Multifunctional, Polymeric Worm-like Vesicles Based on Triblock Copolymers for Tumor-targeted Imaging and Drug Delivery

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Statement of Purpose: Theranostics has the potential to revolutionize the healthcare industry. The development of tumor-targeting, multifunctional nanocarriers for the combined delivery of diagnostic and therapeutic agents to cancer cells would make cancer theranostics possible. In particular, nanocarriers that offer multiple functions, such as active tumor-targeting, imaging ultrasensitivity, and chemotherapy in one system, represent a new paradigm in nanomedicine.

Polymer vesicles are promising nanocarriers for the combined delivery of diagnostic and therapeutic agents because they can simultaneously encapsulate multiple hydrophilic and hydrophobic agents in the aqueous core and hydrophobic membrane, respectively, as liposomes do. However, compared with liposomes, polymer vesicles offer numerous possibilities to control the physical, chemical, and biological properties by tailoring the block lengths, block chemistry, and functionalization (Discher D E et al., Science 2002; 297: 967-73). In this work, we report, for the first time, multifunctional, worm-like polymeric vesicles made of heterofunctional triblock copolymer maleimide-poly(ethylene glycol)(Mw: 5000)-poly(D,L-lactide)-poly(ethylene glycol)(Mw: 3000)-acrylate (Mal-PEG(long)-PDLA-PEG(short)-Acrylate) for tumor-targeting cancer theranostics applications.

A previous study on polymer vesicles formed by amphiphilic block copolymers found that long hydrophilic segments are mostly segregated into the outer hydrophilic layer (~90% or better) while short hydrophilic segments are mostly segregated into the inner hydrophilic layer as a result of thermodynamic stabilization (Luo et al. J. Am. Chem. Soc. 2001; 123: 1012-3.) Thus, it is hypothesized that the maleimide end-group functionalized long hydrophilic PEG chains of the triblock copolymers would preferentially segregate to the outside of the vesicles, making conjugation of various tumor-targeting ligands such as cRGD possible. Also, the acrylate end-group functionalized short hydrophilic PEG chains would preferentially segregate to the aqueous core of the vesicles, making it possible to cross-link the inner PEG shell of the vesicles in order to significantly enhance their *in vivo* stability. For this study, the anticancer drug, doxorubicin (DOX), was loaded into the hydrophobic membrane and hydrophilic superparamagnetic iron oxide (SPIO) nanoparticles (NPs) serving as the MRI contrast-enhancing agents were encapsulated into the cross-linked aqueous core of the worm-like vesicles.

Methods: The triblock Mal-PEG(long)-PDLA-PEG(short)-Acrylate copolymers were synthesized by a ring-opening polymerization of D,L-lactide using Mal-PEG-NH_2 (Mw: 5000) as a macroinitiator, followed by conjugation with HOOC-PEG-Acrylate (Mw: 3000) at room temperature under a nitrogen atmosphere. The worm-like DOX/SPIO-loaded vesicles formed by the triblock copolymer were prepared using a double-emulsion method. The inner PEG shell cross-linking reaction was carried out in the vesicle aqueous solution using $K_2S_2O_8$ as the initiator at 55°C.

Results: The triblock copolymer Mal-PEG(long)-PDLA-PEG(short)-Acrylate was successfully synthesized as confirmed by the ^{1}H NMR spectra. The resulting triblock copolymers can form stable, worm-like vesicles using a double emulsion method. The vesicle morphology was studied by TEM, as shown in Figure 1a. TEM also clearly shows the hydrophilic SPIO NPs were encapsulated in the aqueous cores of the inner-shell cross-linked vesicles (Figure 1b). Moreover, the size and size distribution of the vesicles did not change before and after the cross-linking reaction, which were measured by DLS. These results supported our hypothesis on the preferential segregation of the long and short PEG chains to the outer and inner shells of the vesicles, respectively, due to thermodynamics stabilization. The tumor-targeting ability and the MRI contrast enhancement effects of this nanosystem are currently under investigation in our lab.

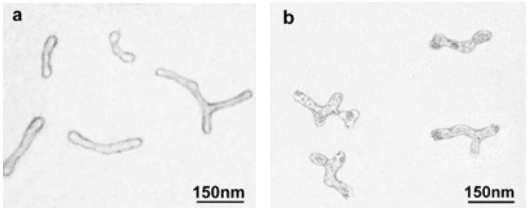

Fig. 1 The TEM micrograph of the blank and DOX/SPIO -loaded wormlike vesicles based on the triblock copolymers with cross-linked PEG inner shells.

Conclusions: Multifunctional, worm-like vesicles made of amphiphilic Mal-PEG(long)-PDLA-PEG(short)-Acrylate triblock copolymers for combined delivery of anticancer drug and MRI contrast agents (i.e., SPIO NPs) were prepared and characterized for cancer theranostics applications. The triblock copolymer also allows easy conjugation of various types of tumor-targeting agents such as cRGD on the outer PEG shells of the vesicles, making active-tumor targeting possible. The inner PEG shells were cross-linked to greatly enhance the *in vivo* stability of the vesicles during blood circulation. Finally, the ability to control the preferential segregation of various functional groups attached to the long and short PEG segments of the triblock copolymers may have significant implications to the development of multifunctional drug nanocarriers in the future.