Comb-shaped Cationic Polycarbonates for Gene Delivery and Antimicrobial Applications

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Statement of Purpose: Aliphatic polycarbonates have emerged as an attractive class of biomaterials owing to their biodegradability and ease of incorporating functional moieties [1,2]. In this study, we describe the design and synthesis of a novel class of comb-shaped polycarbonate-based block copolymers with well-defined molecular weights and narrow polydispersities by metal-free organocatalytic ring-opening polymerization (ROP) of functional cyclic carbonates containing benzyl chloride side using a polycarbonate backbone bearing pendant alcohols as the macroinitiator. Subsequent functionalization with bis-tertiary amines conferred quaternary and tertiary amines for gene binding and endosomal escape, respectively. The amphiphilic character of the resultant cationic polycarbonates was also expected to impart membrane lytic activities against disease-causing microorganisms. The effects of increased hydrophobic content in the comb-shaped block copolymer on gene delivery and antimicrobial activities were studied by copolymerizing with L-lactide and trimethylene carbonate (TMC) monomers to obtain **Polymers 1 and 2**, respectively, as a comparison to the polymer lacking a hydrophobic block in the pendant chains (**Polymer 3**).

Methods: Polymer/DNA complexes were prepared by mixing equivolumes of polymer and DNA in 10 mM phosphate buffer (pH 6.0), followed by incubation at room temperature for 30 min. *In vitro* gene transfection efficiencies of the polycarbonate/DNA complexes, in comparison with 25 kDa PEI, were evaluated in HepG2 using the 6.4 kb firefly luciferase gene driven by the cytomegalovirus promoter (Carl Wheeler, Vical, USA). Cellular toxicity was evaluated in HepG2 using the standard MTT protocol. The minimum inhibitory concentrations (MICs) of the polymers against S. aureus (ATCC29737), *E. coli* (ATCC25922) and *C. albicans* (ATCC10231) were determined using the broth microdilution method. Hemolysis testing was performed using 4% v/v rat red blood cell suspension [2]. The antimicrobial mechanism was investigated in *E. coli* using a double staining procedure with FITC-dextran (500 kDa) and Hoechst 33342 following polymer treatment.

Results:

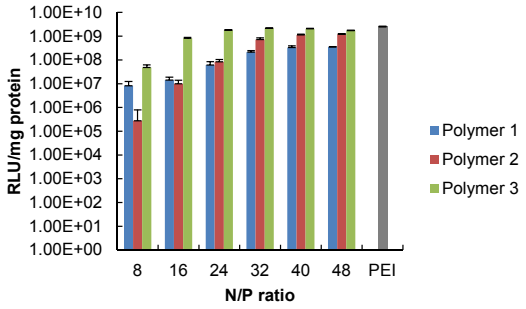

Figure 1. Luciferase gene expression of comb-shaped cationic polycarbonate/DNA complexes in HepG2. PEI/DNA complexes (N/P 10) were formed in HPLC water.

As seen in Figure 1, all three polymers induced high luciferase gene expression in a N/P ratio dependent manner. As a general trend, the gene expression levels induced by the polymers follow the order: **Polymer 3** > **Polymer 2** > **Polymer 1**, indicating that the incorporation of hydrophobic poly(L-lactide) or poly(TMC) blocks reduced gene transfection efficiencies. Despite differences in gene transfection profiles, the luciferase gene expression levels induced by all three polymers were in the same order of magnitude as that of the 'gold standard' PEI/DNA complexes at N/P 10. Interestingly, the incorporation of pendant hydrophobic blocks in **Polymers 1 and 2** led to a significant reduction in cytotoxicities, with cell viabilities of >72% up to N/P 48. **Polymer 3**, on the other hand, had >90% cell viability up to N/P 16, beyond which the cell viability decreases to <70%.

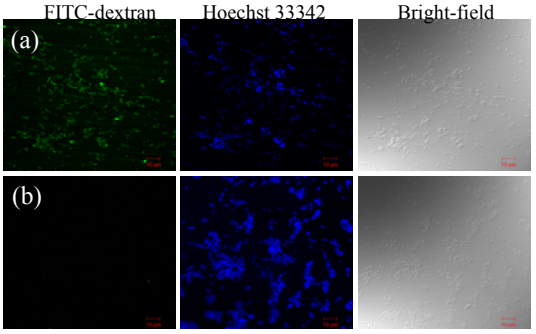

Figure 2. Confocal images showing permeation of FTIC-dextran in *E. coli* treated for 1 h with (a) 250 mg/L of **Polymer 3** as compared to the (b) unstained control.

The cationic polycarbonates demonstrated broad spectrum antimicrobial activities against the various microbes tested (MICs ranging from 62.5 to 1000 mg/L). Importantly, the polymers were not hemolytic at the various MICs (HC_{50} values > 2000 mg/L). As seen in Figure 2, treatment of *E. coli* with Polymer 3 led to significant permeation of microbial membranes by FITC-dextran, unlike in the intact untreated control.

Conclusions: Well-defined comb-shaped block copolymers comprising of a polycarbonate backbone with pendant chains incorporating a block of hydrophobic L-lactide or TMC and polycarbonate with quaternary and tertiary amines effectively mediated high luciferase gene expression levels without inducing overt cytotoxicities. The polymers also possessed good selectivities for microbial membranes. Thus, the fully degradable comb-shaped cationic polycarbonates present as a highly attractive class of material for gene delivery and antimicrobial applications.

References:

[1] Ong ZY. *et al.* J. Control. Rel. 2011;152:120-126.
[2] Nederberg F. *et al.* Nat. Chem. 2011;3:409-414.