PEG Bottle Brush Copolymers for Lysis of Microbial Membrane Mimics by Entropic Templating

Amit Garle[†‡], and Bridgette Budhlall[†].
[†]University of Massachusetts, Lowell, MA and [‡]Mayo Clinic, Rochester, MN

Statement of Purpose: Infections pose serious concerns in a variety of settings such as hospitals, water purification and food packaging and storage. Hospital acquired infections are one of the leading causes of death in USA.[1] In the United States alone, an estimated 100,000 deaths and $6.5 billion[2] cost is attributed to infections. The overuse of antibiotics has led to the development of drug resistant bacteria which are the primary cause of these infection related deaths.[3] Various amphiphilic polymers have been studied to treat infections. The drawbacks of these systems are poor solubility, low selectivity between antimicrobial and mammalian cells, toxicity and slow rate of action. Thus, novel approaches are urgently needed to treat these infections. We propose using entropic templating[4] as a mechanism for lysis of microbial membranes by hydrophilic bottle brush architecture as explained in Figure 1. Using this approach, amphiphilic architecture for antimicrobial action is not needed and biocompatible, hydrophilic PEG can be used to design the polymers.

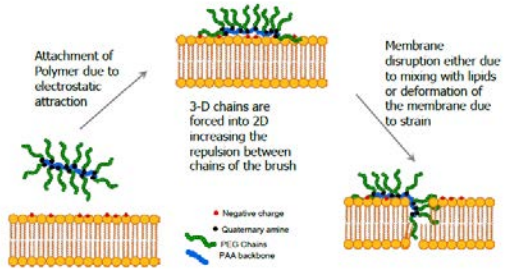

Figure 1: Membrane mimics lysis by entropic templating.

Methods:. PEG monohydroxy polymer was reacted with 4-Methylmorpholine-2,6-dione to obtain PEG with tertiary amine and carboxylic acid end group which were coupled to polyallylamine by carbodiimide chemistry to obtain bottle brush polymers. Positive charge was introduced in the polymers by reacting with ethyl iodide. Polymers were tested for membrane lysis potential using Calcein loaded unilamellar vesicles comprised of 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC) which is used as a red-blood cell membrane mimic, and a 20:80 (mol/mol) mixture of 1,2-dioleoyl-sn-glycero-3-phosphatidyl ethanolamine (DOPE), and 1,2-dioleoylsn-glycero-3-[phospho-rac-(1-glycerol)] (DOPG), which is used to mimic the outer membrane of gram positive bacteria, E.coli. Vesicles were prepared by extrusion according to method reported by Tew's group.[5] Excess Triton-X is added to completely destroy the vesciles (positive control).

Results: A copolymer with a polyallylamine backbone and PEG side chain, giving bottle brush architecture (QPEG), was successfully synthesized by carbodiimide coupling and characterized by NMR, DLS and zeta potential. Quaternization with ethyl iodide resulted in a positively charged copolymer with colloidal size of 65.16 + 1.42 nm (at 1 mg/mL concentration in water) and a zeta potential of 17.55 + 0.55 mV. To prove our hypothesis that aggregates with bottle brush architecture was needed for effective cell lysis, a polymer without PEG side chains (Quat) was synthesized and tested as a control experiment. Quaternization of this control polymer resulted in colloidal aggregates with a size of 24 nm and zeta potential 30 ± 6 mV. The QPEG copolymer structure comprised of design features for binding to phospholipid cell membranes as well as for undergoing transformations that disrupt the cell wall of microbial mimics for selective lysis.

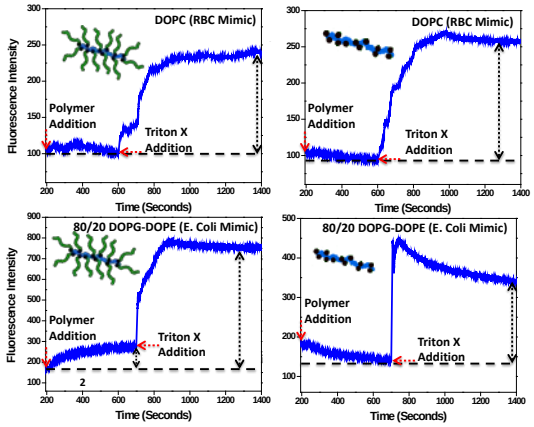

Figure 2: Calcein dye leakage from vesicles on treatment with control and bottle brush polymers.

The interaction between the bottle brush polymer and the surface of the microbial mimics was demonstrated using a calcein dye release assay. As seen in Figure 2 (right graphs), the bottle brush polymer systems had no effect on the red blood cell mimic vesicles but dye leakage was observed in the *E.Coli* mimicking membrane demonstrating selectivity of the system. The control polymer system had no effect on both RBC as well as *E.Coli* mimic vesicles. To further probe the mechanism of membrane lysis, DLS experiments were performed under the dye release assay conditions. *E.Coli* mimic vesicles along with QPEG polymer showed no change in the size of the system (Figure 3). It can be seen, in presence of Triton–X, the vesicles are completely destroyed as seen by the shift in size distribution. Thus, the release of dye n presence of bottle brush polymer is not caused by destruction of vesicles but perforation of the vesicles.

Conclusions: We have successfully demonstrated the lysis of microbial membrane mimics by entropic templating. Using the bottle brush architecture, selective lysis of bacterial cell membrane mimics was successfully demonstrated with no effect on the membranes of RBC mimics. Our study demonstrates that amphiphilic architecture is not needed for membrane lysis.

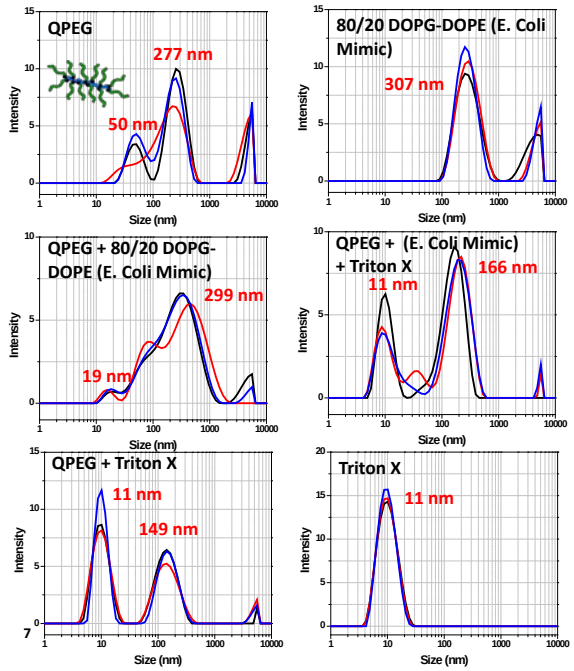

Figure 3: Z-average size analysis of vesicles on treatment with control and bottle brush polymers by Dynamic light scattering (DLS).

References:

(1) *2014 National and State Healthcare-Associated Infections Progress Report*; Centers for Disease Control and Prevention, **2016**; p 147.
(2) Perencevich, E. N.;. *Jama* **2009**, *301* (12), 1285–1287.
(3) Tavernise, S.; Grady, D. Infection Raises Specter of Superbugs Resistant to All Antibiotics. The New York Times. May 26, 2016.
(4) Szleifer, I; Nat. Mater. 2013, 12, 693–694.
(5) Tew, G. N.; Biomacromolecules, 2008, 9 (10), 2805–2810.