Surface Membrane of Rationally Designed Block Copolymer Assembled around Polyplex Core for Intercellular Delivery of Nucleic Acids

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ABSTRACT SUMMARY

We report a non-covalent assembly of a unilamellar tri-block copolymer membrane around a siRNA-loading polyplex core to prolong in vivo circulation, prevent siRNA leaking, and immobilize cell-targeting agent on the particle in optimized surface population. This tri-block copolymer, named as ABCOO hereafter, consists of a steric stabilization block (polyethylene glycol, PEG), a hydrophobic block (polycaprolactone, PCL), and a surface guiding block (surge conjugated multiple carboxylic groups). When being added to a solution of polyplex, ABCOO aligned on the surface of polyplex core to form a unilamellar membrane consisting a hydrophobic PCL layer and a steric stabilizing PEG out layer. Selected cell-targeting agent may be conjugated to the distal end of the PEG block and immobilized on polyplex surface with optimized surface population simply by mixing with non-conjugated ABCOO in refined fractions prior to surface assembly. Non-covalent surface assembly of a functional polymer membrane is superior over covalent conjugation of functional groups to polyplex-forming polymers for better prevention of siRNA leaking, flexibility in selecting cell targeting agent, and convenience in optimizing surface population of the targeting agent.

INTRODUCTION

A therapeutically feasible synthetic carrier for siRNA must accomplish all the essential tasks along the in vivo pathway of siRNA as (A) packing siRNA into nanoparticles to avoid enzymatic degradation; (B) adsorbing onto diseased cells selectively; (C) escaping from endosomes after phagocytosis; (D) releasing siRNA in cytoplasm; and (E) metabolizing itself to nontoxic species before invoking toxicity or immunogenicity (Fig.1.) [1].

For specific cell targeting, a synthetic carrier of siRNA should be endowed with a multifunctional surface to protect the nucleic acids against degradation and leaking, prevent non-specific adsorption to cells or tissues, and direct the particulate to the target cells. Such a surface membrane should carry cell targeting moieties and steric stabilization agent, and best be attached on the carrier particle non-covalently in order to gain the flexibility to adjustable surface population of the functional agents. Unlike the so-called lipopolyplexes, the non-covalent attachment of the surface membrane must be sufficiently stable to avoid dissociation in vivo.

To meet these criteria above, we choose to assemble a monolayer of rationally designed tri-block copolymers over the polyplex core to neutralize its surface charges, prevent siRNA leaking, and immobilize cell-targeting and steric stabilization agents. Surface density of targeting molecules, a critical nature of a nano-particle carrier for cell specific delivery, may easily be optimized by mixing the tri-block copolymers conjugated with and without the cell-targeting agent prior to adding them to the polyplex solution (see Fig. 2).

Fig. 1. Biological pathways for siRNA delivery

Fig. 2. Schematic description of polywraplex formation using rationally designed tri-block copolymer to form the functional surface around a polyplex

EXPERIMENTAL METHODS IN Brief

The tri-block copolymer, ABCOO', was synthesized by ring opening reaction of caprolactone using a PEG as the initiator, followed by conjugating sugar block to the PCL side. For targeting tumor cells, biotin was conjugated to the distal end of the PEG block of the copolymer via activated amidation. The copolymers were added into a solution of siRNA-polyplex to form the surface membrane, and characterized for particle size and Zeta potential changes, as well as fluorescent imaging.

To visualize formation of the surface membrane around polyplexes, fluorescent dyes, Nile red (red) and FAM (green), were used to label the surface membrane and the siRNA in the core, respectively. The surface assembled polyplexes were immobilized in a polyvinyl alcohol hydrogel matrix and subjected to microscopic imaging using two different exciting lasers concurrently.

The ability of the surface membrane to prevent siRNA leaking and degrading under in vivo condition was
examined by co-incubation with calf thymus DNA and with calf serum, respectively, followed by electrophoresis. Improved in vivo circulation and tumor targeting by wrapping the block copolymer membrane were examined by injecting the nano-particle system in mice, followed by fluorescent imaging.

RESULTS AND DISCUSSION
To confirm the formation of the surface membrane around the polyplex, the particles labeled with the hydrophobic Nile red and hydrophilic FAM were immobilized in a polyvinyl alcohol hydrogel prior to microscopic imaging. As shown in Fig. 3, the fluorescent images taken concurrently using exciting lasers of different wave length overlapped exactly, suggesting that the hydrophobic PCL domain was attached to the polyplex leaded with labeled siRNA. Zeta potential measurement was consistent with and fluorescent imaging in that adding the copolymer neutralized the positive charge of the polyplexes, suggesting surface attachment (Fig. 4).

![Fluorescence images of hydrogel-immobilized polyplexes wrapped by a copolymer membrane.](image)

Fig. 3. Fluorescence images of hydrogel-immobilized polyplexes wrapped by a copolymer membrane. The PEI-siRNA polyplexes were labelled with FAM-siRNA (green) by covalent conjugation, and the PEG-PCL-Maltotriose-COOmembrane was labelled with Nile red (red) by hydrophobic partition. The nanoparticles were immobilized in PVA hydrogel prior to photographing consequently.

![Size and Zeta potential measured in water and saline (mass ratio: ABCOO: PEI: siRNA= 1.5:1:1)](image)

Fig. 4. Size and Zeta potential measured in water and saline (mass ratio: ABCOO: PEI: siRNA= 1.5:1:1)

To examine the ability of the block copolymer membrane to prevent siRNA leaking, naked polyplexes and polywrapplexes (both loaded with siRNA at 1:1 mass ratio over PEI) were mixed with calf thymus DNA (1.7 times of siRNA in mass), and subjected to electrophoresis. As shown in Figure 5a, some siRNA leaked from naked polyplexes but not from the copolymer wrapped polyplexes.

![Zeta potential measurement results of polyplexes and polywrapplexes](image)

The ability of the polymer membrane to improve in vivo circulation and tumor targeting of polyplex was examined by injecting the nano-particles to nude BALB/c mice implanted with SMMC-7721 tumor cells. Four formulations, naked fluorescent Cy3-siRNA, naked polyplex formed of PEI-25K and Cy3-siRNA, as well as polyplex surrounded by ABCOO membrane and by a 90:10 mixture of ABCOO and biotin-ABCOO, were injected to each group of the mice through tail vein. As summarized in Fig 6, the fluorescent intensity of the dissected tumors from the mice received different formulations aligned in such an order as NaCl = naked siRNA < polyplex = polywrapplex < Biotin-polywrapplex.

![Fluorescence images of hydrogel-immobilized polyplexes wrapped by a copolymer membrane.](image)

Fig. 5. Calf thymus DNA extraction test. M: marker. A: naked siRNA. B: polywrapplexes alone (PEI : siRNA = 1:1 w/w). C: polywrapplexes extracted by calf thymus DNA. D: polyplexes alone (PEI : siRNA = 1:1 w/w). E: polyplexes extracted by calf thymus DNA. F: calf thymus DNA.

![In vivo imaging in SMMC-7721 tumor-bearing BALB/c nude mice after intravenous injection of (a) 0.9% NaCl, (b) Naked Cy3-siRNA, (c) Polyplexes, (d) ABCOO® polywrapplexes and (e) 10%Biotin-ABCOO® after 24h.](image)

Fig. 6. In vivo imaging in SMMC-7721 tumor-bearing BALB/c nude mice after intravenous injection of (a) 0.9% NaCl, (b) Naked Cy3-siRNA, (c) Polyplexes, (d) ABCOO® polywrapplexes and (e) 10%Biotin-ABCOO® after 24h.

CONCLUSION
Non-covalent assembling of rationally designed block copolymer around polyplexes is a convenient way to prevent siRNA leaking, improve in vivo circulation, and immobilize cell targeting agent with optimized surface population.

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