Surface analysis of nitrogen plasma-treated C₆₀/PS nanocomposite films for antibacterial activity

Naglaa M. EL-Sayed¹ · Fifi M. Reda² · Omar F. Farag¹ ·
Doaa A. Nasrallah¹

Received: 10 August 2016 / Accepted: 24 February 2017 / Published online: 4 May 2017
© Springer Science+Business Media Dordrecht 2017

Abstract The influence of N₂ plasma on the antibacterial properties of polystyrene/fullerene (C₆₀/PS) nanocomposite films with two concentrations is investigated. A comparison is made between the surface characteristics of the films before and after plasma irradiation for different time intervals. The alterations induced on the surface of the films after treatment are analyzed by contact angle and surface energy measurements, FTIR spectroscopy, and atomic force microscopy. The antibacterial properties, growth, biofilm formation, and adhesion of the nanocomposite films against two multidrug-resistant bacterial strains, *Staphylococcus aureus* KT337489 and *Pseudomonas aeruginosa* KT337488, are investigated before and after plasma irradiation. The results indicate that *P. aeruginosa* is more sensitive to treatment than *S. aureus* as well as an enhancement of the anti-adhesion of both strains to treated surfaces through exposure.

Keywords Antibacterial · N₂-plasma · Surface modification · C₆₀ · PS · Nanocomposite

✉ Naglaa M. EL-Sayed
nagla68mohammed@gmail.com

Fifi M. Reda
fifi.reda133@yahoo.com

Omar F. Farag
faragfarag66@yahoo.com

Doaa A. Nasrallah
doaa582012@gmail.com

¹ Physics Department Faculty of Science, Zagazig University, Zagazig, Egypt

² Botany and Microbiology Department, Faculty of Science, Zagazig University, Zagazig, Egypt

1 Introduction

Upon exposing a polymer surface to a plasma environment for a period of time, the plasma constituents contribute to the polymer surface treatment, resulting in surface etching, activation, and/or crosslinking. These effects change the surface properties, e.g., from hydrophobic to hydrophilic or vice versa depending on the kind of plasma working gas [1].

In the last three decades, there has been a strong emphasis on the development of polymeric nanocomposites. This development is supporting plasma surface modification as an attractive method for improving polymer surfaces to be suitable for various applications. Many polymers have good bulk properties such as optical, thermal, electrical, mechanical etc.; however, their surface properties, like wettability, are inconvenient for many applications. Plasma treatment is a successful method for altering the physical, chemical, and biocompatibility properties of the polymer surface without affecting the bulk material [2–8].

Plasma is an ionized gas composed of electrons, ions, neutrals, radicals, and UV radiations. Results from important research activity could broaden the applications of polymers in almost all technical disciplines such as sporting goods, electronics, automotive parts, medicine, and pharmacology, as these utilize high-performance materials that exhibit unusual property combinations and unique design possibilities.

The biocompatibility of polymeric nanocomposites is receiving great interest due to their increasing applications in the field of medicine, including drug transporters, antiseptics, and antioxidant [9] C₆₀/PS nanocomposites, which are known as one of the bioactive (antibacterial) materials [10–12]. They inhibit protein and bacterial adhesion [13], interrupt bacterial colonization, biofilm formation [14], and bacterial growth. C₆₀ is known to be biologically active due to the presence of double bonds and bending of sp₂-hybridized carbon atoms. It has a harmful effect on fungi, bacteria, and viruses. This is due to the fact that C₆₀ is considered a semiconductor of either a donor or acceptor, which upon irradiation with UV or visible light can convert molecular oxygen into highly reactive singlet oxygen, which has the ability to penetrate and damage biological systems [15, 16].

In the present work, C₆₀ is incorporated into PS, which has a wide range of industrial applications with two concentrations to form nanocomposite films. It is known that biomaterials interact with biological systems through their surfaces. Therefore, it is important to control the surface properties. The goal of this research is to investigate the effect of plasma radiation on the antibacterial property of the nanocomposite films by comparing their surface characteristics and their biological activity against two types of bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, before and after plasma irradiation for different time periods.

2 Materials and methods

2.1 Discharge chamber

The DC-discharge unit consists of a Pyrex tube 18 cm long and 13 cm in diameter closed at each end with an aluminum plate. Two plane circular stainless-steel disks of 5-cm radius represent the two electrodes that are connected to each other with four isolated rods keeping the spacing constant at 7 cm. The two electrodes move axially forward and backward as a unit, controlled by a handle outside the tube. The base pressure is achieved with a tube at 10^{−3} Torr by using a rotary pump (Edwards vacuum pump, model ED 200) and then N₂ gas enters the tube through a

controllable needle valve. Plasma properties were kept constant during the experiment; the gas pressure was 0.4 Torr and the discharge power was approximately 3.5 W. The nanocomposite film samples were positioned in front of the cathode, at the edge of the negative glow.

2.2 Sample preparation

The samples were prepared by applying the solvent casting method. PS granules (MW 192,000 g.mol⁻¹), C₆₀ (MW 72,000 g mol⁻¹), and Toluene (with purity 99.99) were purchased from Sigma-Aldrich. Two concentrations of C₆₀/PS composite films were prepared; 1×10^{-3} and 1×10^{-5} mol%. The desired amounts of PS and C₆₀ were dissolved in toluene separately and then added to each other, mixed with a magnetic stirrer. Finally, it was placed into a Petri dish and left to dry at room temperature for a week.

After drying, the samples were cut into rectangular substrates (1 cm × 2 cm) with a thickness range of 0.22–0.25 mm. C₆₀/PS composites films are optically transparent with a violet color and their intensity depends on the C₆₀ concentration, while the pure PS sample is colorless.

2.3 Sample surface analysis

2.3.1 Structural analysis (FTIR spectroscopy)

FTIR spectroscopic analysis determined the chemical functional groups that may be formed on the surface of the composite films upon plasma exposure. The samples under investigation were examined before and after plasma exposure for different time intervals using an infrared spectrometer device (Vertex 70, Bruker Optics).

2.3.2 Contact angle measurements

The modification of hydrophilicity of the nanocomposite films was examined by water contact angle measurements using a traveling microscope (measurements were carried out with distilled water at room temperature). The volume of the liquid drop was about 5 μl using a microsyringe. Measurements were repeated 5–8 times at different points on the surface of the same sample to check the accuracy. The surface free energies for the substrates, using the water and glycerol contact angles before and after plasma exposure, were evaluated by applying the Owens–Wendt method [17],

$$\gamma_l(1 + \cos\theta) = 2(\gamma_l^d\gamma_s^d)^{\frac{1}{2}} + 2(\gamma_l^p\gamma_s^p)^{\frac{1}{2}} \quad (1)$$

where γ_l , γ_l^p , and γ_l^d are the total surface energy, polar and dispersion components of the surface free energy of the liquid, respectively. Similarly, γ_s , γ_s^p , γ_s^d are the values for the nanocomposite films. θ is the contact angle between the sample and the liquid. The values of γ_l , γ_l^p and γ_l^d , required for the solid surface energy calculation, were obtained from the literature [8].

2.3.3 AFM

Surface topography and roughness analysis of the N₂ plasma-treated surfaces, for different exposure times, were carried out by means of atomic force microscopy (AFM) using a WET-SPM scanning probe microscope (Shimadzu, Japan).; model: WET-SPM, scanning probe microscope, Shimadzu, Japan. Measurements were performed in contact mode.

2.3.4 Microbiological studies

Collection of samples Medical specimens of wound infections, sputum, and urine were collected from patients admitted to Sidnawy Hospital, Zagazig, Egypt, in the period from October to December 2014. The specimens were collected and transported according to Miller [18].

Isolation and purification of bacteria The swabs were streaked on a nutrient agar surface and different diagnostic and selective media to isolate *Staphylococcus aureus* and *Pseudomonas aeruginosa* namely Baird Parker, mannitol salt agar and King B agar media until pure single colonies were obtained according to Murray [19].

Antibiotic susceptibility test Susceptibility of the collected isolates to twelve antibiotics (amoxicillin (AX), vancomycin (VA), Chloramphenicol (C), ciprofloxacin (CIP), cefotaxime (CTX), rifampicin (RF), amikacin (AK), tetracycline (T), ampicillin (AM), imipenem (IMP), methicillin (MET) and norfloxacin (NOR)) was assessed using the disk diffusion technique according to NCCLS [20]. The antibiotic disks were purchased from the Oxoid Company.

Detection of biofilm production by bacterial isolates Detection of biofilm production by bacterial isolates was performed by the Congo red agar method [21, 22].

Identification of the selected multidrug-resistant (MDR) and strong biofilm producer bacterial isolates The purified cultures of the selected MDR and strong biofilm producer isolates were identified after investigating the morphological characters and by biochemical tests according to standard clinical laboratory methods reported and recommended by Bergey's manual of Systematic Bacteriology [23–25]. The identification of MDR isolates was molecularly confirmed by an investigation of 16S rRNA gene sequencing according to Zang et al. [26] and Liu et al. [27].

Bacterial adhesion and growth assay The treated and untreated PS films were immersed in bacterial suspensions that were incubated at 37 °C for 24h. After incubation, the samples were washed with 20 ml of 0.87% NaCl solution containing polysorbate 80 at pH 7 [28]. Afterwards, the bacterial growth and its adherence to PS films were assessed by measuring the OD of growth in a spectrophotometer at 600 nm and examination of PS films under a scanning electron microscope (SEM).

3 Results and discussion

3.1 FTIR analysis

FTIR was employed to determine the surface chemical changes due to plasma treatment. Figure 1 represents the IR spectra of C₆₀/PS composite films before and after N₂ plasma treatment with different treatment times for the two samples. PS has been reported to have about 14–16 main absorbing bands, which are depicted in the figures. The bands at 3059 and 3025 cm⁻¹ are due to the C–H aromatic stretching vibration and the bands at 2921 and 2849 cm⁻¹ are correlated to the stretching vibration of the aliphatic C–H bonds. The bands

Fig. 1 FTIR spectra of nitrogen plasma-treated PS/C₆₀ nanocomposites with treatment times 0 min (a), 15 min (b), 30 min (c), 60 min (d), and 120 min (e)

at 1600 cm^{-1} and 1492 and 1450 cm^{-1} are assigned to the phenyl ring stretching and the bands at 1027 , 752 , and 695 cm^{-1} correspond to aromatic C–H deformation and skeletal vibration. Due to the interaction between C₆₀ and the aromatic ring of the PS, there may be overlaps between bands that correspond to C₆₀, which are reported to be at 1429 , 1181 , 577 , and 528 [29] and phenyl ring bands of PS within these ranges [30].

The FTIR spectrum shows a significant difference between untreated and treated samples. Two bands appeared in the range $3200\text{--}3700\text{ cm}^{-1}$; this region belongs to the $\nu(\text{N-H})$ imine group and $\nu(\text{-OH})$ hydroxyl group. At 1260 cm^{-1} , an intermediate peak appeared in all samples, which is probably attributed to the C–N stretch with increasing intensity as the treatment time increases to 30 min; it decreases for 1×10^{-3} samples and vanishes for the 1×10^{-5} samples.

The incorporation of O₂ and N₂ functionalities on the sample surfaces is an indication of surface activation by N₂ plasma and the formation of free radical sights on the nanocomposite film's surfaces. The activated surface either adsorbs moisture from ambient air just after removing it from the plasma chamber or abstracts residual O₂ in the plasma chamber [31, 32]. The appearance of imine, hydroxyl, and C–N functionalities on the examined samples is the main reason for wetting enhancement.

3.2 Surface topography

The surface topography of the nanocomposite surface could play an important role in improving the antibacterial behavior of the films. The plasma exposure and subsequent roughness result in a removal of the top surface molecules of the polymer, consequently exposing the fullerene C₆₀ nanoparticles, which have an antibacterial action. The surface topology of the nanocomposite films was examined with AFM (Fig. 2) for the pure PS untreated sample, untreated nanocomposite film with 10^{-3} mol% and those treated for 30 and 120 min with the same concentration. Hillocks on the surface of the untreated film are observed [10]; this may be because when fullerene is added to polystyrene it can accelerate the formation of primary aggregates due to reorganization of polystyrene. By comparing the topographic image of the untreated and plasma-treated samples for 30 min, the average roughness of the treated sample was found to increase from $5.05\text{ \AA}$ to $11.74\text{ \AA}$, which means that the roughness is doubled upon

Fig. 2 AFM images of N_2 plasma-treated C_{60} /PS films: (1) pure untreated PS; (2) untreated C_{60} /PS films; (3) treated for 30 min; (4) treated for 120 min

plasma exposure for 30 min, while increasing the time to 120 min leads to a slight increase in the average roughness to $11.83 \text{ \AA}$, which means that prolonged exposure is insignificant.

3.3 Contact angle analysis

After plasma surface modification of polymeric material, the water contact angle is affected by two factors: the chemical surface changes in the form of newly formed functional groups and surface roughness. Evaluation of the surface hydrophilicity of the C_{60} /PS composite films was carried out by measuring the water contact angle for both concentrations before and after plasma treatment for different exposure times and then calculating the subsequent surface energy. Changes of contact angle and surface energies with plasma treatment time are specified in Table 1. It was observed that the contact angles do not change according to C_{60} concentration, while their values decrease when increasing the plasma treatment time.

The values of total surface energy, γ_s , were estimated for the C_{60} /PS films by adding polar and dispersive components, γ_s^p , γ_s^d . It was found that values of γ_s increase as the treatment time increases, escalating from 23 mJ/m^2 and reaching a m as the changes of the dispersive component are relatively unappreciable and almost constant after 30 min.

Table 1 Values of water (W) and glycerol (G) CA and total surface energy of plasma-treated samples

Concentration	Exposure time (min)	Contact angle (degree)		Surface energy components (mJ/m ²)		
		W	G	Υ_s^D	Υ_s^P	Υ_s
1×10^{-5}	0	85.0	77	14.84	8.98	23.81
	15	67.8	55	25.87	13.43	39.3
	30	52	42	24.42	24.81	49.23
	60	35	29.2	21.79	38.58	60.37
	120	22.16	15	22.75	44.75	67.508
1×10^{-3}	0	86	77.5	15.186	8.300	23.486
	15	67	59	19.377	17.55	36.933
	30	50	43	21.316	28.53	49.85
	60	39	32	22.46	35.28	57.74
	120	19.9	15	21.734	46.75	68.49

This result is common and agrees with previous works [33]. Decreasing the liquid's contact angle and the subsequent escalating total surface energy by increasing treatment time is good evidence of the formation of polar functional groups and increasing surface roughness.

3.4 Antibacterial activity

Most hospital infections are caused by *S. aureus* and *E. coli* [34]. Urinary tract infections are mostly caused by Gram-negative bacteria such as *Klebsiella* spp., *Enterobacter* spp., *P. aeruginosa*, *Proteus* spp. and by Gram-positive bacteria such as *Enterococcus* spp., *Staphylococci*, and *Streptococci* [35]. Our investigation focused on isolation of *S. aureus* (Gram-positive bacteria) and *P. aeruginosa* (Gram-negative bacteria) from clinical hospital infections. In the present study, the results demonstrate that Gram-positive bacteria were the most common pathogens in the examined clinical specimens (Table 2). Thirty bacterial isolates were studied, with as 17 Gram-positive bacterial and 13 Gram-negative bacterial isolates, representing 56.7 and 43.3%, respectively. These results are in agreement with Hassanein et al. [36] who demonstrated that the most predominant microorganisms isolated from swab, blood, urinary, and sputum cultures were Gram-positive bacteria that represented 59.5%, while Gram-negative bacteria were 40.5%.

Table 2 shows the antibiotic susceptibility of the collected isolates and biofilm formation. The results show that MDR bacterial isolates no. 15 and 20 represent 92% resistance against tested antibiotics and exhibit strong biofilm formation. According to the National Institutes of Health, 80% of hospital-acquired infections worldwide are attributed to bacteria-forming biofilms [37]. *Staphylococcus aureus* is known to be a pathogen involved in nosocomial infections on medical devices and its resistance to methicillin has been held accountable for increased hospital stays and health care costs [38].

The MDR and strong biofilm forming bacterial isolates no. 15 and 20 were preliminary identified as *Staphylococcus aureus* 15 and *Pseudomonas aeruginosa* 20 based on the morphological and biochemical characteristics described in Bergey's manual of Systematic Bacteriology. PCR amplification of 16S rRNA genes confirmed the identity (99%) of the selected potent strains as *S. aureus* 15 and *P. aeruginosa* 20. The partial nucleotide sequences of amplified genes were submitted in GenBank (<http://www.ncbi.nlm.nih.gov/GenBank/update.htm>) under accession numbers KT337489 and KT337488, respectively.

Table 2 Isolation of bacterial isolates from different sources and their screening for biofilm formation and antibiotic sensitivity tests

Source of isolation	Gram stain	Shape of cell and arrangement	Biofilm formation	Antibiotic sensitivity										of Resistance		
				AX	VA	C	CIP	CTX	RF	AK	T	AM	IMP		MET	NOR
Urine	+ ve	Cocci in cluster	+++	I	R	R	S	R	R	S	S	R	S	R	S	50
	- ve	Bacilli short rods	-	I	R	R	S	R	R	S	S	S	S	R	S	67
	+ ve	Cocci in cluster	++	I	R	R	S	R	R	S	S	S	S	R	S	17
	- ve	Bacilli short rods	+	I	R	R	S	R	R	S	S	S	S	R	S	33
	+ ve	Cocci in cluster	++	I	R	R	S	R	R	S	S	S	S	R	S	42
	- ve	Bacilli short rods	+	I	R	R	S	R	R	S	S	S	S	R	S	33
	- ve	Bacilli short rods	-	I	R	R	S	R	R	S	S	S	S	R	S	42
	+ ve	Cocci in cluster	+	I	R	R	S	R	R	S	S	S	S	R	S	58
	- ve	Bacilli short rods	++	I	R	R	S	R	R	S	S	S	S	R	S	42
	+ ve	Cocci in cluster	++	I	R	R	S	R	R	S	S	S	S	R	S	17
Wound	+ ve	Cocci in cluster	-	I	R	R	S	R	R	S	S	S	S	R	S	67
	- ve	Bacilli short rods	+	R	R	R	S	R	R	S	S	R	R	R	S	33
	+ ve	Cocci in cluster	+++	R	R	R	S	R	R	S	S	R	R	R	S	92
	+ ve	Bacilli short rods	++	R	R	R	S	R	R	S	S	R	R	R	S	67
	- ve	Bacilli short rods	+	I	R	R	S	R	R	S	S	R	R	R	S	42
	- ve	Cocci in cluster	-	R	R	R	S	R	R	S	S	R	R	R	S	75
	+ ve	Bacilli short rods	++	R	R	R	S	R	R	S	S	R	R	R	S	50
	- ve	Bacilli short rods	+++	R	R	R	S	R	R	S	S	R	R	R	S	92
	- ve	Cocci in cluster	++	R	R	R	S	R	R	S	S	R	R	R	S	75
	+ ve	Bacilli short rods	++	R	R	R	S	R	R	S	S	R	R	R	S	17
Sputum	+ ve	Cocci in cluster	-	I	R	R	S	R	R	S	S	R	R	R	S	83
	- ve	Bacilli short rods	++	I	R	R	S	R	R	S	S	R	R	R	S	50
	+ ve	Cocci in cluster	++	I	R	R	S	R	R	S	S	R	R	R	S	25
	- ve	Bacilli short rods	-	I	R	R	S	R	R	S	S	R	R	R	S	58
	+ ve	Cocci in cluster	+++	I	R	R	S	R	R	S	S	R	R	R	S	42
	+ ve	Bacilli short rods	+	I	R	R	S	R	R	S	S	R	R	R	S	67
	+ ve	Cocci in cluster	-	I	R	R	S	R	R	S	S	R	R	R	S	17
	- ve	Bacilli short rods	+	I	R	R	S	R	R	S	S	R	R	R	S	33
	- ve	Cocci in cluster	-	I	R	R	S	R	R	S	S	R	R	R	S	33
	30 isolates															

R = Resistant, I = Intermediate, S = Susceptible

AX = amoxicillin (20 µg/disc), VA = vancomycin (30 µg/disc), C = chloramphenicol (30 µg/disc), CIP = ciprofloxacin (10 µg/disc), CTX = ceftaxime (10 µg/disc), RF = rifampicin (10 µg/disc), AK = amikacin (30 µg/disc), T = tetracycline (30 µg/disc), AM = ampicillin (30 µg/disc), IMP = imipenem (10 µg/disc), MET = methicillin (5 µg/disc) and NOR = norfloxacin (10 µg/disc).

+++ = Strong biofilm formation

++ = Moderate biofilm formation

+ = Weak biofilm formation

+ ve Gram positive bacteria

- ve Gram negative bacteria

Polystyrene (PS) is a broadly used polymer in cell culture applications because of its non-toxicity and high transparency [39]. Hence, the present investigation studied the antibacterial and anti-adhesion effects of treated and untreated pure PS films and C₆₀/PS composites with concentrations 1×10^{-3} and 1×10^{-5} against *S. aureus* 15 (KT337489), and *P. aeruginosa* 20 (KT337488) strains. The results in Table 3 reveal that PS film filled with fullerene was more affected than pure PS film especially at higher concentrations. The growth of both tested strains decreased by increasing plasma exposure times and fullerene concentrations; highest inhibitions were after 30 min and 1×10^{-3} C₆₀ concentration compared with untreated samples. The antibacterial activity of PS and C₆₀/PS composite films decreased by further increasing the plasma exposure times up to 120 min. It was found that *P. aeruginosa* 20 was more sensitive than *S. aureus* 15. Also, the production of pyocyanin pigment by *P. aeruginosa* 20 was decreased by increasing plasma treatment time and was completely inhibited by fullerene treatment. According to FTIR and AFM analysis, the presence of O₂ and N₂-containing functional groups, which were responsible for the hydrophilicity improvement of the plasma-treated samples, could enhance the antibacterial properties of the material. These results are in agreement with Pawde et al. [40] and Pandiyaraj et al. [28].

Table 3 Effect of different plasma exposure time and fullerene concentration on the growth of *S. aureus* 15 and *P. aeruginosa* 20 strains

Tested organism		<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
Sample treatment		Optical density (600 nm)	Pyocyanin pigment production
Bacterial growth alone		0.750	0.698
Untreated	Pure PS	0.459	0.432
15-min plasma		0.420	0.418
30-min plasma		0.409	0.353
60-min plasma		0.411	0.365
120-min plasma		0.464	0.420
Untreated	1×10^{-5} Fullerene	0.382	0.358
15-min plasma	conc	0.360	0.347
30-min plasma		0.322	0.305
60-min plasma		0.412	0.371
120-min plasma		0.436	0.392
Untreated	1×10^{-3} Fullerene	0.316	±
15-min plasma	conc	0.272	–
30-min plasma		0.205	–
60-min plasma		0.332	–
120-min plasma		0.364	–

Fig. 3 SEM photograph showing the adhesion and growth of the *S. aureus* 15 strain on plasma-treated and untreated films with different fullerene concentrations

Fig. 4 SEM photograph showing the adhesion and growth of the *P. aeruginosa* 20 strain on plasma-treated and untreated films with different fullerene concentrations

Due to plasma etching, some polymer molecules left the surface of the samples, leaving a large number of fullerene molecules on the surface. Fullerene's antibacterial effect is possibly due to its ability to inhibit bacterial growth by impairing the respiratory chain and inhibition of the energy metabolism. It has also been suggested that it causes cell membrane disruption [40, 41]. Alekseeva et al. [11] stated that polystyrene/fullerene composite film manifests bacteriostatic and fungistatic effects, whereas polystyrene films do not show any effect against bacteria and fungi. They explained that the reason for the microorganism's inactivation is interaction of fullerenes with the functional groups of the amino acids composing of bacterial proteins. This causes cell membrane damage and destruction of the cell wall leading to their death. Furthermore, Da Ros [42] demonstrated that the biological abilities of fullerenes are due to their lipophilic properties that facilitate cell penetration and lack of electrons, helping to react with free radicals and generate active oxygen species [43].

The scanning electron microcopy of the present study presented in Figs. 3 and 4 shows the antibacterial activity and anti-adhesion of plasma and fullerene treatments. These figures illustrate that the cell loading and consequently the adhesion of *S. aureus* 15 and *P. aeruginosa* 20 strains on the PS surface was decreased by increasing plasma exposure times and fullerene concentrations, with most inhibition at 30 min. The antibacterial activity of PS and PS/C₆₀ composite films decreased by further increasing plasma exposure times up to 120 min; this behavior may be attributed to increasing hydrophilicity and roughness over the action of C₆₀ concentration and functional groups and consequently, more bacterial attachments.

The *P. aeruginosa* 20 strain was more sensitive than the *S. aureus* 15 strain for all treatments. Modification of the biomaterial surface gave not only anti-adhesive properties and the ability to oppose biofilm formation but also to combine anti-adhesive and antimicrobial effects in the same coating [44, 45]. Moreover, Emerson et al. [46] confirmed the decrease in bacterial growth with increased surface roughness when nano/micrometer scales are considered.

4 Conclusions

By optimizing the processing parameters of a plasma chamber and using N₂ gas, C₆₀/PS nanocomposite films were treated and surface properties were investigated in comparison with those before treatment. FTIR, AFM, and contact angle analysis confirm that the increase in surface hydrophilicity—in a way that enhances antibacterial performance—is restricted to the plasma exposure time. Prolonged time is not recommended, as it is known that antibacterial enhancement is always accompanied by surface biocompatibility, especially in the case when antimicrobial reagents, such as triclosan or bronopol, are not used.

Acknowledgments The authors would like to acknowledge Prof. Mohammed H. Elgazaly, The Physics Department Faculty of Science, Zagazig University, for his valuable guidance, advice, and discussion.

Compliance with ethical standards

Conflict of interests The corresponding author certifies on behalf of all authors that there are no conflicts of interests to disclose.

References

- Cordeiro, A.L., Nitschke, M., Janke, A., Helbig, R., D'Souza, F., Donnelly, G.T., Willemsen, P.R., Werner, C.: Fluorination of poly (dimethylsiloxane) surfaces by low pressure CF₄ plasma—physicochemical and antifouling properties. *Express Polymer Letters* **3**, 70–83 (2009)
- Chu, P.K., Chen, J.Y., Wang, L.P., Huang, N.: Plasma-surface modification of biomaterials. *Mat. Sci. Eng. Res.* **36**, 143–206 (2002)
- Chou, N.J., Chang, C.A.: *Surface Modification of Polymers*. Butterworth-Heinemann Inc, Boston (1994)
- Ozdemir, M., Yurteri, C.U., Sadikoglu, H.: Surface treatment of food packaging polymers by plasmas. *Food Technology* **53**, 54–58 (1999)
- Pelagade, S.M., Singh, N.L., Rane, R.S., Mukherjee, S., Deshpande, U.P., Ganesan, V., Shripathi, T.: Investigation of surface free energy for PTFE polymer by bipolar argon plasma treatment. *J. of Surf. Eng. Mater. and Adv. Technol.* **2**, 132–136 (2012)
- Inagaki, N.: *Plasma Surface Modification and Plasma Polymerization*. Technomic Publishing Company, Inc. (1996)
- Goddard, J.M., Hotchkiss, J.H.: Polymer surface modification for the attachment of bioactive compounds. *Prog. Polym. Sci.* **32**, 698–725 (2007)
- Elsayed, N.M., Mansour, M.M., Farag, F.O., Elghazaly, H.M.: N₂, N₂-Ar and N₂-He DC Plasmas for the improvement of polymethylmethacrylate surface wettability. *Adv. Appl. Sci. Res.* **3**, 1327–1334 (2012)
- Alekdeeva, O.V., Bagrovskaya, N.A., Kuz'min, S.M., Noskov, A.V., Mlikhov, I.V., Rudin, V.N.: The influence of fullerene additives on the structure of polystyrene films. *Russ. J. Phys. Ch. A* **83**, 1170–1175 (2009)
- Alekseeva, O.V., Bagrovskaya, N.A., Noskov, A.V.: Polystyrene film composite filled with fullerenes. *Proc. Int. Conf., Nanomaterials: applications and properties.* **2**, 03NCNN24(pp3) (2013)
- Alekseeva, O.V., Krestov, G.A., Bagrovskaya, N.A., Noskov, A.V.: Effect of polystyrene/fullerene composites on free-radical processes in biological fluid. *J. of Res. & Dev. in Chem.* (2015). doi:[10.5171/2015.304820](https://doi.org/10.5171/2015.304820)
- Alekseeva, O.V., Bagrovskaya, N.A., Noskov, A.V.: Effect of C₆₀ filling on structure and properties of composite films based on polystyrene. *Arab. J. Chem.* (2014). doi:[10.1016/j.arabjc.2014.09.008](https://doi.org/10.1016/j.arabjc.2014.09.008)
- Neoh, K.C., Kang, E.T.: Combating bacterial colonization on metals via polymer coating: relevance to marine and medical applications. *ACS Appl. Mater Interfaces.* **3**, 22808–19 (2011)
- Arciola, C.R., Campoccia, D., Speziale, P., Montanaro, L., Costerton, J.W.: Biofilm formation in staphylococcus implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials. *Biomaterials* **33**, 5967–82 (2012)
- Bakry, R., Vallant, R.M., Najam-ul-Haq, M., Rainer, M., Szabo, Z., Huck, C.W., Bonn, G.K.: Medicinal applications of fullerenes. *Int. J. Nanomedicine* **2**, 639–649 (2007)
- Kumar, A.: Fullerenes for biomedical applications. *J. Environ. Appl. Biores.* **3**, 175–191 (2015)
- Hejda, F., Solaf, P., Kousal, J.: Surface free energy determination by contact angle measurements—a comparison of various approaches. *WDS'10 Proceedings of Contributed Papers. Part III*, 25–30 (2010)
- Miller, J.M.: *A Guide to Specimen Management in Clinical Microbiology*. ASM Press, Washington, D.C. (1999)
- Murray, C.J., Lopez, A.D.: Mortality by cause for eight regions of the world: Global burden of disease study. *Lancet* **349**, 1269–1276 (1997)
- NCCLS: National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. Documents M2-A6 and M7-A4. Pennsylvania, USA (1999)
- Mather, T., Singhal, S., Khan, S., Upadhyay, D.J., Fatma, T., Rattan, A.: Detection of biofilm formation among the clinical isolates of Staphylococci: An evaluation of three different screening methods. *Indian J. Med. Microbiol.* **24**, 25–29 (2006)
- Arciola, C.R., Baldassari, L., Montanaro, L.: Presence of ica A and ica D genes and slim production in a collection of Staphylococcal strains from catheter associated infections. *J. Clin. Microbiol.* **39**, 2151–2156 (2001)
- Holt, J.G., Krieg, N.R., Sneath, P.H., Staley, J.T., Williams, T., Hensyl, W.R.: *Bergey's Manual of Determinative Bacteriology*. Williams and Wilkins, Baltimore, Maryland, USA (1994)
- Garrity, G.M., Brenner, D.J., Krieg, N.R., Staley, J.T.: *Bergey's Manual of Systematic Bacteriology. The Proteobacteria. Part B: The Gammaproteobacteria*. Springer, New York (2005)
- De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.-H., Whitman, W.B.: *Bergey's Manual of Systematic Bacteriology. The Firmicutes*. Springer, New York (2009)
- Zhang, Z., Schwartz, S., Wagner, L., Miller, W.: A greedy algorithm for aligning DNA sequences. *J. Comput. Biol.* **7**, 203–214 (2000)
- Liu, L.T., Coenye, J.L., Burns, P.W., Whitby, T.L., LiPuma, J.J.: Ribosomal DNA-directed PCR for identification of *Achromobacter* (Alcaligenes) *xylooxidans* recovered from sputum samples from cystic fibrosis patients. *J. Clin. Microbiol.* **40**, 1210–1213 (2002)

28. Pandiyaraj, K.N., Deshmukh, R.R., Ram, B.S., Mahendiran, R.: Improvement of surface and biocompatible properties of flexible transparent nano-TiO₂ films using glow discharge plasma. *J. Nanosci. Nanotechnol.* **2**, 436–441 (2014)
29. Donald, S.B., Gerard, M., Wade, C.T., Hal, J.R., William, G.G., Seki, H., Charles, A.B., Mattanjah de Vries, S.: Vibrational Raman and infrared spectra of chromatographically separated C₆₀, and C₇₀ fullerene clusters. *Chem. Phys. Lett.* **179**, 181–186 (1991)
30. Jung-Woon, K., Kun-Ji, K., Sooyeon, P., Kwang-Un, J., Myong-Hoon, L.: Preparation and characterization of C₆₀/polystyrene composite particle containing pristine C₆₀ clusters. *Bull. Korean Chem. Soc.* **33**, 2966–2970 (2012)
31. Török, T., Urbán, P., Lassú, G.: Surface cleaning and corrosion protection using plasma technology. *Int. J. Corros. Scale Inhib.* **4**, 116–124 (2015)
32. Idage, S.B., Badrinarayanan, S.: Surface modification of polystyrene using nitrogen plasma. An X-ray photoelectron spectroscopy study. *Langmuir* **14**, 2780–2785 (1998)
33. Pandiyaraj, K., Deshmukh, R.R., Mahendiran, R., Su, P.-G., Yassitepe, E., Shahd, I., Perni, S., Prokopoviche, P., Nadagouda, M.N.: Influence of operating parameters on surface properties of RF glow discharge oxygen plasma-treated TiO₂/PET film for biomedical application. *Mat. Sci. Eng. C* **36**, 309–319 (2014)
34. Vaidieki, K., Jyakumer, S., Thilagavathi, G.: Investigation on the antimicrobial activity of RF air plasma and Azadirachtin treated cotton fabric. *J. Instrum. Soc. India* **37**, 258–266 (2007)
35. Akram, M., Shahid, M., Khan, A.U.: Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in J N M C Hospital, Aligarh, India. *Ann. Clin. Microbiol. Antimicrob.* **6**, 4–8 (2007)
36. Hassanein, W.A., EL-Zawahry, Y.A., Reda, F.M., Abd El-Rahman, R.A.: Molecular and microbiological studies on vancomycin-resistant *Staphylococcus aureus* strains isolated from burned patients. *Egypt. J. Exp. Biol. (Bot.)* **9**, 219–231 (2013)
37. Davies, D.: Understanding biofilm resistance to antibacterial agents. *Nat. Rev. Drug Discov.* **2**, 114–22 (2003)
38. Cameron, D.R., Howden, B.P., Peleg, A.Y.: The interface between antibiotic resistance and virulence in *Staphylococcus aureus* and its impact upon clinical outcomes. *Clin Infect Dis.* **53**, 576–82 (2011)
39. Pavithra, D., Doble, M.: Biofilm formation, bacterial adhesion and host response on polymeric implants-issues and prevention. *Biomed. Mater.* **3**, 1–13 (2008)
40. Pawde, S.M., Parab, S.S.: Spectroscopic and antibacterial studies of polystyrene thin films under air plasma and He-Ne laser treatment. *Pramana* **70**, 935–948 (2008)
41. Dizaj, S.M., Mennati, A., Jafari, S., Khezri, K., Adibkia, K.: Antimicrobial activity of carbon-based nanoparticles. *Adv. Pharm. Bull.* **5**, 19–23 (2015)
42. Da Ros, T.: Twenty years of promises: fullerene in medicinal chemistry, Vol. 1 of series carbon materials: Chemistry and Physics. Cataldo, F. and Da Ros, T. (eds), Springer (2008)
43. Deryabin, D.G., Davydova, O.K., Yankina, Z.Z., Vasilchenko, A.S., Miroshnikov, S.A., Kornev, A.B.: The activity of [60] fullerene derivatives bearing amine and carboxylic solubilizing groups against *Escherichia coli*: A comparative study. *J. Nanomater.* **2014**, 1–9 (2014)
44. Ariciola, C.R., Maltarello, M.C., Cenni, E., Pizzoferrato, A.: Disposable contact lenses and bacterial adhesion. In vitro comparison between ionic/high-water content and non ionic/low-water content lenses. *Biomaterials* **16**, 685–690 (1995)
45. Arciola, C.R., Bustanji, Y., Conti, M., Campoccia, D., Baldassarri, L., Samori, B.: *Staphylococcus epidermidis* fibronectin binding and its inhibition by heparin. *Biomaterials* **24**, 3013–19 (2003)
46. Emerson, R.J., Bergstrom, T.S., Liu, Y., Soto, E.R., Brown, C.A., McGimpsey, W.G., Camesano, T.A.: Microscale correlation between surface chemistry, texture, and the adhesive strength of *Staphylococcus epidermidis*. *Langmuir* **22**, 11311–11321 (2006)