid) (PLGA) cores loaded with IR780 molecules. As illustrated in Fig. 1a, for the IR780-loaded nanoparticles, due to the acid-triggered protonation of imidazole groups, the resulting positively charged HMA residues tend to form electrostatic complexes with negatively charged MAA residues, thus facilitating the embedding of outer PEG segments into the neutral and gel-like surfaces and thus the formation of inter-particle aggregation. Through the acid-activated dramatic structural transformation, the IR780-loaded nanoparticles exhibited appreciably increased cellular uptake by TRAMP-C1 cells and prolonged tumor retention time.

2. Experimental part

Synthetic route and characterization of zwitterionic diblock copolymers, and additional experimental methods are provided in the Supplementary Material.

3. Results and discussion

At first, the carbamate imidazole (CI)-modified mPEG and amine-containing PMAA segments were synthesized and characterized, respectively, as illustrated in Fig. S1 and S2. Then the mPEG-b-PMAA was attained by successful conjugation of mPEG-CI and PMAA-NH_2 segments, as evidenced by gel permeation chromatography (GPC) profiles (Fig. S3). Through partial modification of MAA residues of mPEG-b-PMAA with histamine via dicyclohexylcarbodiimide/4-(dimethyl amino)pyridine mediated aminolysis, the mPEG-b-P(MAA-co-HMA) used in this work was acquired. Based on the ^{1}H NMR spectrum of mPEG-b-P(MAA-co-HMA) (Fig. S4), the histamine content was determined to be 18.2 mol % with respect to the initial MAA residues.

We adopted one-step nanoprecipitation approach to prepare the IR780-loaded mPEG-b-P(MAA-co-HMA)/PLGA nanoparticles (denoted herein as PMHPNs) upon the co-assembly of PLGA, mPEG-b-P(MAA-co-HMA) and hydrophobic IR780 molecules in pH 7.4 aqueous solution. For comparison, the pristine PMHPNs and IR780-loaded mPEG-b-PLGA-constituted nanoparticles (PPNs) were also fabricated in a similar manner. The PMHPNs either loaded with IR780 or not showed comparable particle size (ca. 200 nm) and zeta potential (ca. −15 mV) at pH 7.4 (Fig. 1b and c). Moreover, for IR780-loaded PMHPNs and PPNs, similar drug loading efficiencies (ca. 60%) and contents (6.6 wt%) were attained (Table S1). TEM images reveal the well dispersion of IR780-loaded PMHPNs as individual spherical particles at pH 7.4 (Fig. 1d). It should be mentioned that both the pristine and IR780-loaded PMHPNs in PBS at 37 °C maintained superior colloidal stability over 7 days (Fig. S5a), indicating the covering of mPEG-b-P(MAA-co-HMA) on the surfaces of PLGA core can effectively stabilize the nanoparticles in aqueous solution by outer hydrophilic mPEG segments and ionized MAA residues. With IR780-loaded PPNs and PMHPNs being incubated in protein-containing cell culture medium at 37 °C for 1 week, the sizes of both nanoparticles were only slightly increased within the first 24 h and then remained unvaried for the next six days (Fig. S5b). The results demonstrate the excellent colloidal stability of both nanoformulations in protein-containing medium. Obviously, the prominent colloidal stability of both nanoparticles is primarily governed by the PEGylation on nanoparticle surfaces rather than the Zwitterionic property of mPEG-b-P(MAA-co-HMA) adopted for the preparation of PMHPNs.

With solution pH being reduced from pH 7.4 to 6.0, an appreciable conversion in the zeta potential of IR780-carrying PMHPNs from −18.9 to +2.4 mV was observed (Fig. 1c). This could be attributed to the acidity-induced protonation of imidazole and carboxylic acid groups from histamine and MAA residues, respectively. Notably, the particle size of IR780-loaded PMHPNs in response to the pH adjustment from 7.4 to 6.0 is remarkably enlarged as reflected in DLS and TEM results (Fig. 1b and e). This is because the positively charged HMA residues have high tendency of forming electrostatic complexes with negatively charged MAA residues on the surfaces of IR780-loaded PMHPNs at pH 6.0, thus causing entrapment of mPEG segments into nearly neutral and gel-like surfaces to facilitate colloidal aggregation with the absence of steric stabilization and electrostatic repulsion force. For IR780-free PMHPNs, a similar acidity-induced particle aggregation and zeta potential alteration was also attained (Fig. 1b and c). By contrast, due to the lack of pH responsive property of PLGA-b-PEG, IR780-loaded PPNs showed only minor change in their zeta potential and particle size in response to pH reduction (Fig. 1b, c and Fig. S5c)

Compared to free IR780 in PBS, the IR780-loaded PMHPNs and PPNs showed a red shift of the feature absorption peak of IR780 from 774 to 784 nm (Fig. 2a), indicating hydrophobic embedding of IR780 molecules into the particle surfaces [13,14]. Moreover, distinct from a remarkable decrease in the absorbance of free IR780 in PBS over 4 days, only a minor reduction in the absorbance was observed for IR780-loaded PMHPNs (Fig. 2b). This suggests that the incorporation of IR780 species into PMHPNs could prevent IR780 from self-aggregation in PBS and degradation, thus boosting its aqueous photostability. Interestingly

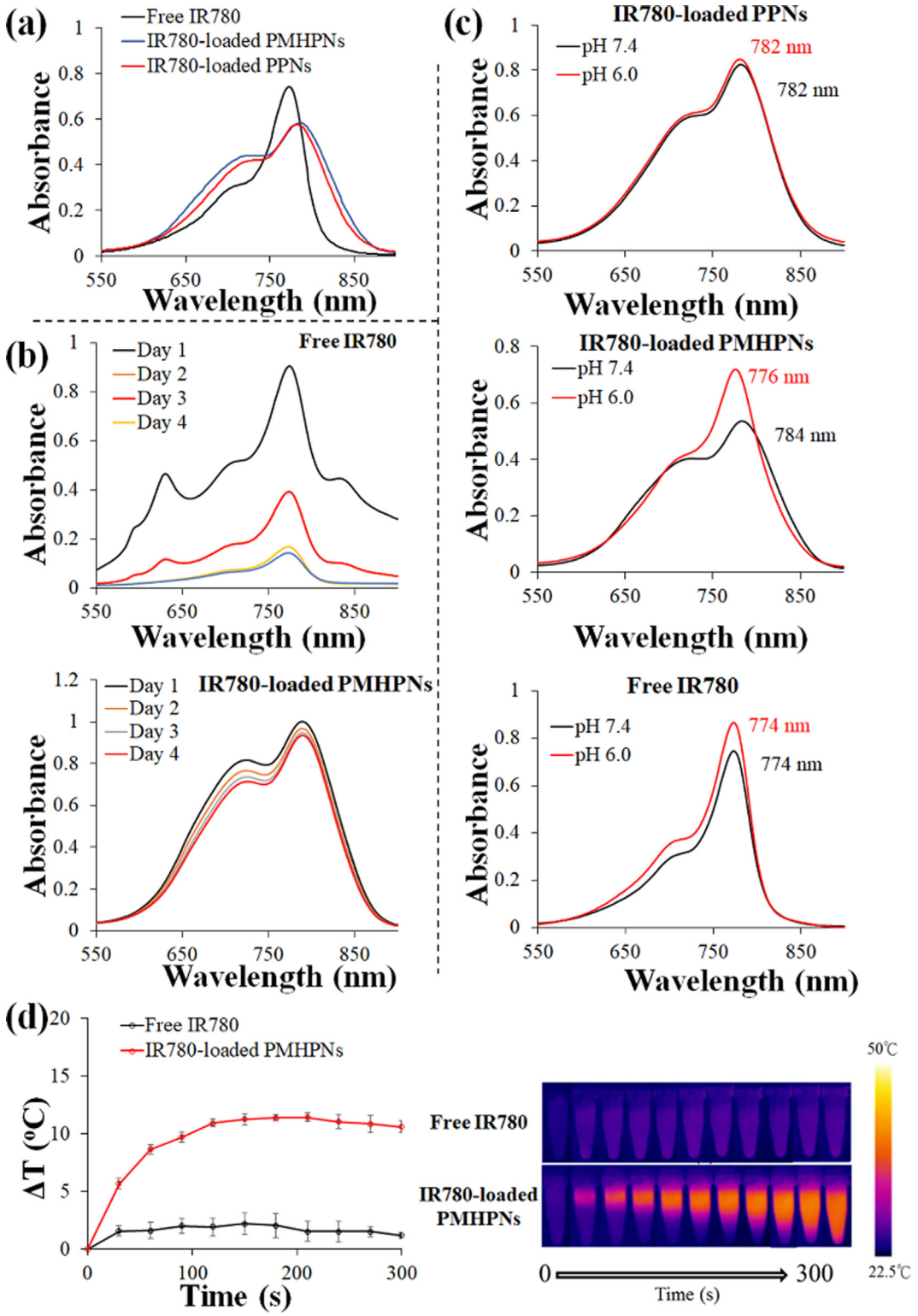

Fig. 2. (a) UV/Vis spectra of free IR780, IR780-loaded PMHPNs and PPNs in PBS. (b) UV/Vis spectra of free IR780 and IR780-loaded PMHPNs in PBS at different time intervals. (c) UV/Vis spectra of free IR780, IR780-loaded PMHPNs and PPNs at pH 7.4 and 6.0. (d) Temperature variation of free IR780 and IR780-loaded PMHPNs in PBS during 808 nm NIR laser irradiation. Error bars represent mean ± s.d (n = 3).

enough, while experiencing a pH decrease from 7.4 to 6.0, the IR780-loaded PMHPNs showed an appreciable blue shift in the absorption peak of IR780 from 784 to 776 nm, whereas free IR780 and IR780-loaded PPNs still retained the wavelength of absorption peak rather invariant (Fig. 2c). A blue shift in absorption peak of the IR780-loaded PMHPNs was ascribed to that the acid-elicited protonation of HMA residues partly hinders the close packing of positively-charged IR780 molecules. Furthermore, at an IR780 concentration of 40 μM, during 808 nm NIR laser irradiation, the temperature of IR780-loaded PMHPN solution at pH 7.4 was significantly higher than that of the free IR780 solution at the same pH (Fig. 2d). Such a difference of the NIR-triggered photothermal effect between free IR780 and IR780-carrying

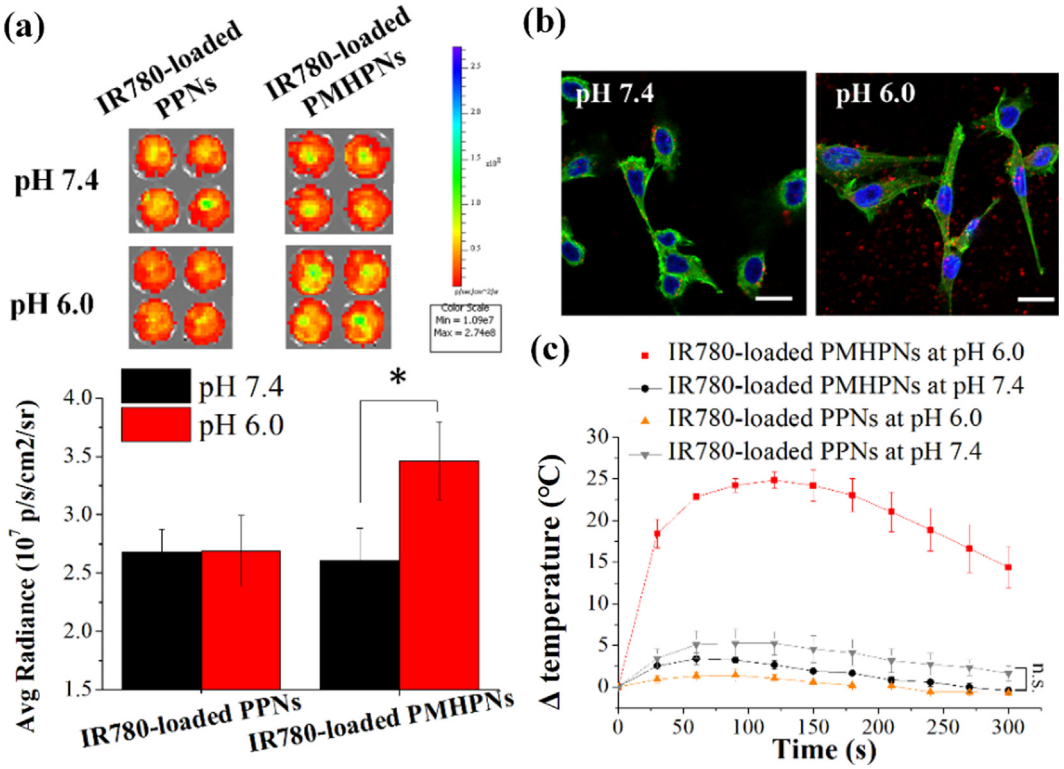

Fig. 3. (a) NIR fluorescence images and intensity of IR780 molecules from TRAMP-C1 cells incubated with IR780-loaded PMHPNs and PPNs (IR780 concentration = 20 μM), respectively, at pH 6.0 and 7.4. *P < 0.05 and n.s. P > 0.05. Error bars represent mean ± s.d (n = 3). (b) Fluorescence images of TRAMP-C1 cells incubated with DiI-labeled PMHPNs at pH 6.0 and 7.4, respectively, for 4 h. Cell nuclei was identified in blue and cytoskeleton in green. DiI signal was shown in red. Scale bars are 20 μm. (c) Temperature change of TRAMP-C1 cells incubated with IR780-loaded PMHPNs and PPNs, respectively, at pH 6.0 and 7.4, during NIR laser irradiation (n = 5).

nanoparticles was also observed elsewhere [13,14]. The poor photothermal effect of free IR780 in aqueous solution was attributed to its water-insoluble nature [14]. On the other hand, as expected, the level of the solution temperature elevation highly depends on the concentrations of IR780-loaded PMHPNs (Fig. S6).

The effects of acidity-activated surface charge conversion of IR780-loaded PMHPNs on their cellular uptake was investigated using TRAMP-C1 cells. With the culture pH being adjusted from 7.4 to 6.0, the increase of the NIR fluorescence intensity of IR780 within TRAMP-C1 cells co-incubated with IR780-loaded PMHPNs was significantly higher than that with the cells being treated with IR780-loaded PPNs (Fig. 3a). Also, the fluorescence images revealed that DiI as a fluorescence dye transported by PMHPNs at pH 6.0 was clearly observed in the cytoplasm of TRAMP-C1 cells whereas only weak signal of DiI delivered at pH 7.4 was found intracellularly (Fig. 3b). These findings definitely demonstrate that the cargo-loaded PMHPNs capable of undergoing acidity-activated surface charge transition as well as colloidal aggregation could effectively promote their cellular uptake. As a result, a significant increase in NIR-elicited hyperthermia of TRAMP-C1 cells incubated with IR780-loaded PMHPNs at pH 6.0 compared to that at pH 7.4 was obtained (Fig. 3c). By contrast, under NIR irradiation, the similar temperature profiles of TRAMP-C1 cells treated with IR780-loaded PPNs at pH 7.4 and 6.0 were observed due to the lack of pH-sensitivity in PLGA-b-PEG (Fig. 3c). Furthermore, significant reductions in the viability of TRAMP-C1 cells treated with IR780-loaded nanoformulations (PMHPNs and PPNs) and NIR laser irradiation were observed (Fig. S7). The best in vitro anticancer efficacy from the photothermal therapy of IR780-loaded PMHPNs at pH 6.0 compared to all other treated groups was ascribed to the acidity-activated agglomeration of the IR780-loaded PMHPNs which further dramatically enhanced the hyperthermia effect as shown in Fig. 3c.

To explore the tumor retention capability of pH_e-responsive IR780-loaded PMHPNs, the in vivo IR780 NIR fluorescence images and intensity of TRAMP-C1 tumor-bearing mice receiving intratumoral injection of IR780-loaded PMHPNs were attained by IVIS. The IR780-loaded PPNs without pH-sensitivity were used as the control. As shown in Fig. 4a and b, at 9 h, 24 h and 72 h post-injection, the tumors receiving IR780-loaded PMHPNs showed remarkably higher IR780 fluorescence intensity in comparison with those receiving IR780-loaded PPNs. The results indicate that the IR780-loaded PMHPNs can effectively reside in tumor by their surface charge conversion and colloidal agglomeration in response to acidic tumor environment. Similar viewpoints have been reported elsewhere [7,8,10,15,16]. Although the PMHPNs are not functionalized with active tumor penetration property, the agglomeration of the PMHPNs within tumor can not only prolong their tumor retention, but also reduce nanoparticle/drug elimination caused by high interstitial fluid pressure of tumor extracellular matrix. The drug molecules once released from the aggregates within tumor are expected to readily penetrate into deep tumor tissues because of their relatively small sizes compared to the conventional drug-loaded nanoparticle systems. Therefore, it becomes highly prominent to promote the therapeutic effect via such a prolonged-retention drug release pattern.

4. Conclusion

In this work, the zwitterionic copolymer mPEG-b-P(MAA-co-HMA) was synthesized and coated on the surfaces of PLGA core to attain the pH_e-responsive PMHPNs employed as the tumor-targeted IR780 delivery system. The IR780-loaded PMHPNs in PBS exhibited superior photostability of IR780 and colloidal stability. Taking advantaging of acidity-triggered surface charge neutralization and colloidal aggregation, the IR780-loaded PMHPNs showed not only the promoted uptake by TRAMP-C1 cells, but also the enhanced tumor retention capability.

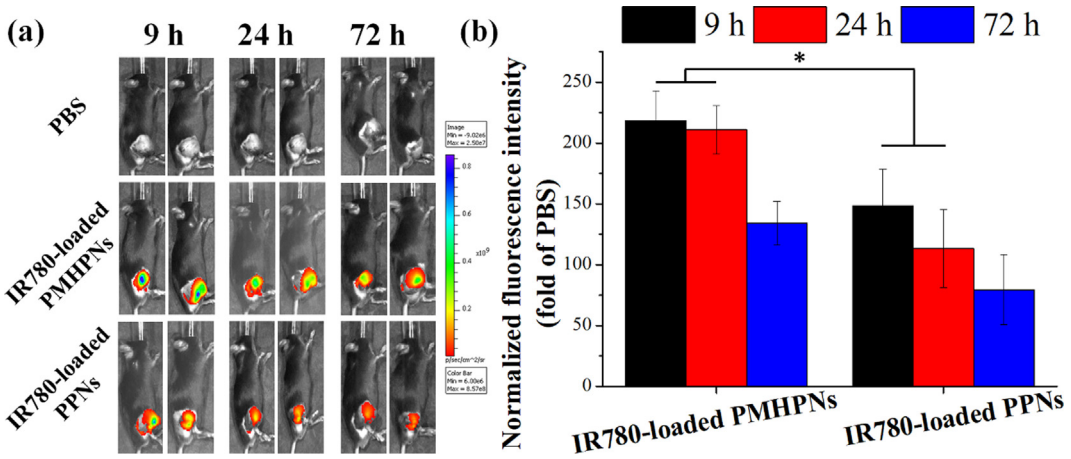

Fig. 4. In vivo NIR fluorescence (a) images and (b) intensity of TRAMP-C1 tumor-bearing mice treated with intratumoral injection of IR780-loaded PMHPNs and PPNs attained by IVIS. Error bars represent mean ± s.d (n = 4).

Although further studies in the in vivo PTT-involved antitumor performance are essential, the PMHPNs designed in this study show great potential to achieve tumor-targeted PTT delivery.

CRediT authorship contribution statement

I.-Lin Lu: Conceptualization, Methodology, Funding acquisition, Writing - original draft. **Te-I Liu:** Investigation, Methodology, Visualization, Writing - original draft. **Hsiao-Chieh Lin:** Investigation, Methodology, Formal analysis. **Siou-Han Chang:** Investigation, Methodology, Formal analysis, Validation. **Chun-Liang Lo:** Resources. **Wen-Hsuan Chiang:** Visualization, Writing - original draft. **Hsin-Cheng Chiu:** Methodology, Investigation, Conceptualization, Supervision, Writing - review & editing.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eurpolymj.2019.109400.

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