Polymersomes formed by phase-guided assembly as carriers for intracellular delivery of anti-tumor therapeutic proteins

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ABSTRACT SUMMARY
We demonstrate a polymersome of asymmetric membrane formed by phase-guided assembly for thermodynamically efficient nano-encapsulation and intracellular delivery of therapeutic proteins of preserved native form for anti-tumor treatment. Instead of random distribution, apoptin, a p-53-independent bcl-2-insensitive apoptotic protein, partitioned preferentially within the interior surrounded by an asymmetric block copolymer membrane which aligned at the interface between a polysaccharide core and a PEG continuous phase. Site-injecting the protein-loaded polymersomes to the mice bearing SMMC-7721 tumor resulted in significant retardation of tumor growth as compared with the mice of control groups to whom naked apoptin as well as apoptin loaded in a solid polymeric nanoparticles were injected on site. This result suggests that phase-guided assembling polymersomes is an ideal strategy to nano-encapsulate therapeutic proteins of native form for intracellular delivery.

EXPERIMENTAL METHODS
Copolymer and protein were added into an aqueous-aqueous two-phase system with dextran forming the dispersed phase and PEG being the continuous phase. After the polymersomes were formed, a free radical initiator and a catalyst, ammonium persulfate and N’, N’, N’, N’-tetramethylethylenediamine (0.2 wt. % and 0.4 wt. % in concentration, respectively), were added to induce cross-linking of the dextran core through the grafted methacrylate bearing a C= C double bond. Then the sample was lyophilized to powder and the PEG continuous phase was washed away using organic solvents. The harvested polymersome particles were re-suspended in buffer and subjected to a number of assays, comprising CLSM micrographs, SEC-HPLC analysis, observation of the cellular localization, and tumor growth retardation effect.

RESULTS AND DISCUSSION
As schematically demonstrated in Figure 1, the triblock copolymer was synthesized by ring opening reaction of caprolactone. MTT assays showed that the triblock copolymers were non-toxic up to a concentration of 1 mg/ml. And, the inhibitory concentration (IC50) of triblock for SMMC-7721 and Hela cells growth was 410μg/ml and 36250μg/ml respectively, which was comparable to PEI 25k of 156μg/ml and 320μg/ml (date not shown).

Fig. 1. Synthetic route to PEG-PCL-Maltotriose triblock copolymers.

To verify the structure of the polymersomes, size distributions of polymersomes were measured by TEM image (Fig. 2.). Then, gaint polymersomes loaded with nile red (red) and FITC-BSA (green) were formed for CISM micrographs test. It was confirmed that confirmed the polymersomal structures with triblock copolymers formed spherical shell between the PEG continuous phase and the dextran dispersed phase by confocal laser scanning microscopy (CLSM).

Due to the preferential partition favoring the dextran phase, our polymersomes employed the PEG-dextran two-
phase system which worked high efficiently for exogenous proteins encapsulation (up to 87%). Also, to determine protein integrity and bioactivity affected by the formulation process, we investigated the SEC-HPLC charts of BSA from original solution and from BSA-loaded polymersomes (Figure 3). Notably, the process for forming BSA-loaded polymersomes did not cause protein aggregation. However, in the case of BSA prepared using the W/O/W method, the portion of aggregated BSA was above 11% of the total protein.

![Fig. 2. CLSM micrographs of gaint polymersome loaded with nile red (A, red) and FITC-BSA (B, green) and overlays of A and B (C), (D) TEM image and (E) Fluorescent intensity profiles of nile red and FITC-Dextran along a diametric line (I: red channel; II: green channel; III: overlay of I and II).](image)

![Fig. 3. The aggregation BSA recovered from various formulations by SEC-HPLC assay. Original BSA; BSA released from polymersome; Control BSA from particles prepared using W/O/W method.](image)

The intracellular uptake and release of proteins was studied by using FITC-BSA protein. The images of SMMC-7721 cells following incubation for 4 h with FITC-BSA loaded polymersomes showed a strong green fluorescence as well as red fluorescence (Fig. 4 C), indicating efficient protein release from endosomes. Remarkably, fluorescence studies revealed that our carriers can efficiently load and transport protein into cells.

To examine intracellular delivery, we investigated in BALB/c athymic mice bearing subcutaneous SMMC-7721 by intratumoral injection. As shown in Fig.5, there was no significant change in tumor growth observed in xenograft mice treated with polymersomes loading EGFP and PBS-treated control group. In contrast, treatment with polymersomes loading apoptin provoked a significant decrease in tumor growth. Next, TUNEL assay implied that the retardation of tumor growth by polymersomes loading apoptin was definitely caused by apoptosis of tumor cells.

![Fig. 4. Direct observation of the cellular localization of polymersomes in SMMC7721 cells. (A) transmittance image; (B) Fluorescence of the cells incubated with NPs loading FITC-BSA (green); (C) Fluorescence of the cells labeled with LysoTracker Red (red); (D) An overlay of (B) and (C) showing co-localization of LysoTracker and NP loading FITC-BSA derived fluorescence (yellow).](image)

![Fig. 5. A: The tumor suppression rate of experimental groups compared with control group. B: Tumor growth retardation effect of different formulation treatments in an SMMC-7721 xenograft mouse model.](image)

**CONCLUSION**

Efficient nano-encapsulation and intracellular delivery of proteins with preserved native form may be achieved by phase-guided assembling of rationally designed block copolymers into polymersomes of asymmetric membrane.

**REFERENCES**


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