Engineering of Polymers and Chemical Complexity

Volume 2 New Approaches, Limitations, and Control

Editors
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ENGINEERING OF POLYMERS AND CHEMICAL COMPLEXITY

Volume II: New Approaches, Limitations, and Control

Edited by

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CHAPTER 16

POLYVINYLCHLORIDE ANTIBACTERIAL PRE-TREATED BY BARRIER PLASMA

IGOR NOVÁK, JÁN MATYAŠOVSKÝ, PETER JURKOVIČ, MARIÁN LEHOCKÝ, ALENKA VESEL, and LADISLAV ŠOLTÉS

CONTENTS

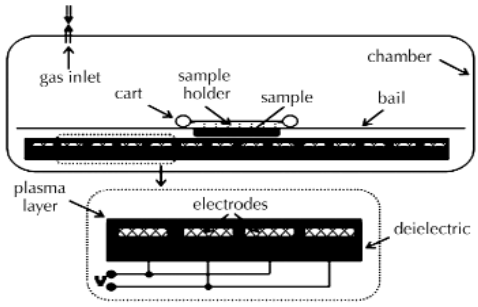
16.1 INTRODUCTION

A multistep physicochemical approach making use of plasma technology combined with wet chemistry has fueled considerable interest in delivery of surface-active anti-adherence materials. In the first step of the approach, concerning an inherent lack of befitting functional groups on pristine substrate, plasma treatment at low temperature and atmospheric pressure has been substantiated to be productive in yielding reactive entities on the surface [1,5]. The highlights the functionality of the adopted multi-step physicochemical approach to bind polysaccharide species onto the medical-grade PVC surface. DCSBD plasma is capable of raising roughness, surface free energy, and introducing oxygen-containing functionalities anchored onto the surface. A structured poly(acrylic acid) brush of high graft density is synthesized using surface-initiated approach to further improve hydrophilicity and develop a stable brush-like assembly to yield a platform for biomolecular binding. In vitro bacterial adhesion and biofilm formation assays indicate incapability of single chitosan layer in hindering the adhesion of Staphylococcus aureus bacterial strain. Chitosan could retard Escherichia coli adhesion and plasma treated and graft copolymerized samples are found effective to diminish the adherence degree of Escherichia Coli.

A new modification method using plasma technology combined with wet chemistry represents an efficient way in delivery of surface-active anti-adherence materials [1-4]. The atmospheric pressure electric discharge plasma has been substantiated to be productive in yielding reactive entities on the surface [5,6]. However, the need for treatment duration to a few seconds remains a pressing obstacle to extensive applications of this type of plasma [7]. A novel technology coined as diffuse coplanar surface barrier discharge (DCSBD) has been developed [8], which enables the generation of a uniform plasma layer under atmospheric pressure with a high surface power density in the very close contact of modified polymer.

16.2 EXPERIMENTAL

- ***Materials***: PVC pellets, extrusion medical-grade RB1/T3M of 1.25 $g \cdot cm^{-3}$ density, were obtained from ModenPlast (Italy) and used as received. Pectin from apple, (BioChemika, with esterification of 70-75%), acrylic acid (AA) (99.0%, anhydrous), and *N*-(3-dimethyl aminopropyl)-*N′*-ethyl carbodiimide hydrochloride (EDAC, 98.0%) were supplied by Fluka (USA). Chitosan from crab shells with medium molecular weight and deacetylation degree of 75-85%.
- ***Plasma Modification***: It was implemented in static conditions by DCSBD plasma technology (Figure 1) of laboratory scale with air as the gaseous medium at atmospheric pressure and room temperature. A schematic profile of the plasma system is given in Scheme 1. It basically comprises a series of parallel metallic electrodes inset inside a ceramic dielectric located in a glass chamber which allows the carrier gases to flow. All samples were treated on both sides with plasma power of 200 W for 15 sec.



SCHEME 1 Scheme of DSBD plasma source.

For grafting by AA PVC substrates were immersed into spacer solutions containing 10 vol.% AA aq. solution. The reaction was allowed to proceed for 24 hr at 30*ºC. PAA grafted PVC samples were immersed into EDAC aq. solution at 4ºC for 6 h*r in order to activate the carboxyl groups on the surface. The highly active key intermediate, O-acylisourea, is produced having potential to react with reducing agents. Subsequently, they were transferred to chitosan and kept there for 24 hr at 30*ºC.*

Sample 1 – pristine PVC, sample 2 –PVC treated by DCSBD plasma, sample 3 – PVC treated by plasma and grafted by AA, sample 4 – PVC treated by plasma, AA and chitosan, sample 5 – PVC treated by plasma AA, chitosan and pectin.

Scanning electron microscopy (SEM) was carried out on VEGA II LMU (TESCAN) operating in the high vacuum/secondary electron imaging mode at an accelerating voltage of 5–20 kV.Bacterial adhesion and biofilm experiments were performed using gram-positive (*S. Aureus* 3953) and gram-negative (*E. Coli* 3954) bacteria. The circular shape specimens (d ≈ 8mm) were cut from the pristine and modified PVC samples before further investigation. After 24 hr incubation at 37*ºC under continuous shaking at 100 rpm. The bacteria adhered on the surface of the specimens were removed by vigorous shaking of the test tube at 2000 rpm for 30 sec and quantified by serial dilutions and spread plate technique*.

16.3 DISCUSSION AND RESULTS

16.3.1 SURFACE ENERGY

Table 1 includes the contact angle values of deionized water (θ_w) recorded on different samples. Each sample has been designated by a number from 1 to 5 whose notation is inserted in the title of Table 1. Based on the given data, sample 1 exhibits a hydrophobic characteristic which after being treated by plasma, an evident change in θ_w arises and hydrophilicity ascends as anticipated. This trend continues as to sample 3 on which polyacrylic acid (PAA) chains are grafted where more hydrophilic propensity is shown inferred from θ_w value. The elevated hydrophilicity upon multistep modifications is assumed to come from the inclusion of superficial hydrophilic entities. The hydrophilicity then decreases as polysaccharides are coated onto the surface, though is well higher than that of sample 1, as the inherent hydrophilicity of chitosan is beyond

doubt. Furthermore, sample 5 exhibits higher wettability than sample 4 implying a more effective binding of chitosan onto the surface, as remarked in other efforts as well. The hydrophilicity then decreases as polysaccharides are coated onto the surface, though is well higher than that of sample 1, as the inherent hydrophilicity of chitosan is beyond doubt. Furthermore, sample 5 exhibits higher wettability than sample 4 implying a more effective binding of chitosan onto the surface, as remarked in other efforts as well.

TABLE 1 Contact angle analysis results of different specimens using deionized water (W), ethylene glycol (E),diiodomethane (D), and formide (F) as wetting agent. Sample 1: pristine control; Sample 2: plasma treated; Smple 3: PAA grafted; Sample 4: chitosan coated; Sample 5: chitosan/pectin coated (mean ± standard deviation)

Specimen	θ_n(°)	θ_E(°)	θ_D (°)	θ_F(°)	$\gamma^{LW/AB}$ (mj/m^2)	$\gamma^{+}_{LW/AB}$ (mj/m^2)	$\gamma^{AB}_{LW/AB}$ (mj/m^2)	$\gamma^{LW}_{LW/AB}$ (mj/m^2)	$\gamma^{tot}_{LW/AB}$ (mj/m^2)	$\gamma^{a)}_{Wa}$ (mj/m^2)	$\gamma^{b)}_{KN}$ (mj/m^2)	$\gamma^{c)}_{LN}$ (mj/m^2)
Sample 1	85.9 (± 2.5)	60.5 (± 3.0)	43.5 (± 3.5)	64.2 (± 6.0)	5.1	0.0	1.0	37.8	38.8	37.8	33.3	33.6
Sample 2	64.9 (± 3.0)	49.4 (± 4.0)	36.2 (± 5.5)	51.0 (± 6.0)	24.9	0.5	6.7	41.5	48.2	41.5	40.4	40.7
Sample 3	46.5 (± 4.0)	51.3 (± 5.5)	38.0 (± 5.0)	7.7 (± 4.5)	62.9	2.7	26.1	40.6	66.7	51.9	43.1	43.4
Sample 4	63.7 (± 5.5)	43.4 (± 3.0)	28.2 (± 2.5)	44.9 (± 5.0)	22.2	0.3	4.9	45.0	49.9	45.0	42.8	43.0
Sample 5	50.5 (± 3.5)	40.0 (± 2.5)	31.5 (± 4.5)	31.0 (± 3.5)	42.2	0.6	10.5	43.6	54.1	50.0	46.4	46.6

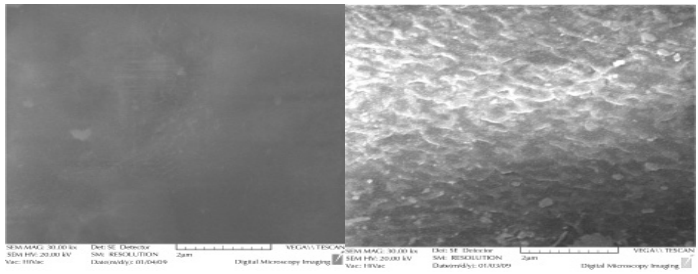
[a)] Surface free energy value according to Wu equation of state [33]. [b)] Surface free energy value according to Kwok-Neumann model [33]. [c)] Surface free energy value according to Li-Neumann Model.

To further explore the physicochemical parameters of the examined surfaces, an extensively used theory, Lifshitz-van der Waals/acid-base (LW/AB), has been exploited for free surface energy evaluation whose outputs with reference to diiodomethane, ethylene glycol, and deionized water as wetting liquids are supplied in Table 1. Sample 1 exhibits a basic character ($\gamma^- > \gamma^+$) as proposed by the data, even though acidity or basicity of neat PVC is yet controversial.

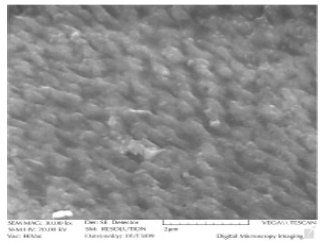
This increase is principally assisted by the polar (acid-base) component (γ^{AB}), rather than the apolar one (γ^{LW}), implying an incorporation of superficial polar oxygen-containing entities thanks to the air plasma treatment. A significant rise in γ^{tot} and γ^{AB} values is noticed for sample 3, in comparison with samples 1 and 2, indicative of the presence of carboxyl-containing units on the surface. As for samples 4 and 5, a reduction in γ^{AB} and γ^{tot} values is observed compared to sample 3, however, their γ^{tot} values rise above that of sample 1. The minimum values of θ_E and θ_F are found for sample 5 which reflect that the surface is seemingly coated by alcoholic and amine containing moieties which in fact points to the more efficient binding of chitosan when compared to sample 4.

16.3.2 SURFACE MORPHOLOGY

The surface topography of samples 1–5 investigated by SEM as a common surface qualitative technique are presented in Figure 2. Sample 1 shows a level and uniform morphology which goes through a significant alteration ensuing the plasma treatment taking on an etched pattern with an unevenly shaped texture. The generated morphology is favorable for next coupling processes due to an enhanced surface area and roughness. The developed pattern on sample 2 is indeed, an outcome of the competing functionalization and ablation phenomena which brings on a reorganization of the surface microstructure.

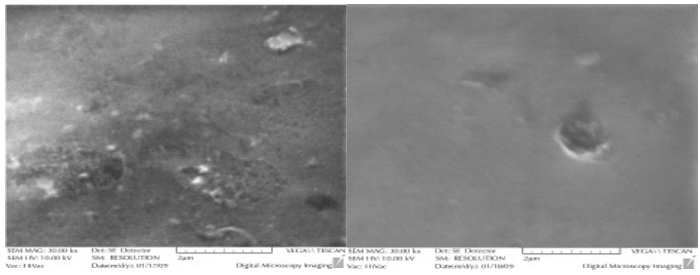


Sample 1 Sample 2



Sample 3

FIGURE 1 *Continued*



Sample 4 Sample 5

FIGURE 1 SEM micrographs of samples 1–5 taken at 3×10^4 magnification.

The incident of the ablation is validated by gravimetric analysis where a weight loss of 4 $\mu g \cdot cm^{-2}$ has been observed due to the plasma treatment for 15 sec implying an approximate etching rate of 2 nm/s in terms of the used PVC grade density. Based on the sample 3 micrograph, PAA chains develop superficial domains of submicron dimension and brush-like features are then recognizable on the surface. As the grafting moves forward, clustering takes place because of the domains size growth. An additional compelling factor in controlling the surface microstructure is the grafting mechanism which is actually initiated by generated surface radicals.

16.3.3 SURFACE CHEMISTRY XPS ANALYSIS

XPS, with a probe depth measuring around 5 nm, has been put to use to more thoroughly monitor the bearings of the surface modifications by picking up a quantitative perception into the surface elemental composition. The recorded survey spectra along with the corresponding surface atomic compositions and ratios of samples 1-5 are all provided in Figure 4. Carbon (C), oxygen (O), chlorine (Cl), and silicon (Si) elements are found on the sample 1 surface whose composition and elemental ratios are presented in the legend of the respective graph. The Cl2p atomic content is substantially lower than the amount found for a neat PVC containing no additives which refers to the existence of several additives and also X-ray degradation. The same rationale accounts for the considerable amount of O1s detected in sample 1 which is not a typical element in standard PVC.Upon binding chitosan on the surface (sample 4), pronounced changes appear in the surface chemistry, as O1s content and O/C fraction increase and also N1s signal emerges, while Cl2p and Si2p bands abate due to the surface coverage by polysaccharide species. This trend yet continues for sample 5 as higher O1s and N1s as well as O/C and N/C atomic rations are detectable compared to sample 4 giving support to the notion that chitosan can be more stably, i.e. in higher quantity, attached onto the surface when layered along with pectin. In other words, use of pectin can promote the quality of chitosan binding.

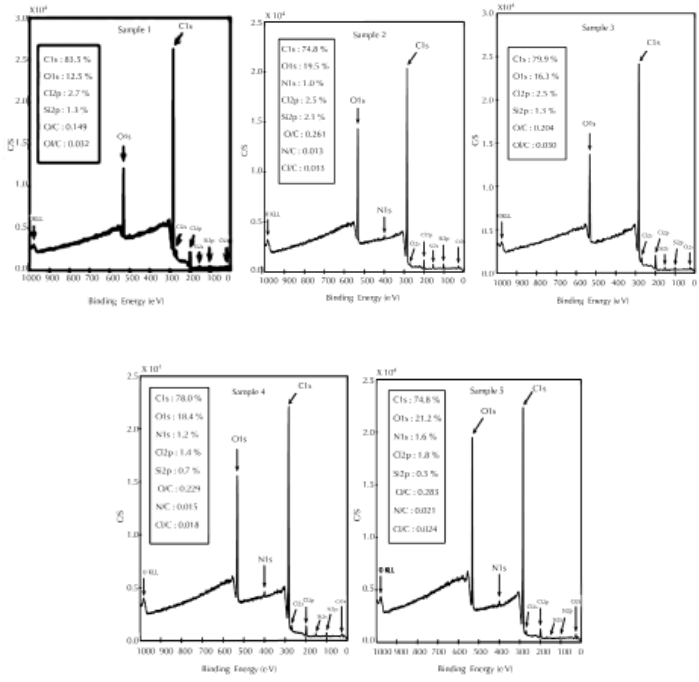


FIGURE 2 XPS survey-scan spectra of samples 1-5 along with atomic compositions.

BACTERIAL ADHESION AND BIOFILM ASSAY

The most crucial step of the biofilm formation is bacterial adhesion considered as a sophisticated topic in biointerface science whose plenty of aspects have not yet been well conceived. As a matter of fact, adhesion phenomenon is an interplay of myriad factors. Figure 5 shows the histograms of bacterial adhesion extent for samples 1-5 after 24 h incubation. As Regards the adherence degree of *S. aureus* onto the samples 2-4, no reduction is evident in the number of viable adhered colonies, compared to sample 1, signifying an inability of the modifications in hampering the *S. aureus* adhesion to the surface. From sample 1 to 3, both hydrophilicity and roughness rise, as remarked earlier, and then decrease in the case of samples 4 and 5. The adhesion degrees vary with a similar trend as well. Considering sample 5, it is inferred that chitosan/pectin assembly imparts biocidal effects against *S. aureus*. Chitosan single layer and chitosan/pectin multilayer restrain the adherence degree by 50% and 20%, respectively. Chitosan/pectin multilayer is found to be effective against both gram-positive and gram-negative strains which can be translated as a higher quality of chitosan coating when it is applied along with pectin.

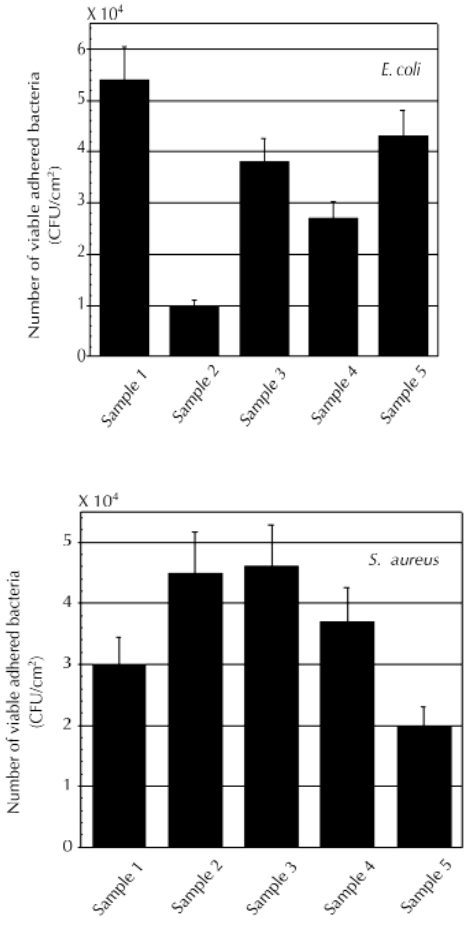


FIGURE 3 Histograms of bacterial adhesion degree for samples 1-5 after 24 h incubation against two microorganisms.

16.4 CONCLUSION

The DCSBD plasma is capable of raising roughness, surface free energy, and introducing oxygen-containing functionalities anchored onto the PVC surface. A structured PAA brush of high graft density is synthesized using surface-initiated approach to further improve hydrophilicity and develop a stable brush-like assembly to yield a platform for biomolecular binding. In vitro bacterial adhesion and biofilm formation assays indicate incapability of single chitosan layer in hindering the adhesion of *S. aureus* bacterial strain, while up to 30% reduction is achieved by chitosan/pectin layered assembly. On the other hand, chitosan and chitosan/pectin multilayer could retard *E. coli* adhesion by 50% and 20%, respectively. Furthermore, plasma treated and graft

copolymerized samples are also found effective to diminish the adherence degree of *E. coli*.

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