Graft and diblock copolymer micelles delivery the hydrophobic protoporphyrin IX for photodynamic cancer therapy
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ABSTRACT SUMMARY
The paper is primarily an investigation of material design. The researchers made use of their imagination and practical skills to evaluate the possible applications and uses of the material design. The primary goal of this paper is a design of a new material. The researchers used their innovation and experimental techniques to assess its practical applications. Two pH/non-pH sensitive graft copolymers, (poly(N-vinyl caprolactam)-g-poly(D,L-lactide) and poly(N-vinyl caprolactam-co-N-vinyl imidazole)-g-poly(D,L-lactide)), were synthesized and utilized for the encapsulation of protoporphyrin IX (PPIX) for in vitro and in vivo PDT in this studies. PPIX-loaded graft and diblock micelles presented prolonged blood circulation and enhanced tumor targeting ability in the animal experiment. In addition, non-pH sensitive micelle-treated mice showed a better repression of tumor growth than PPIX-treated mice, which was likely due to the larger amount of photosensitizer (PS) localized in the tumor region exhibiting therapeutic effects. Finally, effective PDT-induced inhibition of tumor growth was found in pH sensitive micelle-treated mice. This work provides insight into PS-loaded block and graft micelles for the PDT of tumors.

INTRODUCTION
To date, a variety of nanocarriers have been developed for PDT, because they showed tumor-selective accumulation due to the enhanced microvascular permeability and impaired lymphatic drainage. The release of PS can be achieved through a biodegradable process or stimuli response from the drug carrier itself, thereby providing maximum therapeutic efficiency. It has been proven that an aggregation of PS will reduce the formation of ROS due the light quenching effect[1].
However, even PS encapsulated inside a drug carrier has exhibited therapeutic efficiency in some case studies. Therefore, a re-evaluation of PS release in graft co-polymeric micelles during in vitro and in vivo photodynamic therapy is warranted. We found that a significant difference of therapeutic efficiency in the pH/non-pH response of the PDT micelles was obtained in an in vitro and in vivo study, while the formation of ROS was similar for both the pH and non-pH PDT micelles[2].

EXPERIMENTAL METHODS
In vivo antitumor activity
A549 cells were transplanted s.c. into the abdomen of bubl/c nude mice (1×10^5 cells/0.1 mL). Seven days later (the size of tumors at this point was approximately 40 mm^3), the mice were treated i.v. once with free PPIX and PPIX-loaded micelles at 3 mg/kg of PPIX. Twenty-four hours after administration, tumor sites were irradiated with a He-Ne laser at a light dose of 10 J/cm^2. The antitumor activity was evaluated in terms of the tumor size, which was estimated by the following equation: V = (a) × (b)^2 / 2, where (a) and (b) are major and minor axes of the tumor measured by a caliper, respectively.

RESULTS AND DISCUSSION
The PS carrier constructed from graft and diblock copolymer micelles was expected to increase the concentration of PS in the tumor tissue because of the enhanced permeability and retention effects. By using a non-invasive optical imaging system, the tumor targeting ability of the non-pH sensitive (g-C) and pH sensitive g-CIM micelles (both contained the Cy 5.5 image moiety) was evaluated and compared in terms of the accumulation of pH and non-pH PDT micelles in tumors. The fluorescence
intensity of the g-C and g-CIM micelles in the tumor region was directly measured by imaging the fluorescence Cy5.5 dye. The fluorescence signal for both the g-C- and g-CIM-treated mice gradually increased over 2 days, indicating that the graft and diblock copolymers prolong the PPIX circulation, as shown in figure 2. A strong accumulation of micelles in the tumor region suggested that those PDT micelles based on graft and diblock copolymers exhibited excellent targeting behavior for the drug.

Figure 2, Near IR imaging of (a) g-C and (b) g-CIM micelle accumulation at the tumor site in A549 tumor-bearing mice.

The in vivo antitumor efficiency of free PPIX, non pH-sensitive PDT micelles (g-C) and pH-sensitive PDT micelles (g-CIM) were validated in A549-bearing mice, as shown in figure 3. The animal with PPIX administration showed little therapeutic efficacy, with slightly reduced tumor growth in comparison to the control group. This result can be explained by hydrophobic PPIX not having a long circulation time in the blood, thereby making it difficult to reach the target tissue. For the mice intravenously injected with g-C micelles, the tumor growth rate was similar to the control and PPIX groups in the first two weeks. However, a significant regression of tumor growth started at 14 days. PDT has multiple effects on cancer cells. It has been reported that a combination of PS and activating light causes an unusual mixture of apoptotic and necrotic cell death. Although PPIX encapsulated in polymeric micelles cannot direct harm cancer cells, the formation of ROS through light irradiating can result in damage to the vessels supplying the tumor or trigger immunity to the tumor tissue [3]. The g-C micelles showed little inhibition of tumor growth when compared with PPIX only. In contrast, the g-CIM micelle-treated mice showed a large amount of dead tumor cells, indicating successful PDT. Taken together, these results indicated that the pH-sensitive PDT micelles efficiently localize in tumors, release PPIX under in that acidic environment, and then produce cytotoxic ROS that induces cell death and reduces tumor volume.

Figure 3, Tumor inhibition test of s.c. A549 xenograft in Balb/c mice. Mice were injected with a 3 mg/kg PPIX equivalent dose, and 24 h after injection, the tumor was irradiated with a He-Ne laser at a power of 10 J/cm² (data are expressed as means ± SD (n=3).

CONCLUSION
PPIX can be encapsulated by the graft and diblock copolymer mixed micelles, theses polymeric micelles offer PPIX effectively targeting on tumor site. Non-pH sensitive PDT micelles still exhibited retardation of tumor growth, these result can be explained by assuming that ROS generated near the tumor site still affect the tumor growth. Non-pH sensitive PDT micelles can be regarded as pH-sensitive PDT during the circulation, no suppression on tumor was found at the beginning of PDT therapy can be assumed that PS encapsulate in graft and diblock copolymer was stable without toxic effect. Therefore we can conclude that g-CIM PDT micelles have great potential to achieve the site select therapy for cancer treatment.

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REFERENCES