EVALUATION OF ANTIMICROBIAL ACTIVITY AND BIOCOMPATIBILITY OF POLYETHYLENIMINE (PEI) DERIVATIVES

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1. Introduction

In the face of rising drug-resistance amongst bacterial and fungal strains of today, antimicrobial polymers (AMPs) have emerged as a promising class of antimicrobial agents that can help combat this global epidemic [1]. While conventional antibiotics tend to target metabolic or biological functions of the pathogens, AMPs, which are generally functionalized by their cationic and amphiphilic characteristics [1], predominantly act via physical disruption of the cell membrane. Such a membrane active mechanism has been shown to reduce the likelihood of pathogens developing resistance. In this study we propose a new series of guanidine-functionalized AMPs, which are synthesized via facile one-step guanylation of amine-containing polymers (Polyethylenimine, PEI) using commercially available starting materials 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*,*N*'-Dicyclohexylcarbodiimide (DCC). This project is significant in helping to evaluate and analyse the hypothesized broad-spectrum antimicrobial properties of these polymers. Ultimately we look towards applying these polymers as antimicrobial agents in consumer care products such as shampoos and hand washes.

2. Research Methods

A rigorous screening process was used to identify the polymers that were most suited as antimicrobials. Polymers were tested against four clinically relevant strains of microbes: Gram-positive bacteria *S. aureus*, Gram-negative bacteria *E. coli* and *P. aeruginosa*, and fungi *C. albicans*.

We first conducted an antimicrobial assay and a hemolysis assay, a standard set of tests [1] to evaluate the polymer's antimicrobial activity as well as toxicity. The two most selective polymers were identified and further tested to investigate its antimicrobial mechanism. This involved conducting a Killing Efficiency Test, Killing Kinetics test, as well as FESEM Imaging.

3. Results

From the antimicrobial assay and hemolysis test, selectivity of the polymer was calculated and compared.

From there, we calculated selectivity:

$$Selectivity = \frac{HC50\ (minimum\ concentration\ for\ 50\%\ hemolysis)}{MIC\ (minimum\ inhibitory\ concentration)}$$

We found that LPEI-EDC$_{12}$ had the highest and most consistent selectivity against all three bacterial strains while bPEI-EDC$_{60}$ was surprisingly effective against the fungi.

***Table 1**. Polymers with highest and most consistent selectivities (desired values in bold)*

Polymer	Selectivity (HC_{50}/MIC)			
	S.A.	***E.C.***	***P.A.***	***C.A.***
bPEI-EDC$_{60}$	> 64	~ 4	> 8	> **256**
LPEI-EDC$_{12}$	> **64**	> **64**	> **64**	> 32

From the Killing Efficiency Test, significant drops in the number of viable microbial cells after treatment with polymers LPEI-EDC$_{12}$ and bPEI-EDC$_{60}$ at minimum inhibitory concentration (MIC) indicated a microbicidal mechanism. The Killing Kinetics test demonstrated that at least 85% kill could be achieved within 2 minutes of treatment with the above polymers at 16 times MIC. Finally, FESEM imaging showed significant morphological changes to *E. coli* after treatment with LPEI-EDC$_{12}$.

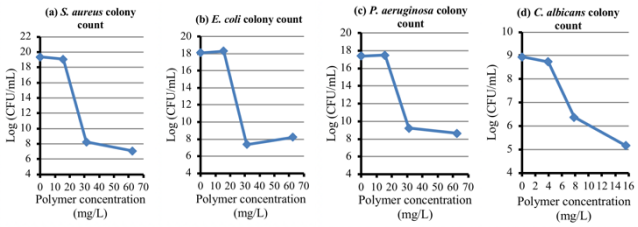

***Figure 1.** Viable microbial colony-forming units (CFUs) after treatment with various polymer concentrations. LPEI-EDC$_{12}$ against (a)SA (b)EC and (c)PA; (d)bPEI-EDC$_{60}$ against CA*

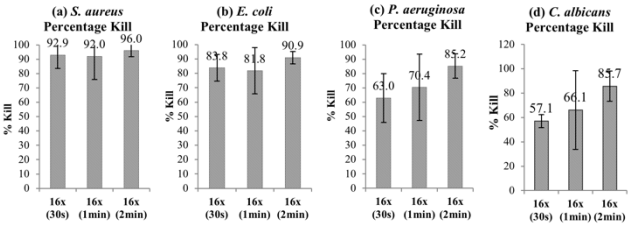

***Figure 2.** Percentage of microbes killed after treatment with polymer solution (16xMIC) for various time periods. LPEI-EDC$_{12}$ against (a)SA (b)EC and (c)PA; (d)bPEI-EDC$_{60}$ against CA*

4. Conclusion

We present in this paper a series of new PEI derivatives, of which polymers LPEI-EDC$_{12}$ and bPEI-EDC$_{60}$ have emerged as the strongest candidates against bacterial and fungal strains respectively, while remaining non-toxic to mammalian erythrocytes. The polymers demonstrated activity against both clinically relevant Gram-positive bacteria S. aureus and Gram-negative bacteria E. coli at 31.25 mg/L and fungi C. albicans at 7.81 mg/L, respectively. It was also determined that a microbicidal mnechanism was present and SEM Imaging visualised the membrane disruption of *E. coli* caused by the cationic polymer. Hence, these guanidine-functionalized PEIs can have potential applications as antimicrobial agents in personal care products such as antimicrobial soaps and shampoo.

5. Literature

[1] Chin, W., Yang, C., Ng, V. W., Huang, Y., Cheng, J., Tong, Y. W., … Yang, Y. Y. (2013). Biodegradable Broad-Spectrum Antimicrobial Polycarbonates: Investigating the Role of Chemical Structure on Activity and Selectivity. Macromolecules, 46(22), 8797-8807.