Fluorescent hyperbranched polymers and cotton fabrics treated with them as agents for antimicrobial photodynamic therapy

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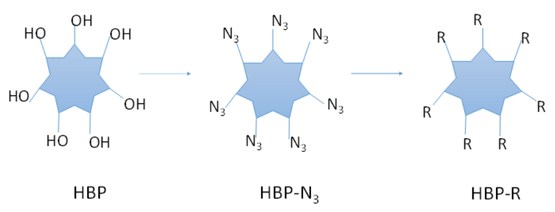
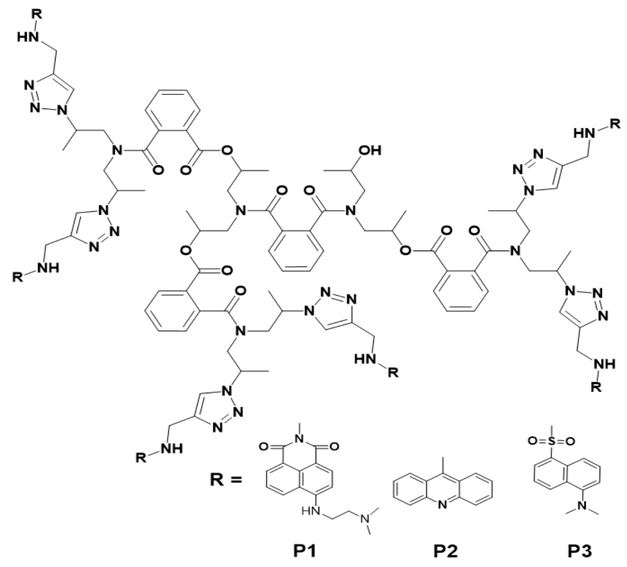
AIMS AND OBJECTIVES

The search for new bioactive substances with microbiological activity is dictated by the increasing resistance of the drugs used in clinical practice against various pathogenic microorganisms. This work presents the results of the first use of hyperbranched photoactive polymers as photodynamic antibacterial agents in solution and after their application on cotton fabric against Gram-positive and *Bacillus cereus* and Gram-negative *Pseudomonas aeruginosa* as model bacterial strains and two respiratory viruses, *HRSV-2* and *HAdV-5*. The generation of singlet oxygen and its role in the inactivation of the growth of pathogenic microorganisms are described.

RESULTS



Scheme 1. Synthetic route of hyperbranced polymers P1-P3.



Scheme 2. Chemical structure of modified photoactive hyperbranched polymers P1-P3.

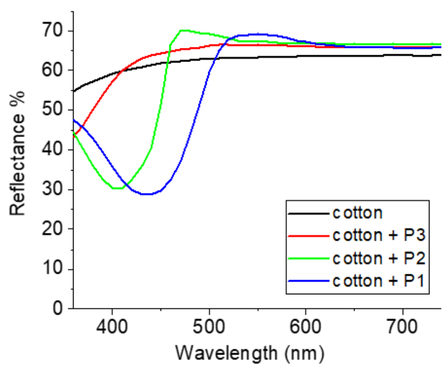


Figure 1. Reflectance curve of cotton fabric and cotton fabric treated with P1-P3.

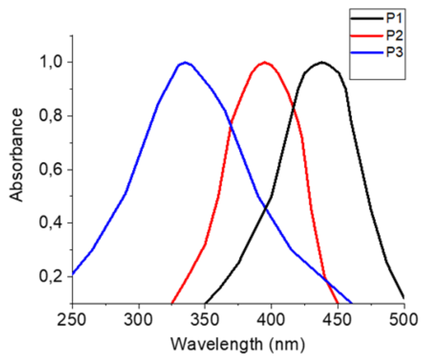


Figure 3. Normalised absorption spectra of polymers P1-P3 in ethanol solution.

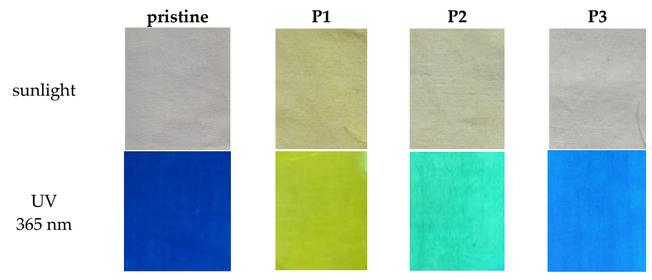


Figure 2. Micrograph of cotton fabrics treated with P1-P3 upon their illumination with sunlight and ultraviolet light at 365 nm.

Table 1. Color characteristics of cotton fabrics treated with P1-P3 polymers

	Before washing				After washing			
Sample	L*	a*	b*	ΔE*	L*	a*	b*	ΔE*
Cotton	83.55	-0.19	1.41	-	83.55	-0.19	1.41	-
Cotton + P1	84.30	-8.76	33.42	33.15	84.24	-7.13	25.83	25.27
Cotton + P2	84.57	-7.98	15.25	15.92	85.53	-7.28	12.95	13.69
Cotton + P3	85.10	-1.00	1.83	1.79	84.86	-0.88	1.69	1.50

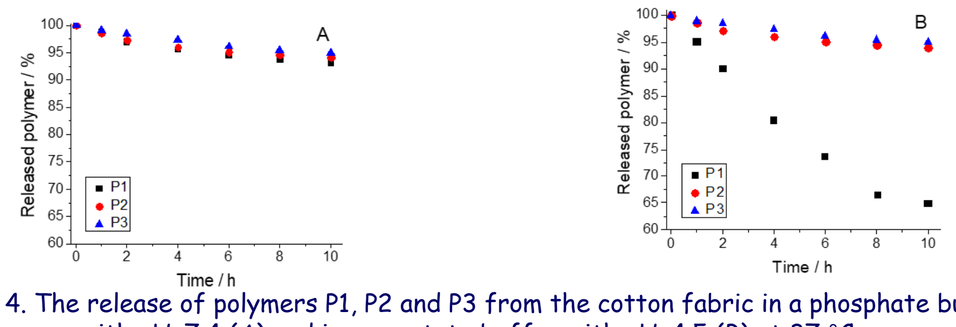


Figure 4. The release of polymers P1, P2 and P3 from the cotton fabric in a phosphate buffer with pH=7.4 (A) and in an acetate buffer with pH=4.5 (B) at 37 °C.

ANTIMICROBIAL ACTIVITY

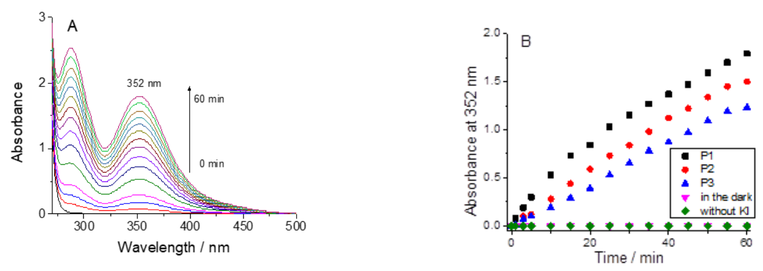


Figure 5. Absorption spectra of 0.5 M KI photo oxidized to I^{3-} in the presence of polymer P1 (A); dependence of the absorption at 352 nm corresponding to I^{3-} caused by polymers P1-P3, polymers in the dark and without KI (B), as a function of irradiation time.

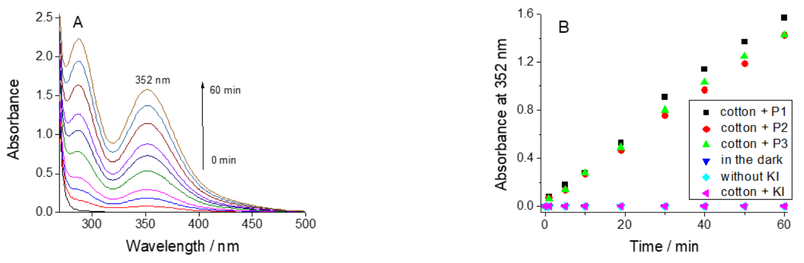


Figure 6. Absorption spectra of 0.5 M KI photooxidized to I^{3-} in the presence of cotton fabrics treated with polymer P1 (A); dependence of the absorption at 352 nm corresponding to I^{3-} caused by cotton fabrics treated with polymers P1-P3, cotton with KI, polymers in the dark and without KI (B), as a function of irradiation time.

Table 2. Virucidal effect against HRSV-2 and HAdV-5 (irradiated with light for 1h and non-irradiated).

	HRSV-2				HAdV-5			
	Irradiated		Non-irradiated		Irradiated		Non-irradiated	
	Δlog 30 min	Δlog 60 min	Δlog 30 min	Δlog 60 min	Δlog 30 min	Δlog 60 min	Δlog 30 min	Δlog 60 min
P1	0	0,2	0	0	0	0,6	0	0
P2	0	0,2	0	0	0	0,3	0	0
P3	0	0,5	0	0	0	0,9	0	0

Table 3. Cotton fabrics treated with the polymers – virucidal effect against (irradiated with light for 1h and non-irradiated).

	HRSV-2				HAdV-5			
	Irradiated		Non-irradiated		Irradiated		Non-irradiated	
	Δlog 30 min	Δlog 60 min	Δlog 30 min	Δlog 60 min	Δlog 30 min	Δlog 60 min	Δlog 30 min	Δlog 60 min
P1	0	0,1	0	0	0	0,2	0	0
P2	0	0,1	0	0	0	0,1	0	0
P3	0	0,3	0	0	0	0,4	0	0

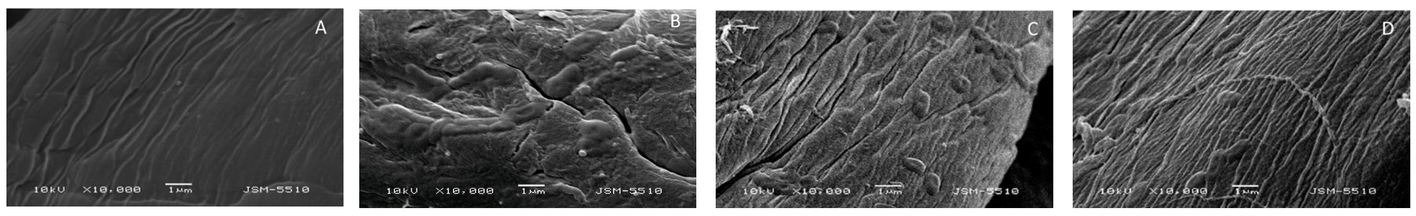
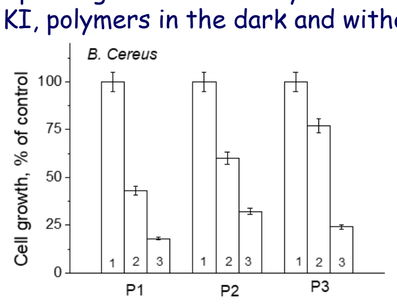


Figure 7. SEM micrographs at 10000x magnification of untreated cotton fabric (A), biofilm of *P. aeruginosa* on untreated cotton fabric (B), cotton fabric treated with P1 and in contact with bacteria in the dark (C), and cotton fabric treated with P1 in contact with bacteria during sunlight irradiation (D).



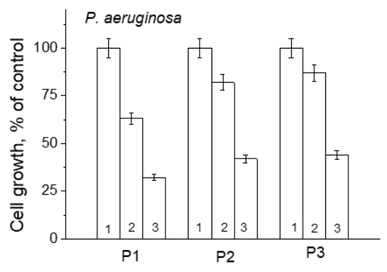
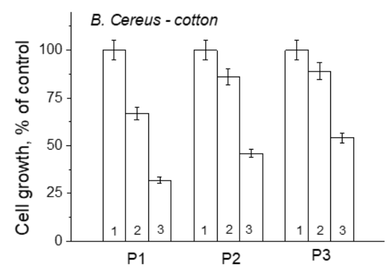


Figure 8. The effect of visible light on model bacteria P. aeruginosa and B. cereus growth in the presence of P1, P2 and P3 (at a concentration of 25 μg ml^{-1}). (1 – control at dark and after illumination, 2- in the dark, and 3- after light illumination).



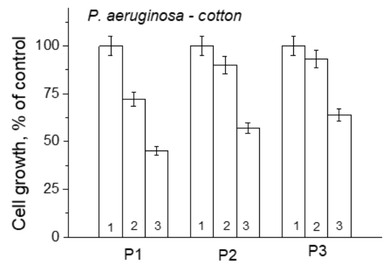


Figure 9. The effect of visible light on the growth of the model strains P. aeruginosa and B. cereus in the presence of cotton fabrics treated with polymers P1, P2 and P3, (1 - control at dark and after illumination, 2- in the dark, and 3 – after light illumination).

CONCLUSIONS

Three new cotton fabrics with antimicrobial properties were obtained by deposition on the fiber surface of photoactive hyperbranched polymers modified with 1,8-naphthalimide (P1), acridine (P2), and dansyl (P3) fragments. It was found that the release of polymers from cotton fabric occurs slowly at pH=7.4 whereas in an acidic medium pH=4.5, due to protonation of the tertiary amino group of 1,8-naphthalimide P1, passes significantly more readily into the aqueous solution.

The *in vitro* microbiological activity of hyperbranched polymers P1, P2, and P3 and cotton fabrics treated with them was investigated against Gram-negative *P. aeruginosa* and Gram-positive *B. cereus* model bacteria and two respiratory viral strains *HRSV-2* and *HAdV-5* in the dark and after irradiation with sunlight. The results show high microbiological activity of all three polymers against Gram-positive and Gram-negative bacterial strains, and their activity is preserved after their application to cotton fabric. The antibacterial effect was enhanced several times after irradiation with light due to the singlet oxygen generated by the polymers. In the dark, the microbiological impact of cotton fabrics treated with P1-P3 is mainly due to their contact with microbial cells. On the other hand, the polymers deposited on the cotton fabric prevented the formation of bacterial biofilms. The virucidal effect against respiratory viruses *HRSV-2* and *HAdV-5* was observed only after irradiation. The results of this study suggest that hyperbranched polymers have promising potential for producing antibacterial and self-disinfecting textiles with biomedical applications.

Acknowledgements

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