Volume 8, Issue 1., 2018, 3053 - 3059 **ISSN 2069-5837**

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

Original Research Article **Open Access Journal**

Received: 12.01.2018 / Revised: 10.02.2018 / Accepted: 12.02.2018 / Published on-line: 15.02.2018

Evaluation of antimicrobial, biofilm inhibitory and cytotoxic activities of a new hiperbranched polymer modified with 1,8-naphthalimide units

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ABSTRACT

New hyperbranched polymer modified with six 1,8-naphthalimide units (P1000-Napht) was tested *in vitro* for antimicrobial activity against Gram-positive bacteria *Bacillus subtilis* and *Bacillus cereus*, Gram-negative bacteria *Pseudomonas aeruginosa* and *Acinetobacter johnsonii*, and the yeasts *Candida lipolytica*. The new modified polymer displayed moderate to good antimicrobial potential against the tested strains. The effect of the P1000-Napht on membrane permeability of bacterial cells was evaluated, and cytotoxicity assay was performed against HEp-2 cell line. The antibacterial finishing of the treated cotton fabric and polylactic acid film was assessed toward Gram-positive and Gram-negative bacteria. The obtained results suggest that the new modified dendritic polymer may be developed as promising antimicrobial alternative for biomedical application.

Keywords: *antimicrobial activity, antibacterial cotton fabrics, cytotoxic activity, hiperbranched polymer, 1,8-naphthalimide, polylactic acid films.*

1. INTRODUCTION

The emergence of microbial resistance to conventional antibiotics is a serious threat to the effectiveness of current antimicrobial therapy [1]. Therefore, the discovery of novel antimicrobial agents to which the pathogenic microbes cannot develop resistance easily is one of the major medical concerns of the 21st century [2]. Nanomaterials could serve as a long-term solution to the growing problem of antimicrobial resistance because they have shown antimicrobial effect against a wide range of drug-resistant pathogens. Dendrimers are a special class of nano-sized, polymeric macromolecules with highly branched three-dimensional architecture consisting of an initiator core, a radiating interior structural layer composed of repeating generations (G0-G10), and end-groups attached on an outer layer of repeat units [3, 4]. These end-groups can be functionalized, thus modifying physicochemical or biological properties of dendrimers [5]. The low polydispersity and the ability to precisely control their surface chemistry make dendrimers especially useful for pharmaceutical and biomedical applications [6-9]. Many researchers are focused on dendrimers as potential antimicrobial compounds or agents improving antibacterial or antifungal activity of existing chemotherapeutics [10]. As the mechanism by which dendrimers kill or inhibit the growth of bacteria depends in particular on the type of dendrimer peripheral groups, special design is required for the synthesis of dendrimers for biomedical applications [11]. It has been found that dendrimer biocides are more potent than both polymeric biocides and small molecule biocides. The significant improvements of biocide action of dendrimers are attributed to the high number of functional groups in a compact space and their polycationic structure [12].

Hyperbranched polymers (HBP) are a class of synthetic tree-like macromolecules called dendritic polymers with densely branched structure and a large number of end functional groups [13]. Recently, a new fluorescent HBP containing 1,8-naphthalimide units in the side-chain of a commercial HBP, designated as P1000-Napht, has been synthesized for the first time through click chemistry, and its photophysical properties have been investigated [14]. In the present study, the antimicrobial, biofilm inhibitory and cytotoxic activities of the newly synthesized P1000-Napht are investigated and some possible applications are discussed.

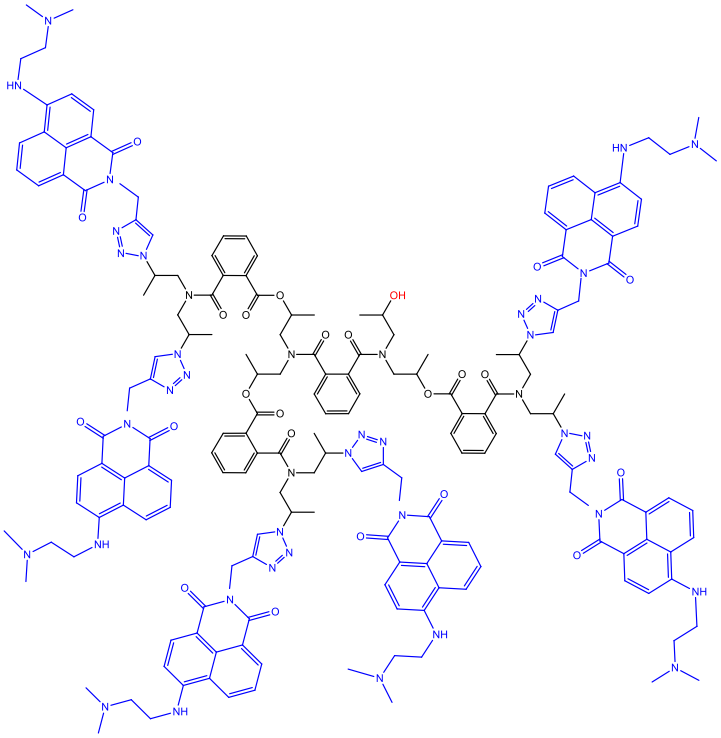
2. EXPERIMENTAL SECTION

The syntesis and characterization of HBP P1000-Napht (Scheme 1) have been described recently [14].

Microorganisms. The antimicrobial activity of the new HBP P1000-Napht was tested against the following model pathogenic strains (Collection of the Institute of Microbiology, Bulgarian Academy of Sciences): Gram-positive bacteria *Bacillus subtilis* and *Bacillus cereus*, Gram-negative bacteria *Pseudomonas aeruginosa* and *Acinetobacter johnsonii*, and the yeasts *Candida lipolytica*. The cultures were maintained at 4°C on Mueller-Hinton agar (MHA) slants and transferred monthly.

Agar diffusion assay. The antimicrobial activity of the P1000-Napht was firstly tested by the agar well diffusion assay. MHA plates of 3-4 mm thickness were seeded with aliquots of overnight grown test cultures. Stock solution of the investigated HBP in DMSO (0.5%) was prepared and equal amounts (30 µl) were added into wells (8 mm in diameter) punched into MHA.

Commercial discs with gentamicin (G, 10 µg) and nystatin (Ns, 100 units/disc) were used as standard antibacterial and antifungal agents, respectively. The plates were incubated overnight at respective temperatures and the growth was monitored for 24-48 h. The antimicrobial activity was indicated by the presence of clear zones around the wells (mm in diameter, including well/disc).



Scheme 1. Chemical structure of hiperbranched polymer P1000-Napht.

Growth inhibitory activity in aqueous solution. Tube dilution assay was performed to evaluate quantitatively the antimicrobial activity of the new HBP. The compound was dissolved in DMSO at a concentration 5 mg/ml (153.3 µM) and was further serially diluted in meat-peptone broth (MPB, pH 7.0) to final concentrations in the range of 122.6-4.6 µM. The inocula were prepared by diluting the overnight cultures with 0.9% NaCl to a 0.5 McFarland standard. After inoculation with 2% (v/v) of each standardized inoculum, the tubes were incubated at appropriate temperature for 24 h under shaking (240 rpm). Growth was assayed by monitoring absorbance at 600 nm (OD_{600}). Experiments were performed with a positive control (HBP and MPB), without inoculum) and a negative control (MPB and inoculum, without HBP). The % growth of the test cultures was determined on the basis of the positive control which was considered as 100%. The lowest concentration of the HBP that was able to inhibit visible growth of microbes was referred to as minimum inhibitory concentration (MIC). All assays were performed in triplicate and mean values are presented.

Antimicrobial test of cotton fabrics. Gram-positive bacteria *B. subtilis* and *B. cereus*, Gram-negative bacteria *P. aeruginosa* and *A. johnsonii*, and the yeasts *C. lipolytica* were used to evaluate the antimicrobial effectiveness of cotton fabrics treated with the new HBP. Test tubes with sterile MPB were inoculated with each overnight grown test culture and sterile square shape cotton speciments (10 mm × 10 mm) were inserted into the test tubes. Tubes with untreated cotton sample and tubes without speciments were also prepared as controls. After 24 h incubation at appropriate temperature, the specimens were removed and the bacterial growth was determined by measuring the turbidity at 600 nm (OD_{600}). To evaluate the antimicrobial activities of the samples, the reduction in cell growth between the untreated and treated samples after incubation was compared. All antimicrobial activity tests were done in triplicate.

Preparation of cotton fabrics for SEM. The adhesion and biofilm formation on cotton fabric were assessed using scanning electron microscopy (SEM). Samples of sterile cotton fabric, untreated and treated with P1000-Napht, were incubated 24 h at 25°C in MPB inoculated with cell suspension of *Acinetobacter johnsonii*. After incubation, the samples were washed with phosphate buffered saline, dried and coated with gold with Jeol JFC-1200 fine coater, and then investigated by a Jeol JSM-5510 scanning electron microscope at different magnification.

Preparation of polylactic acid films. Pure polylactic acid (PLA) and HBP-PLA films were prepared by solvent casting method. Polylactic acid (0.5 g) was dissolved in 10 ml chloroform and 5×10^{-4} g of the new HBP was added. After 30 min stirring, the homogeneous mixture was poured into a Petri dish and the solvent was evaporated slowly. Thus a stable polymer film with a thickness of 80 µm was obtained.

Test of film antimicrobial activity. The antimicrobial effect of the obtained PLA film with incorporated P1000-Napht was investigated against the strains *A. johnsonii*, *P. aeruginosa*, *B. subtilis*, *B. cereus* and *C. lipolytica*. For antimicrobial tests, square shape speciments of 10 mm × 10 mm were cut from pure PLA and HBP-PLA films under aseptic conditions. The test tubes with sterile MPB medium were inoculated with each overnight grown test culture and left at room temperature for 15 min. Then, the PLA specimens were inserted into the test tubes. Tubes without film speciments were also prepared for each bacterial culture. After 24 h incubation at 25°C under shaking, the specimens were removed and the microbial growth was determined by measuring OD_{600}.

Assessment of hydrophilicity of cotton fabrics. Hydrophilicity of the untreated and treated with P1000-Napht cotton fabrics was assessed using static immersion test reported in ATCC Technical Manual 2001. This is a test used to measure the amount of water absorbed by the fabric. The tested samples were weighed and immersed to a depth of 10 cm in a beaker with distilled water. The cotton fabrics were removed after 20 min and tapped to remove excess water and then weighed once again. The absorption percentage was determined by the following formula [15]:
Absorption (%) = (mass of water absorbed/original mass) × 100

Cell surface hydrophobicity assay. Cell surface hydrophobicity was assessed using the microbial adhesion assay to hydrocarbons [16]. Briefly, model microbial cells were cultured in MPB till the growth reached mid-log phase. After centrifugation, the cells were washed twice with PUM buffer and resuspended to reach OD_{600} nm between 0.4 and 0.5 (A0). Aliquots of each cell suspension (1.5 ml) were overlaid by 0.2 ml of the hydrophobic hydrocarbon

n-hexadecane. After vigorous vortexing, phases were allowed to separate for 10 min at 30°C and the OD_{600} nm of the aqueous phase was measured (A1). The percentage of hydrophobicity was calculated as follows: hydrophobicity (%) = [1−(A1/A0)] × 100. All assays are average of three separate experiments.

***In vitro* cytotoxicity assay.** HEp-2 cell line was used for cytotoxicity assays. Cells were incubated in 96 well plates using the appropriate culture medium. Monolayer cell cultures were inoculated with 0.1 mL/well maintenance medium containing different concentrations of the samples in 0.5 lg intervals. The controls consisted of cells were incubated with DMEM only. After 48 h the maintenance medium containing the test compound was removed, cells were washed and 0.1 mL maintenance medium supplemented with 0.005% neutral red dye was added to each well and cells were incubated at 37°C for 3 h. After incubation, the red dye was removed, and cells were washed once with PBS and 0.15 mL/well desorbs solution (1% glacial acetic acid, 49% ethanol, 50% distilled water) was added. The optical density (OD) of each well was read at 540 nm in a microplate reader (Organon Teknika reader 530). The 50% cytotoxic concentration (CC_{50}) was defined as the material concentration that reduced the cell viability by 50% when compared to untreated controls.

Assay for protein leakage. The effect of the new HBP P1000-Napht on protein leakage was determined by treating *B. cereus* and *P. aeruginosa* cells with P1000-Naph solution. Strains were grown in MPB until the mid-log phase. Cells were then harvested, washed and re-suspended in 0.5% NaCl to a final OD_{600} of 1.0. Sample solution (100 µL) in concentrations 9.8, 19.9 and 38.3 µM, arrived at from bacterial growth inhibition assays, were mixed with 900 µL of each bacterial suspension it test tubes and incubated with shaking at room temperature for 1 h. Control was carried out with 0.5% NaCl alone. The amount of protein released in the suspension of the treated cells was estimated using the bicinchoninic acid assay (BCA assay) [17], and compared with that of the control samples without adding P1000-Napht.

3. RESULTS SECTION

Growth inhibitory activity in agar and in aqueous solution. The antimicrobial activity of 1,8-naphthalimide-modified dendritic polymer was evaluated against five pathogenic strains. It was initially determined by the agar well diffusion assay using 153.3 µM solution of the studied compound. The results showed inhibition zones in the range of 11-14 mm against all test cultures except *E. coli* and *S. cerevisiae*, which were found less sensitive to the studied polymer (Figure 1).

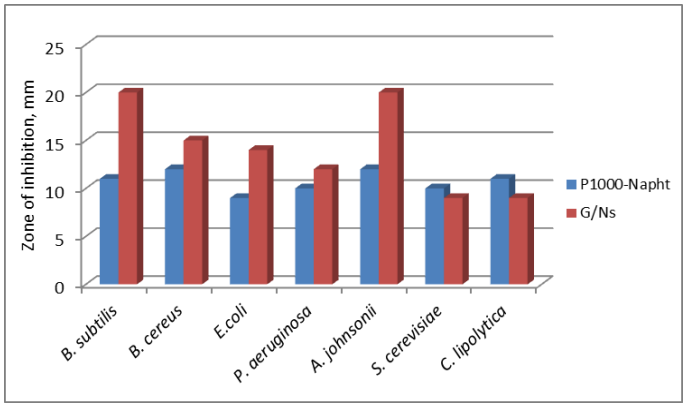


Figure 1. Zones of inhibition of model bacteria and yeasts by 153.3 µM solution of P1000-Napht polymer.

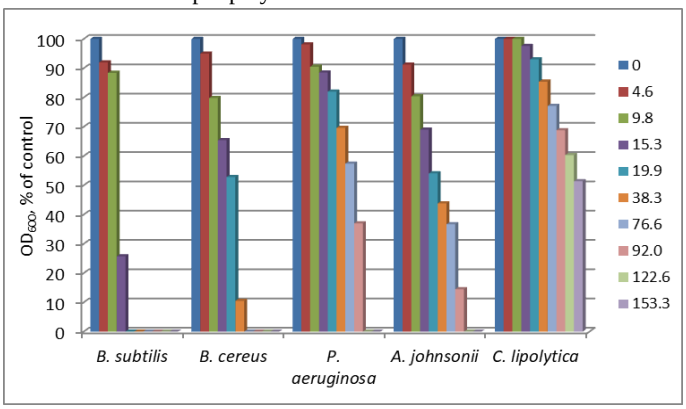


Figure 2. Growth of model microbial strains in presence of different concentrations (in µM) of P1000-Napht polymer.

A quantitative evaluation of the antimicrobial activity of the new HBP was carried out by the shaking flask test against bacterial strains *P. aeruginosa*, *A. johnsonii*, *B. subtilis* and *B. cereus*, and the yeasts *C. lipolytica*. Figure 2 shows the toxicity of the P1000-Napht at concentrations ranging from 4.6 µM to 153.3 µM. The presence of the new P1000-Napht affected the cell growth of all test cultures as compared to the negative control. Cell growth was reduced with increasing the concentrations of P1000-Napht. The relative order of sensitivity to P1000-Napht was found to be a function of the strain. In the case of Gram-positive bacteria *B. subtilis* and *B. cereus*, P1000-Napht exhibited stronger antimicrobial efficiency than towards Gram-negative bacteria (MIC_{90} values at 19.9 µM and 38.3 µM, respectively). In the case of Gram-negative *P. aeruginosa* and *A. johnsonii*, no growth was observed in presence of 122.6 µM P1000-Napht. *C. lipolytica* demonstrated higher resistance to the P1000-Napht with MIC_{50} determined in concentration 153.3 µM.

It is suggested that antimicrobial properties of dendrimers may be determined by their ability to bind to a negatively charged bacterial cell surface and disrupt membrane integrity but the mechanism of their action is still unclear [12, 18]. The same mechanism is proposed for interactions between dendrimers and eukaryotic cells [19]. It has been shown in model lipid bilayers that neutral dendrimers adsorbed to the edges of existing holes and removed lipids forming dendrimer–lipid aggregates [20]. Morgan et al. [21] suggested that dendrimers interact with membrane proteins and phospholipids, which leads to cell membrane disruption. Cationicity and hydrophobicity play an important role in the membrane-lytic activity of dendrimers. Dendrimers have various surface charges from the cationic charge to neutral [22]; in neutral and alkaline solutions they are neutral and hydrophobic interactions dominate. Hydrophobicity can force the insertion of antimicrobials into the hydrophobic bacterial membranes. In this study, the addition of naphthalimide provides the P1000 polymer

more hydrophobic properties and contributed to the membranelytic activity of the compound. In addition, as recently published, very low molecular weight dendrimers plausibly induced antimicrobial activity by diffusing across the cellular membrane affecting intracellular pathways. This occurred at a more efficient rate compared to the medium generation numbers of dendrimers, which might not be able to penetrate bacterial membranes [23]. In this case, the very low molecular weight of P1000-Napht could improve the efficient internalization of the polymer and then its better performance as antimicrobial agent.

We have demonstrated that P1000-Napht HBP was more toxic to Gram-positive *Bacillus* strains than to the test Gram-negative bacteria. Similar observations have been reported for modified with maltotriose PPI dendrimers with positively charged amino surface groups [24]. The differences in biological activity of the P1000-Napht to Gram-positive and Gram-negative bacteria could be explained by the differences in their cell wall structure and thus in cell permeability. As a whole, Gram-positive bacteria exhibit lower resistance to biocides compared to Gram-negative bacteria [25]. More hydrophobic cell surface determined for the test *B. subtilis* and *B. cereus* cells (62% and 78% hydrophobicity, respectively; Figure 3) promote the adhesion of the P1000-Napht. Gram-negative bacteria contain a thin peptidoglycane layer but have an additional outer membrane, which acts as a strong permeability barrier to macromolecules and hydrophilic substances [26]. This multilayered cell structure and more hydrophilic nature of Gram-negative *A. johnsonii* and *P. aeruginosa* cells (12% and 33%, respectively, Figure 3) determine their higher resistance to the studied P1000-Napht.

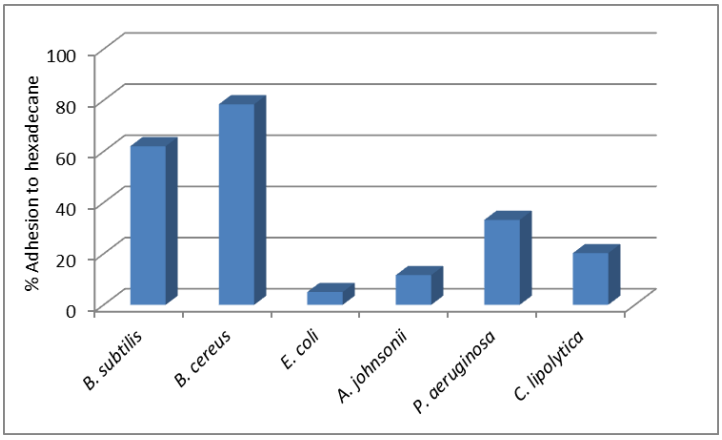


Figure 3. Cell surface hydrophobicity of the test microbial strains grown in MPB.

P1000-Naph-induced leakage of proteins. The extent of membrane permeabilization by P1000-Napht was assessed by measuring the general protein release from the cells. As can be seen in Figure 4, treatment of both types of cells with P1000-Napht resulted in a significant increase in the amount of protein released in the cell suspension, suggesting that P1000-Napht increased the permeability of the bacterial cell membrane.

Antimicrobial activity of modified cotton fabric. The antimicrobial activity of cotton fabric treated with P1000-Napht polymer was evaluated by the reduction in bacterial growth. We found that the treated cotton textile reduced the growth of *A. johnsonii* and *P. aeruginosa* by about 50% and 15%, respectively, and no growth reduction of the other test strains was observed (Figure 5). It can be assumed that both, slow diffusion of dendrimers from the cotton textile into the medium and direct contact with bacterial cells contributed to the antibacterial effect of treated cotton fabrics.

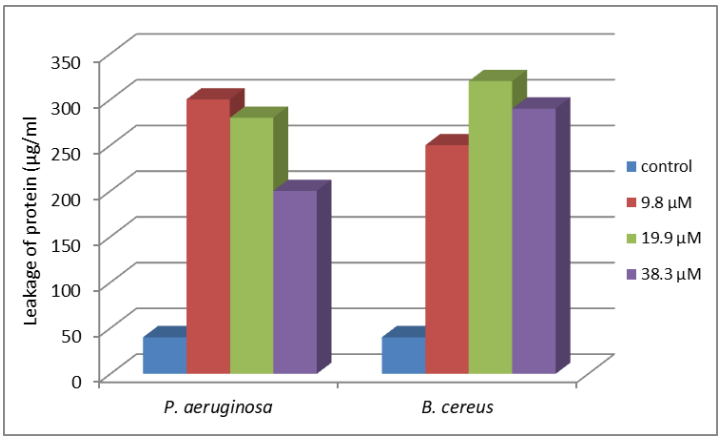


Figure 4. Effect of P1000-Napht polymer on leakage of protein by *P. aeruginosa* and *B. cereus* cells.

Antimicrobial modification of material surfaces is an alternative way of preventing the formation of highly resistant biofilms and can be achieved by different chemical and physical approaches [27]. Dendritic polymers have also been proposed to develop antimicrobial properties for application to textiles [28,29].

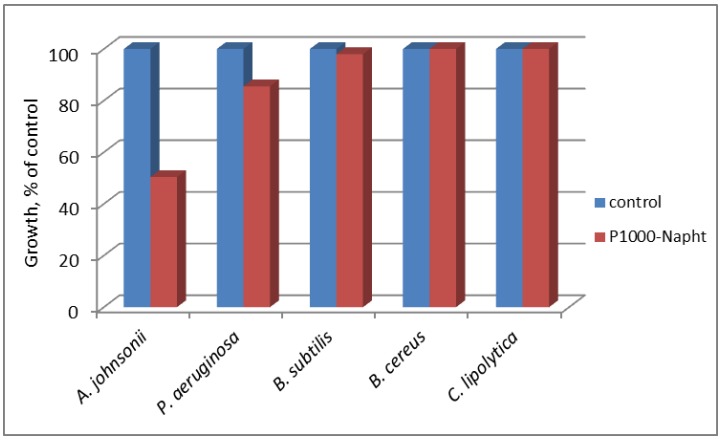


Figure 5. Effect of untreated (control) and treated with P1000-Napht cotton fabrics on the growth of the test microbial strains.

It has been shown that low-generation and flexible dendrimers can adsorb and form a film on surfaces *via* multiple interactions [30]. The antimicrobial effect of textiles may be influenced by several factors such as mechanical retention of microbial cells on textiles depending on the surface morphology; dispersion of the antimicrobial material on the textile surface, in low concentrations avoiding agglomeration in coarse particles, thus reducing contact surface of the agent; variation of the hydrophobic/hydrophilic nature of textiles, which may influence the contact degree of the microbial inoculum with the textiles [31].

Hydrophilicity of cotton fabrics. Hydrophilicity of the treated and untreated cotton fabrics was measured by static immersion test. The absorption of water by the untreated cotton sample and the sample treated with P1000-Napht was determined to be 112% and 96%, respectively. The results clearly show an increase in the hydrophobicity of the treated when compared to the untreated cotton fabrics. In the used MPB medium with neutral pH, the studied polymer is electroneutral, and applied on the cotton surface makes it more hydrophobic. The increase in hydrophobicity of the cotton textile treated with P1000-Napht is due mainly to 1,8-naphthalimide residues attached to the polymer ligand.

The surface charge and surface hydrophobicity of bacterial cells are important physical factors for their adhesion. Bacteria in aqueous suspension are negatively charged; a high surface charge is accompanied by a hydrophilic character of the bacteria [32]. Multilayered cell wall structure and hydrophilic nature (about 12% cell hydrophobicity) confers higher resistance and limited adhesion of the test Gram-negative *A. johnsonii* cells to the more hydrophobic P1000-Napht-treated cotton surface.

SEM investigations. The presence of the P1000-Napht particles on the cotton surface was confirmed by SEM observation. Figure 6 presents SEM micrographs of cotton fabric treated with P1000-Napht at different amplification. It can be seen that P1000-Napht nanoparticles are spread almost evenly across the cotton surface; they are nearly spherical with small size and are located in the gaps of cotton threads. On the other hand it is seen that the P1000-Napht nanoparticles present as clusters with tendency to form agglomerates with higher dimensions.

The efficacy of cotton fabric treated with the new P1000-Napht in preventing the adhesion and formation of bacterial biofilm was investigated by SEM. The micrographs of untreated cotton fabric and cotton fabric treated with P1000-Naph in two magnifications are presented in Figure 7. After 24 h incubation, SEM images revealed the formation of *A. johnsonii* biofilm on untreated cotton sample showing bacterial cells adhering to the cotton surface and embedded in an extracellular matrix (Figure 7a). In cotton sample treated with P1000-Napht, only single cells are attached to the cotton surface and no biofilm formation is visible (Figure 7b). Therefore, deposition of the studied polymer on the cotton fabric prevents the formation and proliferation of bacterial biofilm.

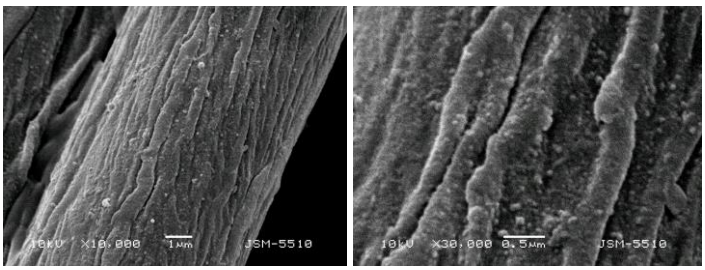
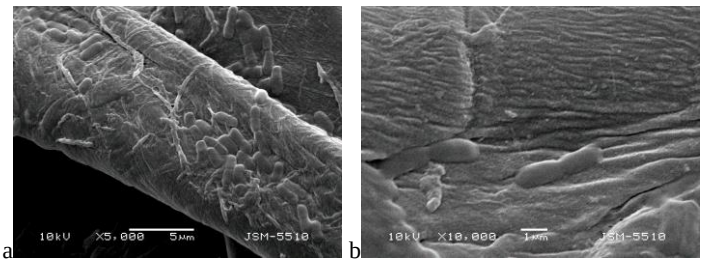


Figure 6. SEM micrographs of cotton fabrics treated with P1000-Napht with different amplification.



a b

Figure 7. SEM images of cotton fabrics tested against *A. johnsonii* at magnification x5000 and x10000: a) biofilm on untreated cotton textile (control sample); b) the absence of biofilm on cotton textile treated with P1000-Napht.

The production of biofilms by bacteria can cause resistance to various antibacterial agents. Thus, the inhibition of biofilm activity may be important for preventing infections and various other disorders. Overall, dendrimers have been shown to be highly effective in biofilm inhibition and reduction. It is hypothesized that microbial adhesion as a first step for biofilm formation could be limited by dendrimer interaction with the cell surface or with ions that promote microbial adhesion changing cell wall properties and avoiding microbial attachment. Similarly to *Legionella pneumophila* biofilm inhibition by PAMAM dendrimers [33], we assume that the presence of the studied P1000-Napht polymer caused the lack of extracellular matrix, resulting in the detachment of cells from the cotton surface.

Antimicrobial activity of PLA films with incorporated P1000-Napht. The results of antibacterial tests of the obtained P1000-Napht-PLA film are shown in Figure 8. As can be seen, no significant inhibition effect of the modified PLA film on the growth of *A. johnsonii*, *P. aeruginosa* and *B. subtilis* was determined (growth inhibition in the range 7-12%), and no inhibition effect was observed against *B. cereus*. The antimicrobial effect should be due mainly to the release of P1000-Napht from the PLA matrix into the medium. As has been reported for incorporated zein films [34], several factors may have effect on the release profile of the immobilized activity such as degree of hydrophilicity of the film surface, the level of immobilized antimicrobial compound retained at the film surface, and the surface area of films.

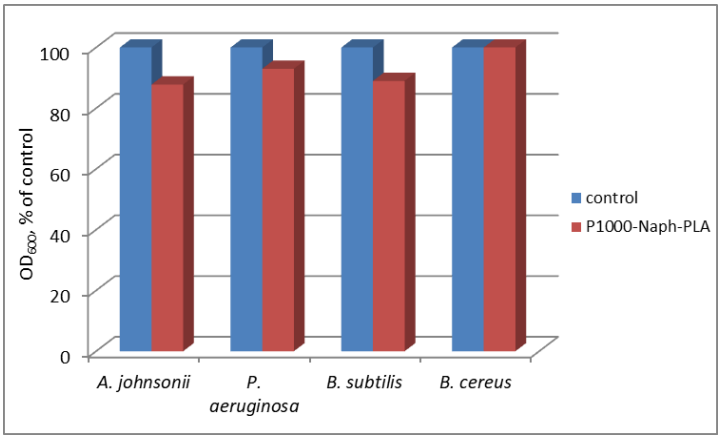


Figure 8. Effect of P1000-Napht -PLA film speciments on the growth of Gram-negative and Gram-positive bacteria tested in MPB.

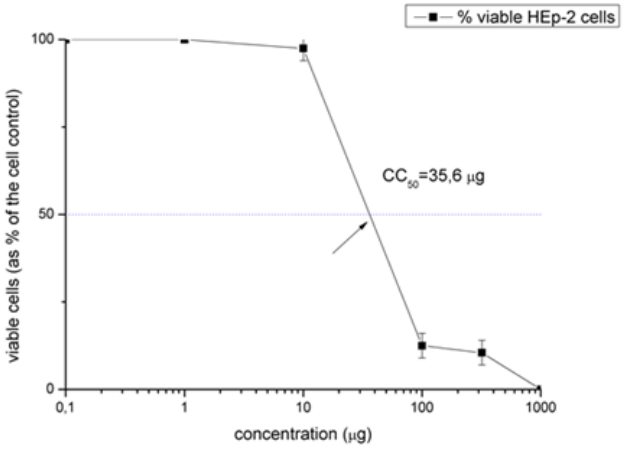


Figure 9. Cytotoxic concentration 50% of P1000-Napht in HEp-2 cell culture.

***In vitro* cytotoxicity assay.** It is well known that unmodified PPI dendrimers are more cytotoxic than modified dendrimers against a variety of eukaryotic cell lines [35]. There are several reports demonstrating that functionalized dendrimers exhibit lower cytotoxicity than unmodified macromolecules [36-39]. Therefore we assumed that naphthalimide-modified HBP may exhibit lower toxicity against eukaryotic cells. We evaluated the P1000-Napht

for its *in vitro* cytotoxicity against HEp-2 cell line. The obtained results showed that modified polymer in 11.2 μM concentration (35.6 μg/mL) induced a 50% decrease in cell viability for the HEp-2 cell line (Figure 9) indicating low cytotoxicity.

4. CONCLUSIONS

New hyperbranched polymer modified with six 1,8-naphthalimide units (P1000-Napht) showed moderate to good antimicrobial activity towards several Gram-positive and Gram-negative bacteria and yeasts. The treated with P1000-Napht cotton fabric effectively inhibited the formation of bacterial biofilms. The obtained results for cytotoxicity and antimicrobial activity make this hyperbranched naphthalimide polymer interesting for biological applications.

5. REFERENCES

[1] Stewart P.S., Costerton J.W., Antibiotic resistance of bacteria in biofilms, *Lancet*, 358, 135-138, **2001.**

[2] Huang K.S., Yang C.H., Huang S.L., Chen C.Y., Lu Y.Y., Lin Y.S., Recent advances in antimicrobial polymers: a mini-review, *Int. J. Mol. Sci*., 17, 1578, **2016.**

[3] Grayson S.M., Fréchet J.M., Convergent dendrons and dendrimers: from synthesis to applications, *Chem. Rev*., 101, 12, 3819-3868, **2001.**

[4] Abbasi E., Aval S.F., Akbarzadeh A., Milani M., Nasrabadi H.T., Joo S.W., Hanifehpour Y., Nejati-Koshki K., Pashaei-Asl R., Dendrimers: synthesis, applications, and properties, *Nanoscale Res. Lett*., 9, 247, **2014.**

[5] Bosman A.W., Meijer E.W., About dendrimers: structure, physical properties, and applications, *Chem Rev*., 9, 1665-1688, **1999.**

[6] Gillies E., Fréchet J.M.J., Dendrimers and dendritic polymers in drug delivery, *Drug Discovery Today*, 10, 35-43, **2005.**

[7] Yang H., Kao W.J., Dendrimers for pharmaceutical and biomedical applications, *Biomater. Sci. Polym*., 17, 3-19, **2006.**

[8] Scholl M., Kadlecova Z., Klok H.-A., Dendritic and hyperbranched polyamides, *Prog. Polym. Sci*., 34, 24-61, **2009.**

[9] Ma N., Ma C., Deng Y., Wang T., He N., Advances in applications of dendritic compounds, *J. Nanosci. Nanotechnol.*, 13, 33-39, **2013.**

[10] Mintzer M.A., Grinstaff M.W., Biomedical applications of dendrimers: a tutorial, Chem. Soc. Rev., 40, 173-190, **2011.**

[11] Castonguay A., Ladd E., van de Ven T.G.M., Kakar A., Dendrimers as bactericides, *New J. Chem*., 36, 199-204, **2012.**

[12] Chen C.Z., Beck-Tan N.C., Dhurjati P., van Dyk T.K., LaRossa R.A., Cooper S.L., Quaternary ammonium functionalized poly(propylene imine) dendrimers as effective antimicrobials: structure-activity studies, *Biomacromolecules*, 1, 473-480, **2000.**

[13] Seiler M., Dendritic Polymers – Interdisciplinary Research and Emerging Applications from Unique Structural Properties, *Chem. Eng. Technol*., 25, 237-253, **2002.**

[14] Medel S., Bosch P., Grabchev I., de la Torre M.C., Pedro Ramírez P., Click chemistry to fluorescent hyperbranched polymeric sensors. 2. Synthesis, spectroscopic and cation-sensing properties of new green fluorescent 1,8-naphthalimides, *Eur. Polym. J*., 74, 241-255, **2016.**

[15] Vaideki K., Jayakumar S., Rajendran R., Thilagavathi G., Investigation on the effect of RF plasma and neem leaf extract treatment on the surface modification and antimicrobial activity of cotton fabric, *Appl. Surf. Sci*., 254, 2472-2478, **2008.**

[16] Rosenberg M., Bacterial adherence to hydrocarbon: a useful technique for studying cell surface hydrophobicity, *FEMS Microbiol. Lett*., 22, 289-295, **1984.**

[17] Walker J.M., The bicinchoninic acid (BCA) assay for protein quantitation, *Methods Mol. Biol*., 32, 5-8, **1994.**

[18] Tülü M., Ertürk A.S., Dendrimers as Antibacterial Agents. In: A Search for Antibacterial Agents, Ed. V. Bobbarala (Rijeka: InTech), 89-106, **2012.**

[19] Kuo J.S., Jan M., Lin Y.-L., Interactions between U-937 human macrophages and poly(propyleneimine) dendrimers, *Journal of Controlled Release*, 120, 51-59, **2007.**

[20] Mecke A., Uppuluri S., Sassanella TM, Lee D.K., Ramamoorthy A., Baker J.R. Jr, Orr B.G., Banaszak Holl M.M., Direct observation of lipid bilayer disruption by poly(amidoamine) dendrimers. *Chem. Phys. Lipids*, 132, 3-14, **2004.**

[21] Morgan D.M., Larvin V.L., Pearson J.D., Biochemical characterisation of polycation-induced cytotoxicity to human vascular endothelial cells, *J. Cell Sci*., 94, 553-559, **1989.**

[22] Fischer M., Appelhans D., Klajnert B., Bryszewska M., Voit B., Rogers M., Influence of Surface Functionality of Poly(propylene imine) Dendrimers on Protease Resistance and Propagation of the Scrapie Prion Protein, *Biomacromolecules*, 11, 1314-1325, **2010.**

[23] Engler A.C., Wiradharma N., Ong Z.Y., Coady D.J., Hedrick J.L., Yang Y.Y., Emerging trends in macromolecular antimicrobials to fight multi-drug-resistant infections, *Nano Today*, 7, 201-222, **2012.**

[24] Felczak A., Wrońska N., Janaszewska A., Klajnert B., Bryszewska M., Appelhans D., Voit B., Różalska S., Lisowska K., Antimicrobial activity of poly(propylene imine) dendrimers, *New J. Chem*., 36:2215–2222, **2012.**

[25] Denyer S.P., Maillard J.Y., Cellular impermeability and uptake of biocides and antibiotics in Gram-negative bacteria, *J. Appl. Microbiol*., 92, 35S-45S, **2002.**

[26] Nikaido H., Vaara M., Molecular basis of bacterial outer membrane permeability, *Microbiol. Rev*., 49, 1-32, **1985.**

[27] Morais D.S., Guedes R.M., Lopes M.A., Antimicrobial approaches for textiles: from research to market, *Materials*, 9, 498, **2016.**

[28] Ghosh S., Yadav S., Vasanthan N., Sekosan G., A study of antimicrobial property of textile fabric treated with modified dendrimers, *J. Appl. Polym. Sci*., 115, 716-722, **2010.**

[29] Akbari S., Application of dendritic material in textile engineering, *Sci. Bull. ESCORENA*, 7, 11-26, **2013.**

[30] Sheiko S.S., Buzin A.I., Muzafarov A.M., E. A. Rebrov E.A., Getmanova E.V., Spreading of carbosilane dendrimers at the air/water interface, *Langmuir*, 14, 7468-7474, **1998.**

[31] Surdu L., Stelescu M.D., Manaila E., Nicula G., Iordache O., Dinca L.C., Berechet M.-D., Vamesu M., Gurau D., The improvement of the resistance to *Candida albicans* and *Trichophyton interdigitale* of some woven fabrics based on cotton,

Bioinorganic Chemistry and Applications, Hindawi Publishing Corporation, 2014, 763269, **2014.**

[32] Hogt A.H., Dankert J., Feijen J., Adhesion of Staphylococcus epidermidis and *Staphylococcus saprophyticus* to hydrophobic biomaterials, *J. Gen. Microbiol*., 131, 2485-2491, **1985.**

[33] Andreozzi E., Barbieri F., Ottaviani M.F., Giorgi L., Bruscolini F., Manti A., Battistelli M., Sabatini L., Pianetti A., Dendrimers and polyamino-phenolic ligands: activity of new molecules against *Legionella pneumophila* biofilms, *Front. Microbiol*., 7, 289, **2016.**

[34] Güçbilmez Ç.M., Yemenicioflu A., Arslanoflu A., Antimicrobial and antioxidant activity of edible zein films incorporated with lysozyme, albumin proteins and disodium EDTA, *Food Res. Int*., 40, 80-91, **2007.**

[35] Hong S., Bielinska A.U., Mecke A., Keszler B., Beals J.L., Shi X., Balogh L., Orr B.G., Baker Jr. J.R., Banaszak Holl M.M., Interaction of poly(amidoamine) dendrimers with supported lipid bilayers and cells: hole formation and the relation to transport, *Bioconjugate Chem*., 15, 774-782, **2004.**

[36] Rojo J., Delgado R., Glycodendritic structures: promising new antiviral drugs, *J. Antimicrob. Chemother*., 54, 579-581, **2004.**

[37] Agashe H.B., Dutta T., Garg M., Jain N.K., Investigations on the toxicological profile of functionalized fifth-generation poly (propylene imine) dendrimer, *J. Pharm. Pharmacol*., 58, 1491-1498, **2006.**

[38] Calabretta M.K., Kumar A., McDermott A.M., Cai C., Antibacterial activities of poly(amidoamine) dendrimers terminated with amino and poly(ethylene glycol) groups, *Biomacromolecules*, 8, 1807-1811, **2007.**

[39] Lopez A.L., Reins R.Y., McDermott A.M., Trautner B.W., Cai C., Antibacterial activity and cytotoxicity of PEGylated poly(amidoamine) dendrimers, *Mol. Biosyst*., 5, 1148-1156, **2009**.

6. ACKNOWLEDGEMENTS

The authors acknowledge Grants № H09/03-2016, Fund "Scientific Research", Ministry of Education and Science of Bulgaria and № MAT2012-31709 from the Spanish Ministry of Economy and Competitivity. IG and PB also acknowledge COST CA15114 "Anti-MIcrobial Coating Innovations to prevent infectious diseases (AMICI)".