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Amphiphilic Antimicrobial Copolymers: exploring bio-based systems built with Amino Acids and Tetrahydrogeraniol

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INTRODUCTION

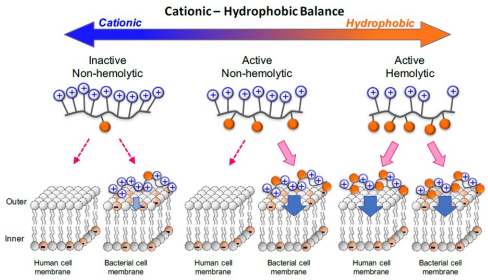

Figure 1. Schematic diagram of the influence of cationic-hydrophobic balance on antimicrobial activity and cytotoxicity in cationic amphiphilic random copolymers [1,2]

With the rise of antibiotic resistance and growing attention to personal health, there is increasing demand for effective antimicrobial materials. Amphiphilic antimicrobial polymers, inspired by antimicrobial peptides and composed of cationic and hydrophobic monomers, have drawn significant interest. In this study, bio-based precursors (amino acids and tetrahydrogeraniol) were used to synthesize a series of novel amphiphilic antimicrobial polymers, including both random and block copolymers. The influence of different amino acid-derived side chains (aliphatic, aromatic, and positively charged) on antimicrobial activity was systematically investigated.

DESIGN STRATEGY

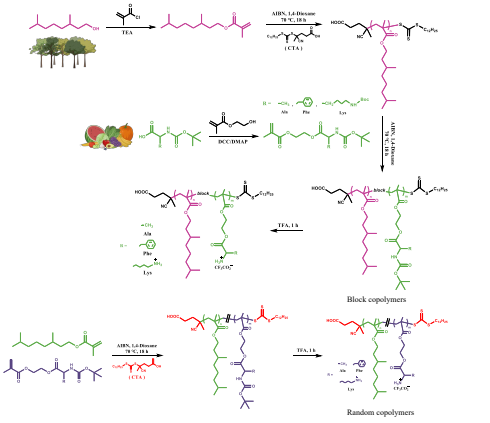

Figure 2. Schematic illustration of the investigation

RESULTS

Table 1. Characterization of amphiphilic polymers by NMR, GPC, DLS, zeta potential analysis, and evaluation of antimicrobial and cytotoxic effects

Amphiphilic polymers	Theoretical composition (%)	Boc-protected						Deprotected						MIC (μg mL^{-1})		MBC (μg mL^{-1})		IC_{50}^{e} (μg mL^{-1})	Selectivity[f]	
		MP_{THGC}[c] (%)	M_n[a]	DP^{an}	M_n[b]	M_n[b]	D^{b}	MP_{THGC}[c] (%)	M_n[a]	DP^{n}	M_n[b]	M_n[b]	D^{b}	D_h^{c} (nm)	ζ^{d} (mV)	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	*L929*	*E. coli*	*S. aureus*
P(A-r-T)7	70 (Ala) : 30 (THGA)	30	5290	17	4940	6170	1.25	34	3960	17	3580	4960	1.38	2.0	n.d	>5000	500	>5000	1000	>5000	/	>10
P(A-r-T)9	90 (Ala) : 10 (THGA)	13	5709	18	5040	6210	1.23	26	3420	16	3460	4880	1.41	1.7	+8.2	2500	>2000	5000	>2000	1250	0.5	<0.625
P(L-r-T)5	50 (Lys) : 50 (THGA)	51	5760	16	5910	7090	1.09	53	nd	nd	nd	nd	nd	48.1	+21.6	1000	1000	5000	>2000	>5000	2.5	2.5
P(L-r-T)7	70 (Lys) : 30 (THGA)	34	7070	18	6620	7170	1.08	29	nd	nd	nd	nd	nd	59.0	+18.5	250	2000	2000	>2000	>5000	10	1.25
P(L-r-T)9	90 (Lys) : 10 (THGA)	19	nd	nd	6310	7290	1.15	13	nd	nd	nd	nd	nd	64.6	+13.4	125	500	2000	500	2500	20	5
P(A(20)-b-T(20))	20 (Ala) : 20 (THGA)	45	6330	22	6820	7590	1.11	/	/	/	3690	4300	1.17	19.32	+48.19	>2000	250	>2000	2000	1250	/	5
P(A(30)-b-T(10))	30 (Ala) : 10 (THGA)	30	9160	31	9580	12260	1.28	/	/	/	/	/	/	20.35	+54.80	>2000	500	>2000	>2000	1250	/	2.5
P(P(30)-b-T(10))	30 (Phe) : 10 (THGA)	26	10100	28	10160	11750	1.16	/	/	/	6140	7290	1.19	90.98	+62.02	2000	1000	>2000	>2000	1250	0.625	1.25
P(L(20)-b-T(20))	20 (Lys) : 20 (THGA)	45	nd	nd	9980	11190	1.12	/	/	/	/	/	/	22.67	+52.02	62.5	125	500	1000	2500	40	20
P(L(30)-b-T(10))	30 (Lys) : 10 (THGA)	27	10700	26	11680	14560	1.25	/	/	/	/	/	/	26.77	+57.53	31.2	250	500	1000	2500	80	10

***MP*$_{THGC}$[c]** (mole percentage of hydrophobic side chains); **M_n[a]** (number average molecular weight); and **DP^n** (degree of polymerization) values were determined by ^{1}H NMR peak integration analysis. **M_n[b]**, **M_w[b]**, Dispersity[b] (**$Đ^b$**) was determined by GPC analysis in DMF against PMMA standards; Hydrodynamic particlesize (**Dh^c**) and zeta potential (**ζ^d**) determined by Malvern Zetasizer Nano ZS at a concentration of 0.1 mg mL^{-1} in PBS; [e]Concentration for a 50 % reduction in cell viability; [f]Selectivity is calculated by IC$_{50}$/MIC

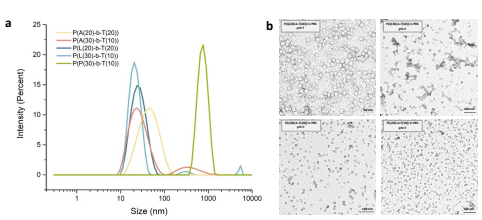

Figure 3. DLS intensity size distributions (**a**) and TEM images (**b**) of block amphiphilic copolymers in PBS (pH=7)

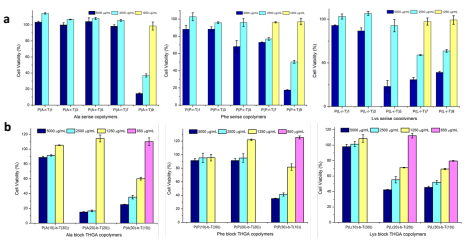

Figure 4. The cytotoxicity of random (**a**) and block (**b**) copolymers with different cationic amino acids and monomer feed ratios on cultured mouse fibroblasts (L929) after 24 h of incubation.

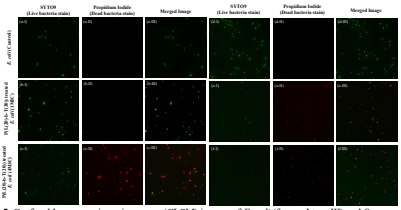

Figure 5. Confocal laser scanning microscopy (CLSM) images of *E. coli* (from a-I to c-III) and *S. aureus* (from d-I to f-III) treated with P(L(20)-b-T(20)) at different concentration (MIC and 4 x MIC).

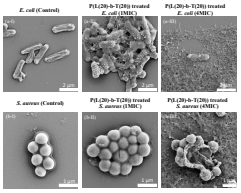

Figure 6. SEM images of E. coli (from a-I to a-III) and S. aureus (from b-I to b-III) treated with P(L(20)-b-T(20)) at different concentration (MIC and 4 x MIC) for 4 h.

CONCLUSIONS & OUTLOOK

- A library of amphiphilic random and block copolymers was synthesized using amino acid-derived cationic monomers (Ala, Phe, Lys) and bio-based THG as the hydrophobic component.
- Antimicrobial activity was closely dependent on the cationic/hydrophobic balance.
- Block copolymers exhibited better selectivity and lower cytotoxicity compared to random copolymers.
- Lys-based block copolymers showed strong activity against *E. coli* and *S. aureus*.
- Random copolymers required higher cationic content, increasing cytotoxicity risk.
- Live/dead staining and SEM confirmed membrane disruption as the primary killing mechanism.

REFERENCES

1. Takahashi H, Palermo E F, Yasuhara K, et al. Macromolecular bioscience, 2013.
2. Takahashi H, Caputo G A, Vemparala S, et al. Bioconjugate chemistry, 2017.

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