

**THERMODYNAMIC INVESTIGATION OF THE
BACTERIAL ADHESION ON TITANIUM DIOXIDE
INCORPORATED DIAMOND-LIKE
CARBON COATINGS**

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Abstract

Thermodynamic approaches were used to investigate the antibacterial property of diamond-like carbon (DLC) films containing titanium dioxide (TiO_2) nanoparticles. The films were grown on 316L stainless steel substrates from a dispersion of TiO_2 in hexane using plasma enhanced chemical vapour deposition. The composition, bonding structure, surface energy, and work of adhesion of these films were evaluated. The results were compared to practical antibacterial tests. As TiO_2 content increased, there was an increasing in I_D/I_G ratio, the films become more hydrophilic, with higher surface free energy and the interfacial energy of bacteria adhesion decreased. The results show that the antibacterial activity of TiO_2 -DLC films is directly influenced not only by the increased concentration of TiO_2 nanoparticles, but also by the I_D/I_G ratio, wettability, and work of adhesion of these films.

1. Introduction

Bacterial adhesion to medical devices is the first step in the development of medical device infections [8]. Once bacteria attach to a medical device, a multistep process starts leading to the formation of a complex and adhering microbial community [8, 23]. An effective and desired approach to reduce bacterial adhesion is to alter the surface properties of the medical devices and to make them less attractive for bacteria by using surface coating techniques [8]. For biomedical applications, the coatings should have a good biocompatible and mechanical property [8].

Diamond-like carbon (DLC) films have been actively studied over the last decade in the field of material engineering. Consisting of dense amorphous carbon or hydrocarbon, DLC mechanical properties fall between those of graphite and diamond [4, 13]. Due to their mechanical, electrical, optical, and chemical properties, the use of DLC coatings in mechanical and electrical fields has increased with recent applications in food, beverage, and medical market segments [3].

Titanium dioxide (TiO_2) has been widely studied as regards various electronic applications, utilizing the photo-catalytic nature and transparent conductivity, which strongly depend on the crystalline

structure, morphology, and crystallite size [11]. Due to TiO_2 photo-semiconductor properties, it may find an application as antibacterial agent for the decomposition of organisms [6, 17]. TiO_2 in the anatase crystalline form is a strong bactericidal agent when exposed to near UV-light [7].

The antibacterial activity of TiO_2 -DLC films was already been reported in our previous publication [10]. In the current paper, thermodynamic approaches were used in order to investigate the influence of the TiO_2 nanoparticles on the bacterial adhesion of TiO_2 -DLC coatings.

2. Experimental Procedures

The 316L stainless steel substrates were mechanically polished to a mirror-like finish surface. All the substrates were cleaned ultrasonically in an acetone bath for 15min, and then dried in nitrogen atmosphere. The clean samples were mounted on a watercooled, 10cm diameter cathode into the plasma chamber.

The cathode was fed by a pulsed DC power supply, with variable pulse voltage from -100 to -1000V , at a frequency of 20kHz , and duty-cycle of 50% .

In the chamber (vacuum base pressure of 1.3mPa), the substrates were additionally cleaned by argon discharge with 1 sccm gas flow at 11.3Pa working pressure and a discharge voltage of -700V for 10min prior to deposition. In order to enhance the DLC film adhesion to metallic surfaces, a thin amorphous silicon interlayer (thickness around 200nm) were deposited using silane as the precursor gas (1 sccm gas flow at 11.3Pa for 12min and a discharge voltage of -700V) [1]. The DLC films were deposited using hexane as the feed gas to a thickness of around $2.0\mu\text{m}$ (at 18.0Pa for 60min and a discharge voltage of -700V).

To produce TiO_2 -DLC films, TiO_2 nanoparticles (Aeroxide[®] TiO_2 P25 from Evonik), in anatase crystalline form with average particle size of 21nm , were dispersed in hexane at 0.1 , 0.5 , and 1.0g/L . These dispersions replaced the pure hexane during the DLC deposition.

The atomic arrangement of the films was analyzed by Raman scattering spectroscopy by using a Renishaw 2000 system with an Ar⁺-ion laser ($\lambda = 514\text{nm}$) in backscattering geometry. The laser power on the sample was ~ 0.6 mW and the laser spot had $2.5\mu\text{m}$ diameter. The Raman shift was calibrated in relation to the diamond peak at 1332cm^{-1} . All measurements were carried out in air at room temperature.

The contact angle θ of the samples was measured by using the sessile drop method with a Kruss Easy Drop contact angle instrument (Easy Drop DSA 100). The liquid was dropped automatically by a computer-controlled system. All measurements were carried out at room temperature.

The surface energy composed of polar and dispersive components of the samples was evaluated by the measurement of contact angle. The interfacial tension between two condensed phases can be determined by Young's equation [22], according to which

$$\cos\theta\gamma_{LV} = \gamma_{SV} - \gamma_{SL}, \quad (1)$$

where θ is the measured contact angle between liquid and solid, and γ_{LV} , γ_{SV} , and γ_{SL} are the interfacial energies of the liquid/vapour, solid/vapour, and solid/liquid interfaces, respectively. This equation can be rewritten as the Young-Duprè equation:

$$W_a = \gamma_{LV}(1 + \cos\theta) = \gamma_{SV} - \gamma_{SL}, \quad (2)$$

where W_a is the adhesion energy per unit area of the solid and liquid surfaces. In the general form of Equations (1), (2) then can be written:

$$\gamma_{LV}(1 + \cos\theta) = 2\sqrt{\gamma_L^P\gamma_S^P} + 2\sqrt{\gamma_L^D\gamma_S^D}, \quad (3)$$

where γ_L^P and γ_S^P are the polar components of the surface energy of liquids phase and solid phase, respectively, while γ_L^D and γ_S^D are the dispersive component of the surface energy of the liquid and solid phase, respectively. Since γ_L^D and γ_S^D have been published for many liquids, it is

possible to approximate γ_S^D and γ_S^P from a single measurement of θ by the use of Equation (3). Therefore, by measuring the contact angles of two different liquids (distilled water and diiodomethane) with well-known polar and dispersive components of surface energy (Table 1), Equation (3) can be solved to determine the polar and dispersive components of the surface energy of the materials [2, 19].

Table 1. Test liquids and their surface tension components [19]

Surface tension data (mN/m)	γ_L^D	γ_L^P	γ_{LV}
Water	21.8	51.0	72.8
Diiodomethane	50.8	0.0	50.8

The liquid was dropped automatically by a computer-controlled system. All measurements were carried out at room temperature. In order to investigate bacterial adhesion mechanism, work of adhesion for bacteria to attach to the coatings was calculated by using thermodynamic approach [16]:

$$\Delta F_{Adh} = \gamma_{SB} - \gamma_{SL} - \gamma_{BL}, \tag{4}$$

where ΔF_{Adh} is the interfacial free energy of adhesion, γ_{SB} is the solid-bacterium interfacial free energy, γ_{SL} is the solid-liquid interfacial free energy, and γ_{BL} is the bacterium-liquid interfacial free energy. They can be calculated by using contact angle data and Van Oss acid-base approach [8, 16, 20].

The following equation is used to determine the interfacial energy of bacteria adhesion to a solid surface [8, 16, 20]:

$$\Delta F_{Adh} = 2 \left(\begin{array}{l} \sqrt{\gamma_S^{LW} \gamma_L^{LW}} + \sqrt{\gamma_S^P \gamma_L^d} + \sqrt{\gamma_S^d \gamma_L^P} \\ + \sqrt{\gamma_B^{LW} \gamma_L^{LW}} + \sqrt{\gamma_B^P \gamma_L^d} + \sqrt{\gamma_B^d \gamma_L^P} \\ - \sqrt{\gamma_S^{LW} \gamma_B^{LW}} - \sqrt{\gamma_S^P \gamma_B^d} - \sqrt{\gamma_S^d \gamma_B^P} - \gamma_L \end{array} \right). \tag{5}$$

According to thermodynamic theory, if ΔF_{Adh} is negative, adhesion is thermodynamically favourable. While if ΔF_{Adh} is positive, adhesion is thermodynamically unfavourable.

All the parameters obtained through thermodynamic approaches were compared with the bactericidal results performed against *E. coli* and reported in our previous publication [10].

3. Results and Discussion

Bacterial colonization on a surface is a complex process and the initial phase is bacterial adhesion to the biomaterial substrate. From a physicochemical point of view, the adhesion of bacteria cells to a surface is determined by the interplay of electrostatic and hydrophobic/hydrophilic interactions [21].

Figure 1 shows the dependence of the water contact angle and the surface energy (γ) of the samples according to the concentration of TiO_2 nanoparticles in DLC films. The water contact angle of the films decreased as the TiO_2 concentration increased. Usually, a hydrophobic surface has a contact angle higher than 70° , while a hydrophilic surface has a contact angle lower than 70° [2]. These results point that TiO_2 -DLC films increased their hydrophilic characteristic due to the presence of amorphous TiO_2 surfaces [18], which leads to an increasing contact between bacteria and the coatings. This can be more evident when the water contact angle values were compared with the antibacterial activity [10], as in Figure 2. This comparative shows that the water contact angle has a significant influence on TiO_2 -DLC antibacterial activity with a good correlation coefficient ($R^2 = 0.9768$). As the concentration of TiO_2 nanoparticles in DLC films increased, the water contact angle decreased from 82 to $\sim 50^\circ$.

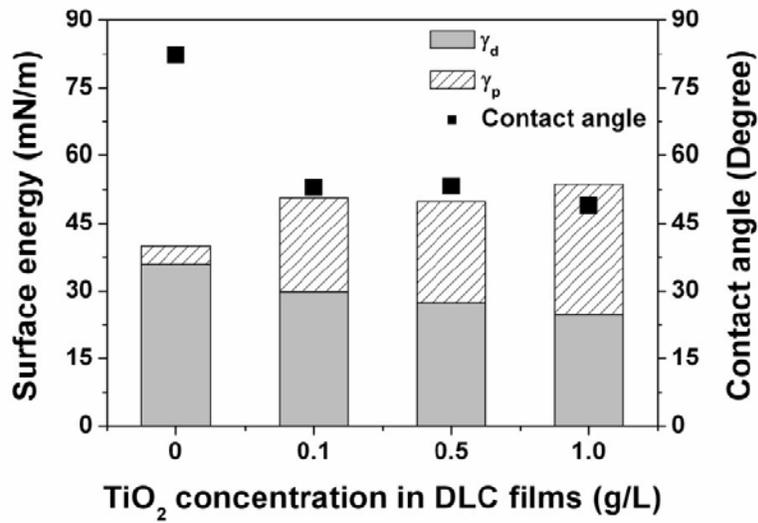


Figure 1. Dependence of the water contact angle and the surface energy of TiO₂- DLC samples according to the TiO₂ concentration in the films.

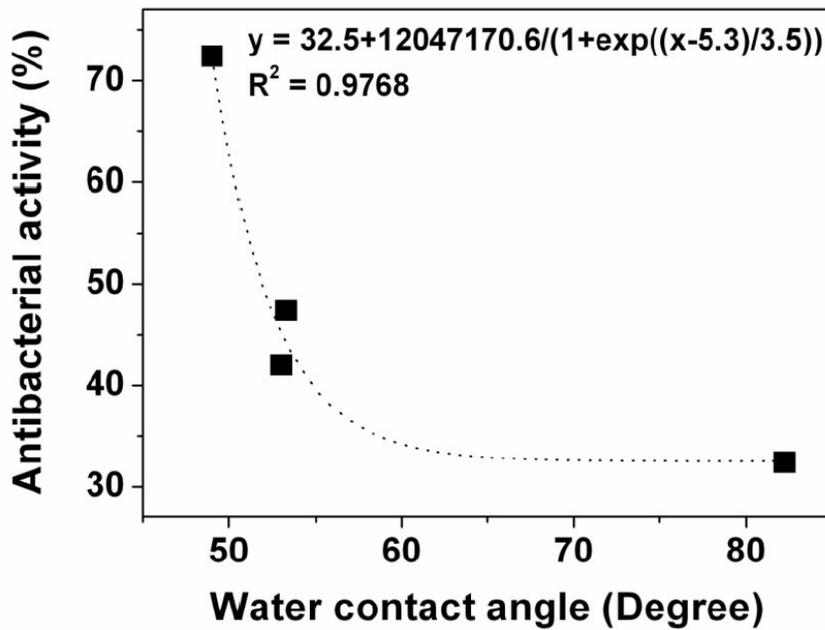


Figure 2. Antibacterial activity [10] vs. water contact angle.

The total surface energy of the samples increased with the increasing TiO₂ nanoparticles. It is clear in Figure 1 that this increasing is attributed to the increasing in the polar component. TiO₂-DLC films have a higher polar component due to the oxide particles on the surface [15]. The polar components attract the electric dipoles of water, which minimizes the interfacial energy and the water contact angle [15]. Electric dipole of water molecule would be attracted by the polar component, which reduces the interfacial energy between the surface and the water and thus the wetting angle of water [14].

The bacterial adhesion was predicted by using thermodynamic approaches [16, 20]. Table 2 presents the interfacial free energy of adhesion (ΔF_{Adh}) of the samples according to the concentration of TiO₂ nanoparticles in DLC films. The as-deposited DLC films presented $\Delta F_{\text{Adh}} = + 17.6\text{mJ/m}^2$. According to the thermodynamic theory, the bacterial adhesion is unfavourable if the work of adhesion is positive. The TiO₂-DLC films produced from 1.0g/L TiO₂ in hexane presented $\Delta F_{\text{Adh}} = - 5.2\text{mJ/m}^2$. In this case, the bacterial adhesion is favourable. This result suggests that as the TiO₂ content in DLC films increased, they become thermodynamically favourable to bacterial adhesion, increasing the direct contact between bacteria and more TiO₂ nanoparticles, promoting the increase in the bactericidal activity.

Table 2. Work of adhesion of the TiO₂-DLC samples according to the TiO₂ concentration in the films

TiO ₂ Concentration (g/L)	Interfacial free energy of adhesion (mJ/m ²)
0.0	17.6
0.1	1.8
0.5	- 0.4
1.0	- 5.2

Figure 3 compared the theoretical values of work of adhesion (ΔF_{Adh}) with the practical results of antibacterial activity [10]. The good correlation coefficient ($R^2 = 0.9999$) shows that the bactericidal mechanism suffers influences not only from the presence of TiO_2 nanoparticles on the DLC surface, but also from the interaction between the nanoparticles and the DLC film, changing its proper characteristics.

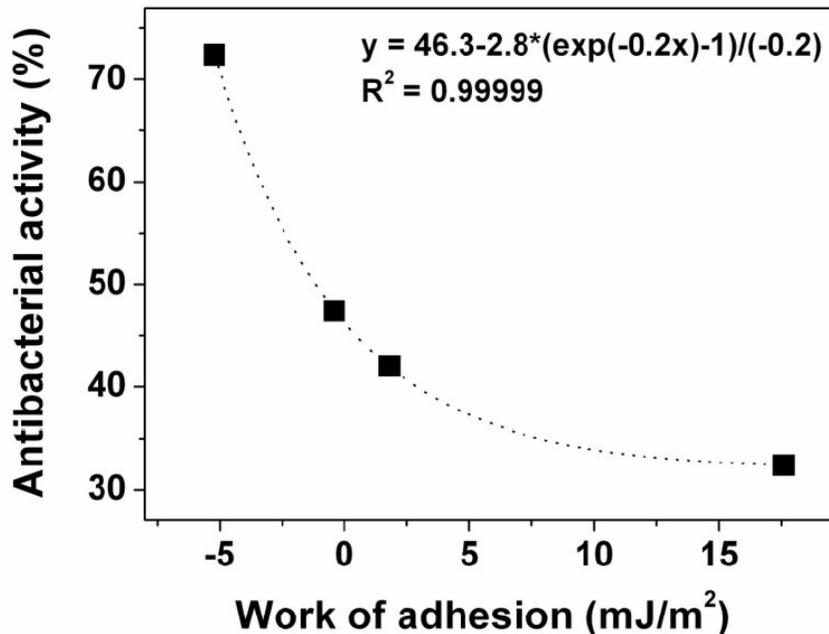


Figure 3. Antibacterial activity [10] vs. work of adhesion.

Raman scattering spectroscopy was used to evaluate the chemical structure of the DLC films. The spectra are composed of two broad bands, centered approximately $\sim 1400\text{cm}^{-1}$ (*D* band) and $\sim 1550\text{cm}^{-1}$ (*G* band). The *D* and *G* band peak positions were determined by subtracting a linear background and fitting a Gaussian function to the peak of the Raman spectrum. The peak position, width, and ratio of the integrated areas under *D* and *G* peaks were used to determine I_D / I_G . Table 3 summarizes the main characteristics of the Raman spectra of DLC films with various TiO_2 concentrations. The incorporation of TiO_2 nanoparticles

in DLC films result in the increase of the intensity ratio of D and G peak (I_D / I_G) and a shifting of D and G bands toward higher frequencies. The shift of the G -band peak position to higher frequencies can be attributed to the increased number of sp^2 bonds and the formation of sp^2 hybridized carbon domains [5]. These characteristics imply the increase of the graphite-like bonds in DLC matrix [13]. In TiO_2 -DLC samples, it was possible to see a third band arising from TiO_2 nanoparticles. The TiO_2 powder Raman spectrum presents a band on 1226.0cm^{-1} . When the TiO_2 nanoparticle concentration increased, the TiO_2 band shifts toward TiO_2 powder peak.

Table 3. Gaussian fitting results of Raman spectra from DLC films with various TiO_2 concentrations

TiO_2 Concentration (g/L)	D Band position (cm^{-1})	G Band position (cm^{-1})	TiO_2 Band position (cm^{-1})	I_D/I_G
0.0	1328.5	1534.1	–	1.35
0.1	1347.5	1538.1	1201.8	1.37
0.5	1377.2	1542.4	1189.3	1.39
1.0	1380.3	1543.0	1158.6	1.48

Figure 4 shows the dependence between I_D / I_G ratio and the antibacterial activity [10]. When I_D / I_G increased, the antibacterial activity also increased. Other authors have already reported this behavior when silver nanoparticles were incorporated in DLC films [2], and when the films were modified with oxygen plasma treatment [9]. The results showed that exists a strong correlation between I_D / I_G and the antibacterial activity, as shown in Figure 4 ($R^2 = 0.9952$). This means I_D / I_G ratio have a significant influence on antibacterial activity and its increasing is in favour of the bactericidal response of the films.

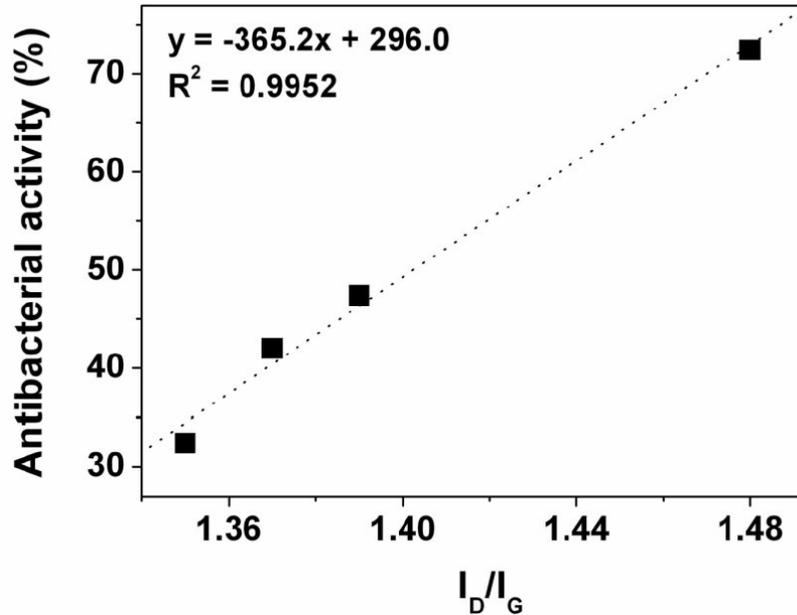


Figure 4. Antibacterial activity [10] vs. I_D / I_G ratio.

4. Conclusion

The incorporation of TiO_2 nanoparticles increased DLC I_D / I_G ratio and water contact angle. As the TiO_2 concentration in DLC films increased, its antibacterial activity increased proportionally to the increase of I_D / I_G ratio. This means I_D / I_G ratio have a significant influence on antibacterial activity and its increasing is in favour of the bactericidal response of the films. The thermodynamic approach also shows the increasing in bacterial adhesion with the increasing of TiO_2 content. The antibacterial activity of TiO_2 -DLC films is directly influenced by I_D / I_G ratio, wettability, and work of adhesion. These experimental results will be helpful to design antibacterial coatings.

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