The effects of amorphous carbon films deposited on polyethylene terephthalate on bacterial adhesion

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Abstract

There is an increasing interest in developing new methods to reduce bacteria adhesion onto polymeric materials that are used in biomedical implants. The antibacterial behavior on polyethylene terephthalate (PET) treated by acetylene (C\textsubscript{2}H\textsubscript{2}) plasma immersion ion implantation-deposition (PIII-D) is investigated. The surface structure of the treated PET is determined by laser Raman spectroscopy, X-ray photoelectron spectroscopy (XPS) and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR). The results show that a thin amorphous polymer-like carbon (PLC) layer is formed on the PET surface. Atomic force micrographs (AFM) show that C\textsubscript{2}H\textsubscript{2} PIII-D significantly changes the surface morphology of PET. The capacities of Staphylococcus aureus (SA) and Staphylococcus epidermidis (SE) to adhere onto PET are quantitatively determined by plate counting and Gamma-ray counting of \textsuperscript{125}I radio labeled bacteria in vitro. The results indicate that the adhesion of the two kinds of bacteria to PET is suppressed by PLC. The adhesion efficiency of SE on the coated surface is only about 14\% of that of the untreated PET surface, and that of SA is about 35\% of that of the virgin surface. The electrokinetic potentials of the bacterial cells and substrates are determined by zeta potential measurement. All the substrates as well as the bacterial strain have negative zeta potentials, and it means that bacterial adhesion is not mediated by electrostatic interactions. The surface energy components of the various substrates and bacteria are calculated based on measurements in water, formamide and diiodomethane. The surface free energies obtained are used to calculate the interfacial free energies of adhesion (\textit{D}_{\text{Adh}}) of SA and SE onto various substrates, and it is found that bacterial adhesion is energetically unfavorable on the PLC deposited on PET by C\textsubscript{2}H\textsubscript{2} PIII-D.

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1. Introduction

Polyethylene terephthalate (PET) is used in cardiovascular implants such as artificial heart valve sewing rings \cite{1,2} and artificial blood vessels \cite{3,4} because of its excellent mechanical properties and moderate biocompatibility. Medical devices such as artificial heart valves and vascular grafts are now designed for life span of the order of 10-years or more. The infection of medical devices is a life threatening complication, leading to significant morbidity and mortality \cite{5,6}. In particular, the incidence of prosthetic valve endocarditis (PVE) is about 2–3\% in patients undergoing valve replacement, with staphylococcus epidermidis (SE) accounting for about 30\% overall of these infections \cite{7}. The bacterial adhesion to the biomaterial substrate is the first event in a series of both host and organismic reaction that leads to PVE \cite{8,9}. This adhesion is mediated by physicochemical interactions between the bacteria and substrate. As a result, a significant number of studies on improving the antibacterial adhesion of polymer have focused on surface modification \cite{10–12}.

Plasma immersion ion implantation-deposition (PIII-D) is a rapidly developing surface modification technique that has been shown to be an effective method for modifying the physicochemical characteristics of thin films and mixing layers \cite{13}. In PIII-D, the target is typically enshrouded in self-excited plasma generated by applying a high negative voltage to the target. The ions
in the plasma bombard the target at normal incidence, providing effective and uniform ion implantation. This technique is therefore highly useful for treating three-dimensional objects, and is also suitable for polymers such as PET. Tanaka et al. [14,15] have successfully coated PET films with amorphous carbon or diamond-like carbon (DLC) using acetylene PIII to improve the gas-barrier properties. On the other hand, the research on amorphous carbon or DLC deposited on PET for antibacterial adhesion has not been frequently reported.

The objectives of the present research are to fabricate amorphous polymer-like carbon (PLC) films on PET employing acetylene PIII-D and to gain insight on the influence of this coating on bacterial adhesion. The physicochemical factors such as zeta potential, surface free energy and interfacial free energies ($\Delta F_{\text{sys}}$) of various kinds of bacterial cells on different substrates are measured and calculated to explain the results of bacterial adhesion. This study may provide a novel approach to design and synthesize carbon coatings on polymers to repel bacteria and consequently reduce the infection risk.

2. Materials and methods

2.1. Materials

The PET films 10 $\mu$m thick supplied by 3M were washed successively and ultrasonically in methanol, acetone and doubly distilled water for 10 min and dried in a purified biological desiccator.

2.2. PIII-D treatment

PET films were laid on stainless-steel substrates attached to an insulated stainless-steel electrode in the center of the vacuum chamber. A negative voltage was then applied to the electrode. Some carbon layers were also deposited onto Si(100) wafers. These wafers were placed on PET films on the stainless substrate to make the same electric contact as the PET samples.

C$_2$H$_2$ PIII-D was performed at 5 kV for 40 min. The base pressure in the vacuum chamber was $2.4 \times 10^{-3}$ Pa. The pressure of vacuum chamber was $1.0 \times 10^{-1}$ Pa after introduction of C$_2$H$_2$ at a flow rate of 30 standard cubic centimeters per minute (SCCM). The sample voltage pulse width was $20 \mu$s and frequency was 100 Hz. This sample is designated as PIII-PET in this work.

2.3. Surface characterization

The film thickness was measured with an Alpha-step$^\text{W}$-500 surface profiler. The structure of the carbon layer was determined by laser Raman spectroscopy (JobinYvon, T64000). The attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) spectra of the PET films were obtained using a Perkin Elmer 16PC. The surface chemical states were determined by XPS (PHI 5802) employing a monochromatic Al K$_\alpha$ radiation operated at 14 kV and 350 W. Atomic force microscopy (AFM) studies were carried out on a Park Scientific Instrument Autoprobe Research System in the non-contact mode.

2.4. Bacteria culture

The bacteria were kept on nutrient agar plates consisting of peptone, beef extract, sodium chloride, and agar for 16 h at 4°C. Single colonies were then transferred to a soy broth and incubated at 37°C for 24 h. 9.9 ml culture media was inoculated with 0.1 ml test culture and 10 $\mu$l 125I-desoxyuridine ($^{125}$I-UDR). Then the bacteria were pelletized by centrifugation for 10–15 min. The supernatant was discarded and the pellet was suspended in a phosphate-buffered solution (PBS). The same procedure was repeated three times. The bacteria cell concentration was adjusted to $1 \times 10^{-5}$ cells/ml by dilution with PBS.

2.5. Quantification of bacteria adhesion

Untreated and modified PET films were sterilized under high temperature and cut into 16 pieces of approximately 2.5 cm$^2$. Untreated and modified films were added to two different flasks containing the cell suspension. A control flask containing the cell suspension without the film was also tested. The flasks were agitated at 100 rpm during incubation. Four films were taken out sequentially from the bacteria suspension every 5 h for a total interval of 20 h and rinsed by PBS three times. Thereafter the adherent bacteria were detached from the PET films by PBS-Tween20 solution ultrasonic cleaning. The PBS-Tween20 washings containing bacteria was sampled to determine the viable counts.

2.6. Contact angle measurements and surface free energy calculations

Contact angle measurements by the sessile drop technique using a contact angle geniometer (JY-82, China) were conducted at 25°C with doubly distilled water, formamide and diiodomethane as wetting agents. In the case of the bacterial cells, the measurements were performed on the bacterial layers deposited on membrane filters according to the method described by Busscher [16]. A series of contact angle data on a given surface yields the surface free energy components by fitting the data by Lifshitz–van der Waals/acid–base approach (LW-AB) [17]. The total surface free energy can be expressed as

$$\gamma = \gamma^{\text{LW}} + \gamma^{\text{AB}},$$

(1)
Unlike $\gamma_{\text{LW}}$, the nonpolar London–van der Waals component, the acid–base component $\gamma_{\text{AB}}$ comprises two non-additive parameters. These are the electron-acceptor surface tension parameter ($\gamma^+$) and the electron-donor surface tension parameter ($\gamma^-$), and the acid–base interactions are complementary in nature. The total acid–base contribution to the surface tension is given by

$$\gamma_{\text{AB}} = 2\sqrt{\gamma^+ \gamma^-}. \quad (2)$$

The total interfacial tension between condensed phases $i$ and $j$ is

$$\gamma_{ij} = \left(\sqrt{\gamma_{ij}^{\text{LW}}} - \sqrt{\gamma_{ij}^{\text{LW}}}\right)^2 + 2\left(\sqrt{\gamma_{ij}^+ \gamma_{ij}^-} + \sqrt{\gamma_{ij}^+ \gamma_{ij}^-} - \sqrt{\gamma_{ij}^+ \gamma_{ij}^-} - \sqrt{\gamma_{ij}^+ \gamma_{ij}^-}\right). \quad (3)$$

The Young equation relates the liquid–vapor ($\gamma_L$), the solid–vapor ($\gamma_S$) and the solid–liquid interfacial free energy with the contact angle $\theta$

$$\gamma_L \cos \theta = \gamma_S - \gamma_{SL}. \quad (4)$$

Expressing the three tensions in Eq. (4) in terms of Eq. (3) gives

$$(1 + \cos \theta)\gamma_L = 2\left(\sqrt{\gamma_{\text{LW}}^{\text{LW}}} - \sqrt{\gamma_{\text{LW}}^{\text{LW}}} + \sqrt{\gamma_{\text{S}}^{\text{S}}} \gamma_{\text{L}}^- + \sqrt{\gamma_{\text{S}}^{\text{S}}} \gamma_{\text{L}}^+\right) \quad (5)$$

Eq. (5) contains three unknown parameters. The contact angles of three liquids with known $\gamma_{\text{LW}}^{\text{LW}}, \gamma_L^+$ and $\gamma_L^-$ (as shown in Table 2) need to be measured on solid substrates and bacterial cell lawns.

### 2.7. Zeta potential measurement

The zeta potential (ZP) of the two kinds of bacteria in a phosphate buffer were measured with a Zeta Sizer 3000HsA (Malvern Instruments, England) at 25°C. Zeta potential measurements were obtained following 4h incubation of $10^8$ cells/ml in the phosphate buffer. The same procedure was followed to obtain the zeta potential of the amorphous carbon and PET. In this case, the amorphous carbon scraping off from the coating on Si and PET had been previously reduced to small particles.

### 3. Results

#### 3.1. Surface morphology

Fig. 1(a) and (b) contrast a $5 \times 5 \mu m^2$ surface topography as measured by AFM without any filtering. The surface of the untreated PET sample exhibits rectangular structures. Pinnacle-like structures can be
observed in the image of PIII-PET. Each AFM image is analyzed in terms of surface average roughness. The PET surface modified by acetylene PIII-D treatment shows decreased average roughness ($R_a$) from 58.9 to 11.2 nm. This result indicates that the surface morphology of PET film is significantly affected by C$_2$H$_2$ PIII-D treatment.

3.2. Raman spectroscopy

The Raman spectrum of the untreated PET film exhibits characteristic sharp peaks at 1292, 1616 and 1727 cm$^{-1}$ (Fig. 2a). The peak at 1292 cm$^{-1}$ is attributed to the ring and C=O stretch, whereas the 1616 and 1727 cm$^{-1}$ peaks can be ascribed to C=C ring and Carbonyl stretch, respectively. The Raman spectrum of the PIII-PET reveals a strong carbon layer band on which the PET Raman bands are superimposed (Fig. 2b). The Raman spectrum of the carbon film deposited on Si shows two broad peaks at 1300–1450 and 1500–1650 cm$^{-1}$ (Fig. 2c). Such a spectrum with an asymmetric broad peak is often seen on carbon deposit that is called amorphous PLC [19].

3.3. ATR-FTIR spectral analysis

Fig. 3 shows that infrared peaks such as alkyne end groups and C=C bonds of fatty hydrocarbon are observed in the PIII-PET film. The creation of alkyne end groups (R–C≡C–H) is evidenced by the new band at 3300 cm$^{-1}$, which is assigned to C–H stretching vibration mode of the alkyne end group. Large peaks are seen at 1630 cm$^{-1}$ due to C=C (sp$^2$ bonding). The intensity of the peaks assigned to sp$^3$ CH$_2$ and sp$^3$ CH$_3$ at around 2850–2950 cm$^{-1}$ is increased. This result suggests that this surface coating is mostly contained in a mixture of sp$^2$, sp$^3$, and a few sp$^2$ coordinated carbon atoms in a disordered network.

3.4. XPS analysis

Fig. 4 shows the XPS surveys of the untreated and PIII-PET. The atomic ratio of Cls to O1s increases from 75/25 to 85/15. Fig. 5(a) displays the XPS spectrum for Cls resolved into three peaks representing the interatomic bonds of carbon: C–C (sp$^3$), C=C (sp$^2$), and C=O peak. The dominant features in the spectrum for the PIII-PET film are sp$^2$ (284.5 eV) and C–H bonding (285 ± 0.2 eV). The spectrum is analyzed in more details by fitting the Gaussian functions for the proportions of sp$^3$, sp$^2$ and C=O as shown in Fig. 5(b). The ratio of sp$^3$ to sp$^2$ and C–H is 0.25. This indicates that the carbon layer is dominantly graphite or amorphous (C–H bonded).

3.5. Contact angle and surface energy components

The values of the contact angles with the various liquids and surface energy components for the bacterial cells and substrates are summarized in Table 2. The surface energy components are obtained by solving
Eq. (5) and using the surface tension components of the reference liquids shown in Table 1. It can be seen that both SE and Staphylococcus aureus (SA) all are predominantly electron-donating, since the component $\gamma_S$ is much higher than $\gamma^+_S$. The free energy components of the untreated PET are in accordance with the results of Van Oss et al. [20], stating that most polymers have a $\gamma_{LW} \approx 40 \text{ mJ/m}^2 \pm 10\%$. Table 2 shows that the PLC film deposited on PET by PIII-D are electron acceptor.

3.6. Bacteria adhesion

Fig. 6 shows that the changes of the adhered numbers of SA and SE on the surface of the PET control and PIII-PET with time. This result suggests that bacteria adhesion on biomaterials is a dynamic process that is the same as the growth curve of bacteria [21]. The number of the same bacteria on the same kind of biomaterials significantly changes and does not remain constant or increase continually. Fig. 7 demonstrates that the adhered capacities of two types of bacteria on PET are all suppressed by PLC film on PET. The adhered concentration of SE on the surface of PIII-PET is as less as 14% on the untreated PET surface. The capacities of SA on the modified surface are 35% comparing with that of the untreated surface.

4. Discussion

It is believed that the reduction in bacterial adhesion is achieved following the deposition of a thin amorphous polymeric-like carbon layer on PET. Bacterial colonization on a surface is a complex process. The initial phase is bacterial adhesion to the biomaterials substrate. From a physical–chemical point of view, the
adhesion of bacteria cells to surface is determined by the interplay of electrostatic and hydrophobic interactions. The surface charge of bacteria may be one important physicochemical factor for bacteria adhesion. Long-range electrostatic forces may influence the initial phase of bacteria adhesion onto biomaterial surface.

All the interacting surfaces are found to have negative zeta potentials as shown in Table 3 and for all combinations, electrostatic repulsion is to be expected. This importance of this fact is to provide evidence that in this case, adhesion is not mediated by direct electrostatic interactions between the bacteria and substrates.

The other important physicochemical factor affecting bacterial adhesion is the hydrophobic interaction. A thermodynamic approach offers a powerful tool to predict bacterial adhesion to solid substrates [16]. On the basis of an interfacial free energy balance, neglecting electrical charge interactions, addition may be expected if

$$\Delta F_{\text{Adh}} = \gamma_{SB} - \gamma_{SL} - \gamma_{BL} < 0,$$

(6)

where $\Delta F_{\text{Adh}}$ is the interfacial free energy of adhesion, $\gamma_{SB}$ the solid–bacterium interfacial free energy, $\gamma_{SL}$ is the solid–liquid interfacial free energy, and $\gamma_{BL}$ is the bacterium–liquid interfacial free energy, whereas adhesion is energetically unfavorable if

$$\Delta F_{\text{Adh}} > 0.$$  

(7)

The values of $\gamma_{SL}$, $\gamma_{BL}$ and $\gamma_{BS}$ can be determined from Eq. (3). The following equation is used to determine the interfacial energy of bacteria adhesion to a solid surface [22]:

$$\Delta F_{\text{Adh}} = \left( \sqrt{\gamma_{SL}^{\text{LW}}} - \sqrt{\gamma_{SB}^{\text{LW}}} \right)^2 - \left( \sqrt{\gamma_{BL}^{\text{LW}}} - \sqrt{\gamma_{SL}^{\text{LW}}} \right)^2$$

$$- \left( \sqrt{\gamma_{SL}^{\text{LW}}} - \sqrt{\gamma_{BL}^{\text{LW}}} \right)^2 + 2 \left( \sqrt{\gamma_{SL}^{\text{LW}}} + \sqrt{\gamma_{BL}^{\text{LW}}} \right)$$

$$- \sqrt{\gamma_{SL}^{\text{LW}}} \sqrt{\gamma_{SB}^{\text{LW}}} - \sqrt{\gamma_{BL}^{\text{LW}}} \sqrt{\gamma_{SL}^{\text{LW}}},$$

(8)
Table 4 shows that the interfacial free energy of adhesion $\Delta F_{\text{Adh}}$ is negative for SA and SE bacterial strains on PET, but $\Delta F_{\text{Adh}}$ is positive for the same bacterial strains on PIII-PET. This result suggests that untreated PET is thermodynamically favorable to SA and SE adhesion and the amorphous PLC film deposited by $\text{C}_2\text{H}_2$ PIII-D mitigates adhesion of SA and SE bacterial strains. On the basis of these results, a drastic reduction in bacterial adhesion of SA and SE can be expected on PIII-PET. This is confirmed by the results of adhesion tests as shown in Fig. 7.

Even on a molecular level, the surface topography of devices used in medicine and industry has been demonstrated to affect the rate of microbial adhesion. Previous studies show that surface roughness influences bacterial adhesion [23,24]. A cause may be that a rough surface has a greater surface area and the troughs in the rough surfaces provide more favorable sites for colonization. The AFM results indeed show a small decrease in the surface roughness from 58.9 to 11.2 nm after $\text{C}_2\text{H}_2$ PIII-D and consequently suppression of microbial adhesion.

5. Conclusion

Amorphous DLC coatings are fabricated on PET films by acetylene PIII-D under different working pressure and characterized using various techniques. The coatings are shown to mitigate bacterial adhesion. Since all the interacting surfaces are found to have negative zeta potentials, the main physicochemical reason for bacteria repulsion from the PLC films deposited by acetylene PIII-D on PET is $\Delta F_{\text{Adh}}<0$. The change in the surface topography may be another factor for the suppressed bacterial adhesion. Our study provides a novel surface engineering approach to reduce the infection risk.

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