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Blood Compatibility of Surface Modified Poly(ethylene terephthalate) (PET) by Plasma Polymerized Acetobromo- α -D-glucose

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ABSTRACT: Poly (ethylene terephthalate) (PET) was surface modified by plasma polymerization of acetobromo- α -D-glucose (ABG) at different radio frequency (RF) powers. Plasma polymerization was carried out by vaporizing ABG in the powder form by heating at 135°C. Surface modification resulted in improved hydrophilicity and smoothness of the surface especially at low RF powers (30–50 W), but at high RF powers, the surface was found to be etched and the hydrophilicity decreased as evidenced by atomic force microscopy (AFM) and contact angle measurements. The plasma polymerized ABG film was found to be extensively cross-linked as evidenced by its insolubility in water.

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Figure 6 appears in color online: <http://jba.sagepub.com>

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Infra red (IR) and X-ray photoelectron spectroscopy (XPS) were employed to characterize the plasma polymerized ABG films. IR studies revealed that at lower RF powers, polymerization was taking place mainly by breaking up of acetoxy group while retaining the ring structures to a major extent during the polymerization process whereas at high RF powers, the rupture of ring structures was indicated. XPS indicated a reduction in the percentage of oxygen in the polymers going from low to high RF powers suggestive of complete destruction of the acetoxy group at high RF powers. Cross-cut tests showed excellent adhesive properties of the plasma polymerized ABG films onto PET. Static platelet adhesion tests using platelet rich human plasma showed significantly reduced adhesion of platelets onto modified PET surface as evidenced by scanning electron microscopy. Polymerization of glucose and its derivatives using RF plasma has not been reported so far and the preliminary results reported in this study shows that this could be an interesting approach in the surface modification of biomaterials.

KEY WORDS: plasma polymerization, surface modification, glucose, PET, platelet adhesion, biocompatibility.

INTRODUCTION

Plasma polymerization has been extensively employed to modify the surface of implants, surgical tools and other medical devices for improved biocompatibility [1–8]. Because of the highly cross-linked nature of plasma polymers, it is insoluble and stable in aggressive environments such as organic solvents, acids and alkalis compared to conventional non-cross-linked polymers [9]. Therefore, surface modification with plasma polymers on implants or medical accessories is a highly promising method to improve their biocompatibility.

PET is widely used in cardiovascular implants such as sewing rings of the mechanical heart valve prostheses, vascular grafts, cardiac patches and angioplasty balloons because of its excellent mechanical properties and moderate biocompatibility [10–12]. These applications clearly suggest the importance of the surface modification of PET to improve its biocompatibility from its moderate level. Plasma polymerization results in pinhole free, thin, substrate independent films and the method can generally be used to modify the surface properties of any material [13–15].

Human tissues consist of many proteins and sugars such as glycosaminoglycans. Glucose is an important sugar present in the body and therefore it would be safe to assume that the molecule is a biocompatible one. Since we found that to develop a polymer from glucose is bit difficult, we paid attention to develop a plasma polymer from a glucose derivative known as acetobromo- α -D-glucose (ABG). To the best of our knowledge, no attempt has been made to prepare a

polymer film from glucose or a derivative of glucose by any method including radio frequency (RF) plasma polymerization method. In this study, we examined the plasma polymerization of ABG onto poly (ethylene terephthalate) (PET) surfaces and evaluated the surface properties before and after modification.

EXPERIMENTAL

PET was purchased from Dupont, Japan. Samples of 3 cm × 1.5 cm × 1 mm size were washed with non-ionic soap solution followed by several rinses using singly distilled water. Specimens were then sonicated in doubly distilled water for 15 min and dried in an air oven at 50°C. ABG (2, 3, 4, 6-tetra-O-acetyl- α -D-glucopyranosyl bromide) was purchased from Sigma and was used as such. After successive washing of the glass slides ultrasonically in acetone and distilled water and then drying them in an air oven at 50°C, we have coated plasma polymerized ABG (PP-ABG) on to it for infra red (IR) studies. For adhesion strength studies, we have used PET substrates coated with PP-ABG.

The schematic diagram of the plasma polymerization chamber (Samco International, model BP-1, Japan) used for preparation of polymer film is shown in Figure 1. The plasma reactor consists of a Pyrex glass bell jar, a pair of parallel disk electrodes (70 mm in diameter) connected to an RFG 200 RF generator, which is operated at 13.56 MHz, through an impedance matching circuit. Plasma polymerization was carried out by vaporizing ABG in the powder form, from the lower electrode by heating at 135°C. The substrate made of glass and/or PET was mounted on the lower side of the upper electrode at the center of the reactor. Thin films of ABG were prepared by the RF discharge at a pressure of 10 Pa, and the flow rate of argon gas was adjusted to 5 mL/min. RF power from 10 to 100 W was introduced into the reactor through a matching box for 3 min.

For structural characterization of ABG films, we employed infrared (IR) spectroscopy (Model JIR 7000 JEOL, Japan) and X-ray photoelectron spectroscopy (XPS) (ESCA 750, Shimadzu, Japan—we have used the software provided along with ESCA 750 for curve fitting to C1s narrow scan and for the same we have used average of Gaussian-Lorentz((G + L)/2). For recording the IR spectra, ABG coated onto the glass substrates was scrapped and pelletized with KBr. For surface characterizations, we employed scanning electron microscopy (SEM) (Model 7400F, JEOL, Japan), and atomic force microscopy (AFM, operated in DFM mode) (Model SPA 300, Seiko Instruments, Japan).

Adhesion to the substrate was estimated by cross cut test in which an adhesive tape is used to peel off a thin film cross cut to 100 squares

(10×10 ; 1×1 mm each. For cutting the thin film we have used a razor blade and to peel off the film we have used a standard adhesive tape (SEKSUI, Japan) from the PET substrate. The adhesive strength is expressed by the index from 0 to 10, depending on the extent of retention of the squares on peeling off the tape, where index 0 means complete detachment of cross cut squares and index 10 refers to no detachment of the cross cut squares [16]. Contact angle measurements were done using a contact angle goniometer (ERMA Inc., Tokyo, Japan). For thickness measurement of the thin films we have made step structures of the thin films on glass substrates and measured the step height using thickness measurement instrument (Dektak II A, USA).

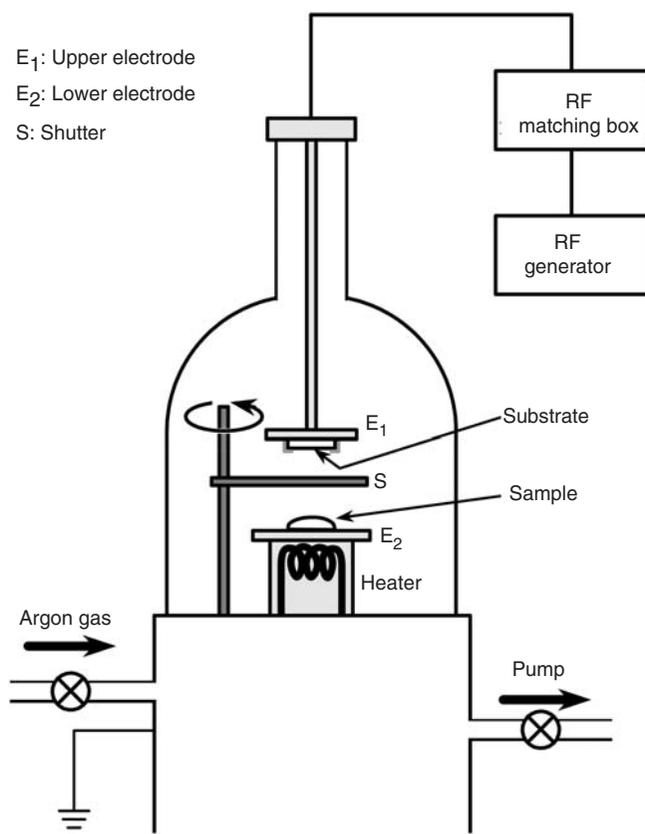


Figure 1. Structure of 2, 3, 4, 6-tetra-O-acetyl- α -D-glucopyranosyl bromide (Acetobromoglucose-ABG).

A preliminary blood-compatibility assessment was done by *in vitro* platelet adhesion studies using platelet rich human plasma. For platelet adhesion studies, ABG-coated and uncoated PET substrates were treated with to platelet rich plasma (PRP) from human blood as reported previously [17]. After washing gently with buffer (phosphate buffered saline (0.1M, pH 7.4)) many times to remove non-adhering platelets, adhered platelets were fixed with 2.5% glutaraldehyde solution, freeze-dried in a laboratory freeze dryer, coated with gold (thickness of 50 Å) and examined in the SEM.

RESULTS AND DISCUSSION

The structure of ABG is shown in Figure 2 Through plasma polymerization of ABG, we can develop polymer films on PET substrates with thickness approximately from 1000 to 4000 Å while using RF power for 3 min from 10 to 100 W. Figure 3 depicts the IR spectra of ABG and plasma polymerized ABG films prepared under different RF powers from 10 to 100 W. A reduction of the peak intensity around 1751 cm^{-1} can be seen while going from monomer to the polymer, and further with increasing RF powers from 10 to 100 W. This particular peak represents the acetoxy group ($\text{CH}_3\text{COO-}$) in the monomer as well as in the polymer [18]. The reduction in the intensity of the peak is believed to be an indication of the rupture of this particular group. Peaks in the range from 1018 to 1442 cm^{-1} indicate the presence of ring structures in the monomer as well as in polymer [18]. At lower RF powers (10 & 30 W) of polymers, we can observe a reduction in the number of peaks and

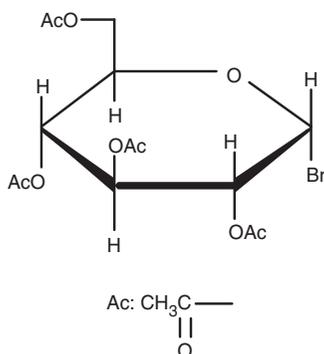


Figure 2. Experimental setup for plasma polymerization. Ar–Argon, RP–Rotary pump, RFG–Radio frequency generator, IM–matching box, U–Upper electrode, M–Monomer, S–Substrate.

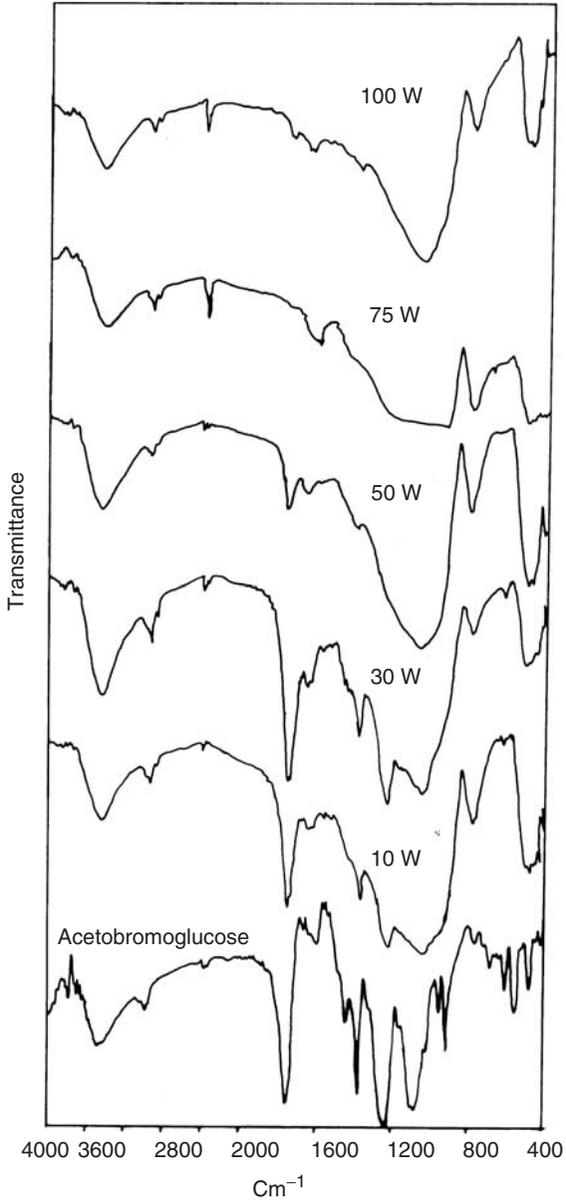


Figure 3. IR spectra of ABG for both monomer and plasma polymerized films.

a broadening of the band in this range. Reduction in the numbers of peaks can be related to the destruction of ring structures in the monomer during plasma polymerization even though the presence of some peaks in this area indicate that the ring structure is retained to a major extent in the polymer. According to Osada et al. [19], broadening of IR peaks can be considered as the characteristic of plasma polymers. At higher RF powers (50, 75, and 100 W) we could not observe any peaks in the range of 1018–1442 cm^{-1} and rather a broad strong peak is observed. This may be an indication of the rupture of ring structures at the higher powers. However, the existence of the broad band in this area shows that ring structure is retained to a certain extent in the polymer. IR studies reveals that at lower RF powers, polymerization is taking place mainly by breaking up of acetoxy group while retaining the ring structures to a major extent during the polymerization process. We can expect polymerization due to very low level cross-linking at this stage. However, at higher RF powers we can see the rupture of the ring structures and a total destruction of the acetoxy group which leads to a highly cross-linked polymer.

To verify our observations further regarding the polymerization of the ABG, we took the ESCA spectra of plasma polymers obtained at different RF powers of 10–100 W. Figure 4 shows the spectra of C1s of plasma polymers from 10 to 100 W. From this figure, it can be seen that the peak at the left shoulder of the C1s spectra is diminishing while moving from low power to high power. To check the indication of this phenomenon, we carried out the curve fitting analysis to C1s peak of the polymers. Figure 5 depicts a curve fitting analysis on C1s peak of polymer obtained at 75 W. Table 1 gives the detailed percentage analysis of the different carbon bondings in the polymers for different powers. It is seen that percentage of CH bonds in the plasma polymers show a slight upward tendency with respect to the increase in the plasma powers. However, percentage of C–O and C(O)O decreases while going from lower power to higher power. This fact would be undoubtedly related to our observation regarding acetoxy group that, at the lower RF power, this group is retained up to a certain extent and gradual destruction of acetoxy group is taking place at the higher RF powers leading to complete destruction at 100 W. This observation clearly shows that at the lower powers, we can expect some order in the polymerization but at higher powers, we can expect a highly cross-linked structure. The percentage of C=O is retained by and large in the polymer, which can be explained due to the higher dissociation energy needed for breaking C=O bonds.

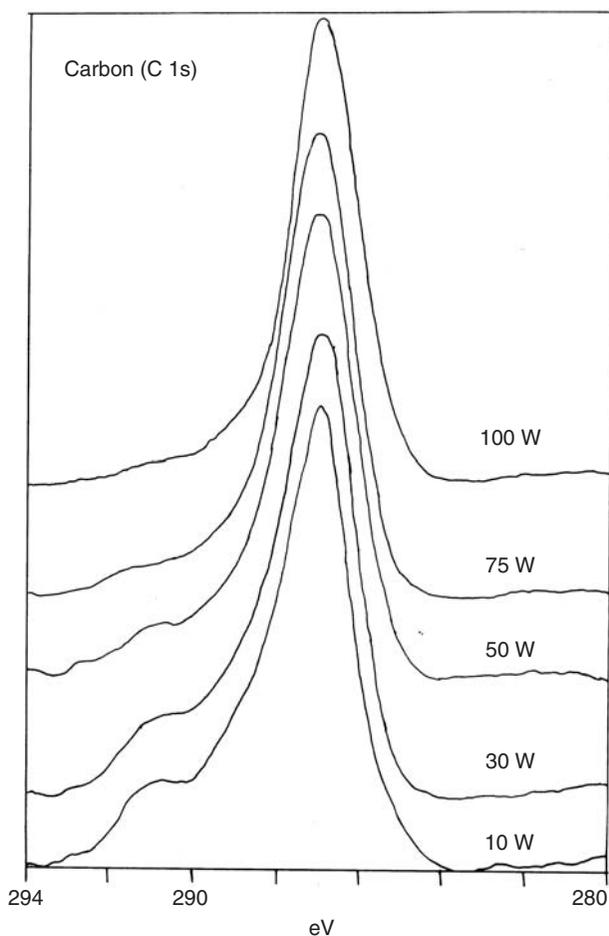


Figure 4. C1s spectra obtained by ESCA.

Table 2 compares different elements in the monomer and polymers obtained by ESCA analysis. This table shows that percentage of carbon in the polymer is increasing in comparison with other elements while going from lower power to higher RF power. We can observe a reduction in the percentage of oxygen in the polymers going from low powers to high powers. This observation can be related to the complete destruction of the acetoxy group while polymerizing the monomer. However, an interesting observation is that the percentage of bromine is the same in the polymer as in the monomer even though dissociation energy is low for C-Br bond.

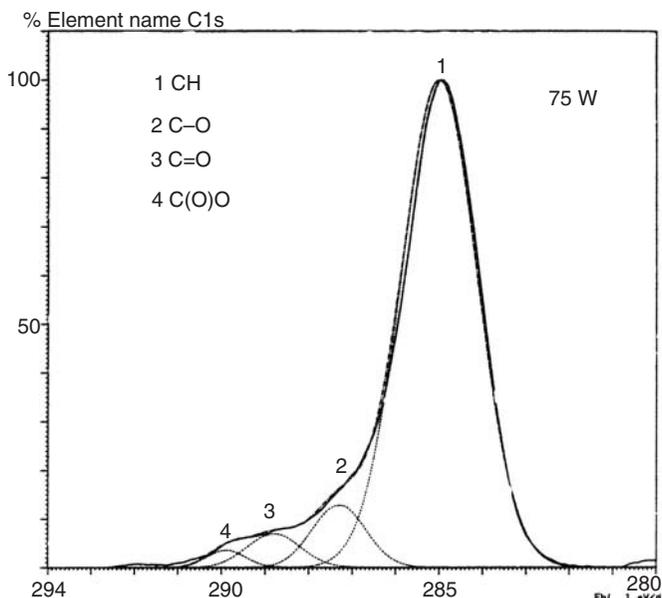


Figure 5. Curve fitted spectra of C1s spectra for plasma polymerized ABG prepared by 75 W RF power.

Table 1. Percentage of different carbon bondings obtained from C1s spectra for plasma polymerized films developed at different RF powers.

RF power	C-H (285) eV	C-O (286) eV	C=O (288) eV	C(O)O (289) eV
10W	61.4	19.9	7.8	10.9
30W	67.1	16.6	7.2	9.1
50W	70.2	15.5	6.8	7.5
75W	72.3	14.8	6.6	6.3
100W	75.4	12.5	6.9	5.2

The foregoing observations lead us to conclude that plasma polymerization is taking place at lower RF powers by breaking some acetoxy groups while retaining by and large the ring structure. However at higher powers, we predict complete destruction of acetoxy group and destruction of ring structures of the monomer which leads to a highly cross-linked polymer by keeping bromine content same. This cross-linking nature is very common in the plasma polymers and therefore, it is practically impossible to arrive at any definite

Table 2. Atomic percentage of different elements in monomer and plasma polymerized films obtained at different RF powers.

↓Materials elements→	C1s	O1s	Br 3d
Monomer (%–Theoretical)	58.33	37.50	4.17
10 W	71.34	24.80	3.86
30 W	73.97	22.25	3.88
50 W	79.55	17.33	3.12
75 W	82.54	13.50	3.96
100 W	85.55	10.73	3.72

Table 3. Adhesive strength of plasma polymerized films obtained at different RF powers.

RF power	10 W	30 W	50 W	75 W	100 W
Adhesive index	8	9	10	10	10

conclusions regarding their structure, considered as one of the drawbacks [9].

A high degree of adhesion to any substrates is considered as the important physical property of the plasma polymerized films [9]. To check the adhesion property of the plasma polymerized film, we carried out cross cut test as described before. Table 3 shows the strength of adhesion to the PET substrate. As can be seen, good adhesion of the polymer is evident at higher powers.

To check the insolubility of the plasma polymerized ABG films at different powers (10–100 W), films were gently scrapped from the surfaces of the glass substrates and examined their solubility in water. All films were found to be insoluble in water and thus suggesting that the films are highly cross-linked.

The static water-in-air contact angle was determined at room temperature. The contact angle formed between a sessile drop of water and the surface of PET and modified PET is directly related to the forces at the liquid-solid interface, indicating the hydrophobic or hydrophilic characteristics of the surface [20]. The measurement was performed using doubly distilled water and values provided are average of 10 measurements.

Table 4 depicts the contact angle data obtained from the plasma polymerized ABG modified and unmodified PET surfaces. The untreated PET has a hydrophobic surface exhibiting a water-in-air contact angle of $82 \pm 1^\circ$. After the surface modification with ABG, the contact angle

Table 4. Water-in air contact angle of unmodified PET and surface modified PET with plasma polymerized films of ABG obtained at different RF powers.

Sample	PET	10 W	30 W	50 W	75 W	100 W
Contact angle (°) ± SD	82 ± 1	36 ± 2	36 ± 2	39 ± 2	42 ± 2	44 ± 2

decreased to $33 \pm 2^\circ$ (for 30 W) and to $44 \pm 2^\circ$ (for 100 W). These results clearly indicate that surface modification of PET with plasma polymerized ABG results in highly hydrophilic surfaces [21]. Hydrophilicity of the surface is considered as one of the desirable property of a biomaterial surface [22,23].

AFM was employed to examine the surface morphology of the PET and plasma polymerized ABG surfaces. Figure 6(a) shows the surface morphology of the PET substrate. Figure 6(b) to (d) show the nature of the surface of the plasma polymerized ABG coated PET at RF powers of 30–100 W. At 30 and 50 W, the surface features were very much similar and therefore we have provided AFM picture of only 30 W. It is seen that the plasma polymerized ABG films are almost smooth and uniform in coverage of the substrate. The surface modification involving plasma polymerization of a polymer itself generates a smooth and uniform surface is particularly noteworthy and could be considered as a pointer to better hemocompatibility [24].

From the AFM pictures, it can be seen that while using higher plasma powers during surface modification, it indirectly causes an etching on the surface of the modified PET. This etching destroys the smoothness of the surfaces. Etching is highly evident on the surface generated using an RF power of 100 W for surface modification (Figure 6(d)). We can see a limited level of etching on the surface of the modified material using an RF power of 75 W. Since a smooth surface is better in preventing adhesion of blood components such as proteins and cells compared to a rough surface with pits and crevices etc., the etching of surfaces leading to surface roughness may not be desirable in the fabrication of biocompatible surfaces. Therefore, surface modification using RF plasma has to be optimized with respect to the RF power employed for the modification.

Static platelet adhesion studies showed significant difference in the platelet adhesion character of untreated PET and plasma polymerized ABG modified surfaces. Figure 7(a) to (e) show the SEM of platelet adhesion onto unmodified PET (Figure 7(a)) and plasma modified samples at different RF powers (Figure 7(b) to (e)).

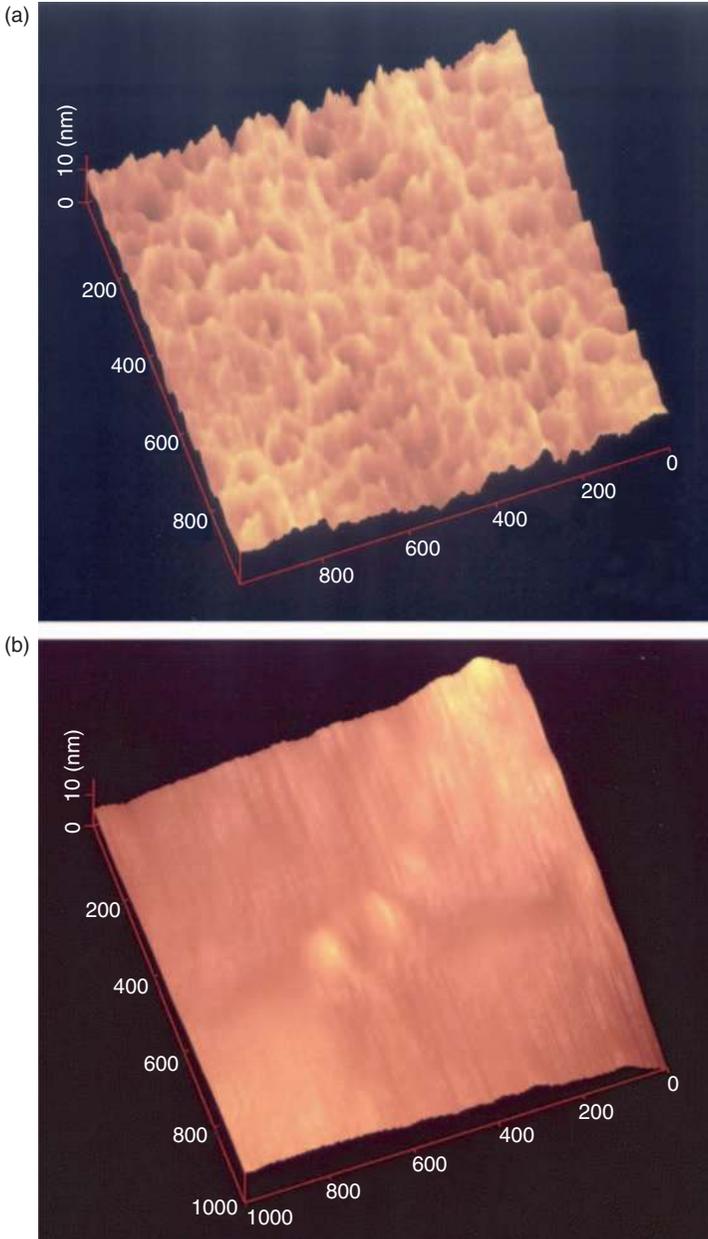


Figure 6. AFM of surface of unmodified PET and modified PET with PP-ABG at different plasma powers (a) unmodified PET (b) Modified by 30 W (c) 75 W, and (d) 100 W.

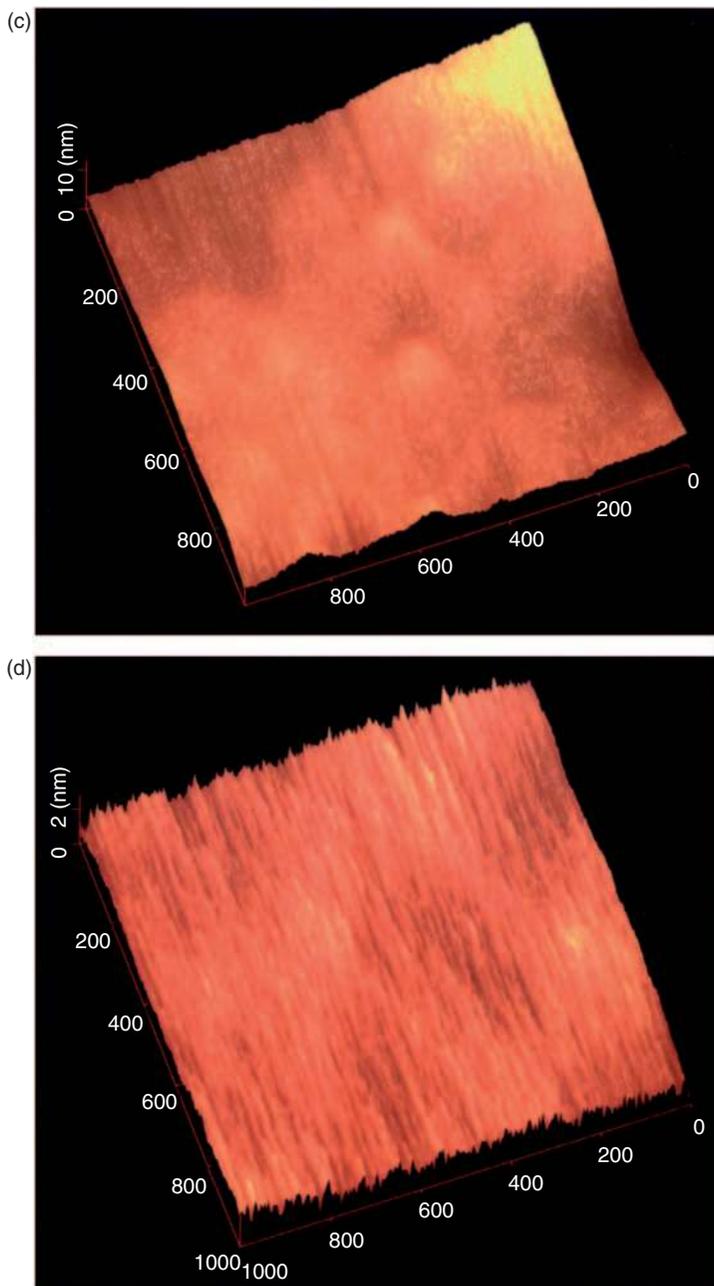


Figure 6. Continued.

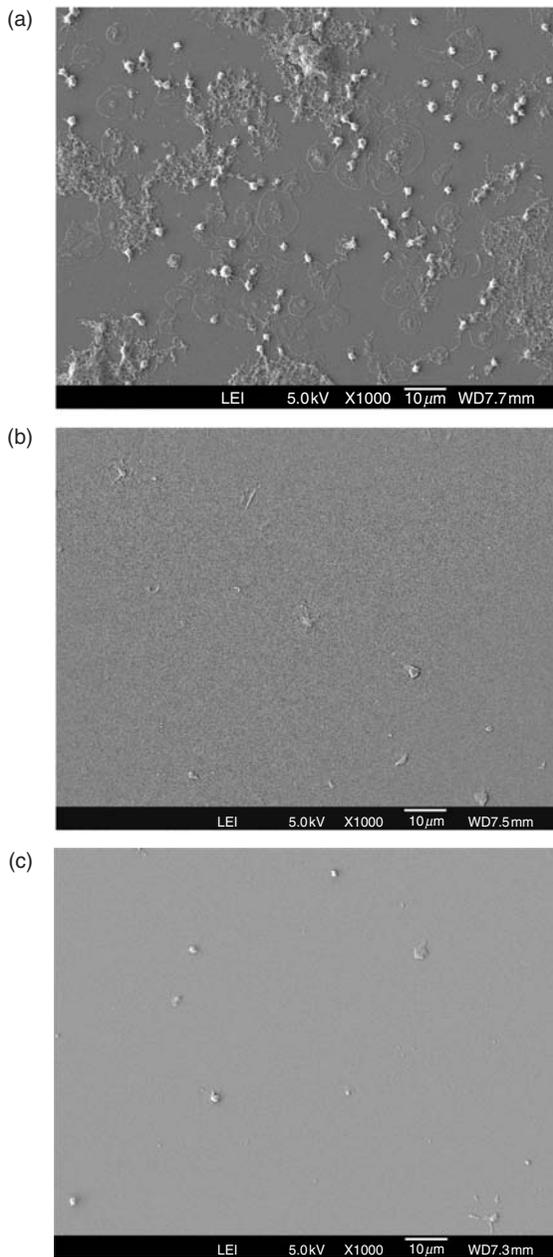


Figure 7. SEM showing platelet adhesion on to unmodified PET surface (a) and PP-ABG deposited on PET surface (b) 30 W (c) 50 W (d) 75 W, and (e) 100 W.

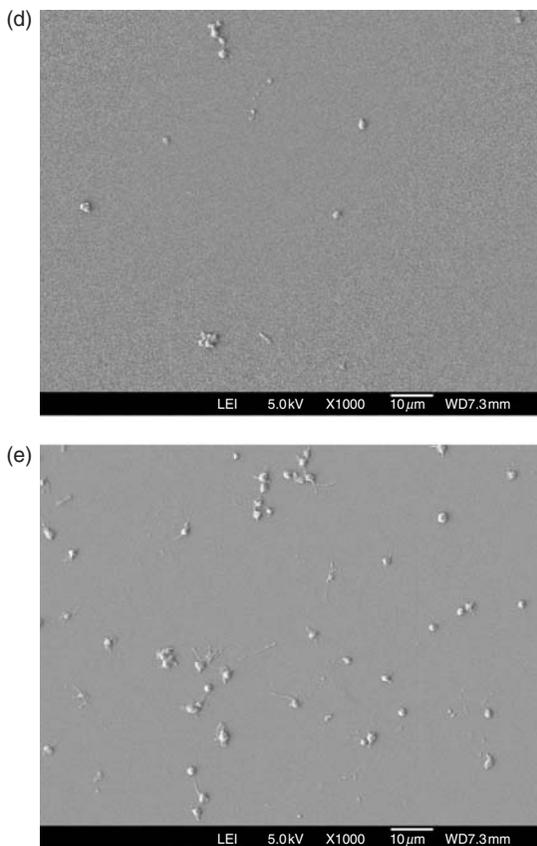


Figure 7. Continued.

Considerably reduced platelet adhesion on the modified surfaces compared to unmodified PET surface indicates the improved nature of the blood compatibility [23,25]. It is also seen that there seems to be a tendency for increased adhesion of platelets on surfaces modified a high RF power. This phenomenon is possibly suggestive of the importance of moderate RF power (30–50 W) for generating a more blood compatible surface. Here it is worthwhile to point out that while going from low power to high RF powers, the hydrophilicity of the surface decreases as evidenced by the contact angle data. Another important observation is that that while going from low RF power to high RF power, we can see that the oxygen content in the plasma polymerized ABG is also decreasing. The oxygen content and hydrophilicity bear a direct relationship due to hydrogen bonding of water. Along with this, the

reduction in the surface smoothness while going from low to high RF powers also could account for the reduction in the blood compatibility.

We are aware that platelet activation is a more important parameter than platelet adhesion for determining the biocompatible nature of the surface. Therefore, this observation of lower platelet adhesion on the modified surface should only be seen as a preliminary test of blood compatibility and more extensive investigations would be required to determine whether ABG-modified surface would be truly biocompatible.

Surface modification of PET using plasma polymerized ABG generates a surface which is hydrophilic and apparently less thrombogenic. Plasma polymerization has the unique advantage that one can obtain a highly uniform surface coverage on the substrate as a thin film which is strongly adhered onto the substrate irrespective of the nature of the substrate. Since this surface modification is solvent free, quick and highly industrial oriented one, modification of surfaces using plasma polymerized ABG has the advantage over any other surface modification methods and could be the subject of an extensive investigation.

CONCLUSIONS

In summary, we have attempted to show in this study that an insoluble polymer from a glucose derivative, ABG can be generated by plasma polymerization of the powder at different RF powers. While going from low to high RF plasma power, oxygen content decreases in the polymer as well as surface hydrophilicity and smoothness of the deposited film. Moderate plasma power (30–50 W) provides very good surface modification leading to a smooth surface and good hydrophilicity. The adhesiveness of the polymer film onto PET surface was found to be excellent. The polymer film was highly cross-linked as demonstrated by its insolubility in water. Preliminary blood compatibility screening using static platelet adhesion tests tend to point to the improved biocompatible nature of the modified surface as compared to bare PET surface. Plasma surface modification using glucose and derivatives of glucose would be an interesting approach in the surface modification of biomaterials.

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