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Influence of surface energy of modified surfaces on bacterial adhesion

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Abstract

There are a number of contrary reports on the effect of surface energy of substrates on bacterial adhesion. Some reports showed that bacterial adhesion decreased with decreasing surface energy of substrates; while other reports showed that bacterial adhesion decreased with increasing surface energy of substrates. In this study *Escherichia coli* adhesion on the Ni–P–PTFE coatings with various surface energies was investigated and the extended DLVO theory was used to calculate the interaction energy between bacteria and the substrates in water. The theory explained the effect of surface energy of substrates on bacterial adhesion.

Keywords: Surface energy; Ni–P–PTFE; Bacterial adhesion; Extended DLVO theory

1. Introduction

Biofilm or biofouling formation on the surfaces of medical devices, food processing equipment, heat exchangers, cooling water systems and ship hulls has been recognized as a widespread problem. Since bacterial adhesion is a prerequisite condition for biofilm formation, prevention of bacterial adhesion on a surface will have a major impact in preventing biofouling. Surface energy is probably the most important physicochemical property of a solid surface. When a surface is immersed in an aqueous solution, molecules or atoms at the surface tend to interact with molecules or atoms in the solution, and the types of forces or interactions depend on the chemistry of both solid and liquid [1]. Therefore the surface energy of a solid surface gives a direct measure of intermolecular or interfacial attractive forces. It would be much more desirable to modify the surface energy of processing equipment and devices to minimize microbial adhesion.

Over the past two decades, bacterial adhesion to surfaces with different surface energies has been investigated. Some reports showed that bacterial adhesion decreased with decreasing surface energy of substrates [2–7]. Fletcher et al. [2] showed that the disk-like attachment base produced on high-energy surfaces was strongly adherent and difficult to remove by gentle brushing, while the more filamentous attachment base produced on low-energy surfaces was more loosely adhered and quite easily detached. Dexter et al. [3,4] and Hamza et al. [5] demonstrated that the low number of marine bacteria were associated with low energy substrates and the high numbers of bacteria were associated with high energy substrates. Milne and Callow [6] reported that fewer bacteria adhered to a low surface energy material compared to a high surface energy material. Bakker et al. [7] showed that bacterial adhesion increased with increasing surface energy of substrates. However a number of contrary reports showed that bacterial adhesion decreased with increasing surface energy of substrates [8–12]. Bacterial adhesion mechanism is complex and many factors affect cell adhesion. In this study the extended DLVO theory was used to calculate the interaction energy between bacteria and the substrates in water. The effect of surface energy on bacterial adhesion was explained using the extended DLVO theory. An anti-bacterial Ni–P–PTFE coating with desired surface energy was developed.

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2. Experimental

2.1. Ni–P–PTFE coatings

Ni–P and Ni–P–PTFE were coated on the stainless steel 304 sheets with the dimension 10 mm × 15 mm × 0.69 mm. The composition and the plating conditions for electroless plating Ni–P and Ni–P–PTFE solutions used in the present investigation are given in Table 1. A 60% PTFE emulsion from Aldrich with particle size in the range 0.05 to 0.5 μm was used. Both the PTFE emulsion and the FC-4 surfactant were diluted with de-mineralized water and stirred for 1 h. Then the solution was filtered with a filter of pore size 0.2 μm before use. An Energy Dispersive X-ray analysis (EDX) (model JEOL T-300) was used to give element composition of electroless Ni–P–PTFE coatings.

2.2. Bacterial adhesion

Escherichia coli XA90 from the Welcome Trust Biocenter of University of Dundee was used for bacterial adhesion tests. After the frozen *E. coli* XA90 was defrosted, they were cultured in Luria-Bertani (LB) medium on solid agar plates at 36.4 °C overnight, and then colonized in 5 ml LB broth at 37 °C for 8 h. After cell culture, the required volume of bacterial suspension was put in a centrifuge tube and was centrifugalized at 5000 rpm for 5–6 min. Then the bacteria were re-suspended in 50 ml sterile deionized water to a concentration of 10^9 cells/ml. The optical density of the bacterial suspensions at 600 nm (OD_{600}) was measured to insure that the same number of bacteria were exposed to the surface in each experiment.

A standard membrane filtration method was used to quantify the number of the bacterial colonies or colonies forming units (CFU) attaching to the treated and untreated surfaces [13]. The coated and uncoated samples were exposed to the 50 ml suspension of *E. coli* XA90 on a shaker 20–30 rpm for 30 min at 22 °C, 5 h at 37 °C and 18 h at 37 °C separately. Then these samples were taken out and were put into 100 ml sterile deionized water under ultrasonic cleaning conditions for 5 min in order to remove the adhered bacteria into the water thoroughly. Then the water passed through a membrane filter with a pore size 0.45 μm and *E. coli* XA90 remained on the filter surface. When the filters were replaced on a growth medium-membrane lauryl sulphate broth (MLSB) in a petri dish, the bacteria formed a small visible colony after 24 h incubation at 44 °C. The incubation at 44 °C is to promote growth of *E. coli* and inhibit growth of other non-thermotolerant bacteria. The number of *E. coli* XA90 colonies on the filter was counted. If the water contains a large amount of bacteria, it needs to be diluted several times with sterile deionized water in order to count easily. Finally, the number of *E. coli* colonies (CFU/cm^2) on the surfaces was calculated. Each experiment was repeated a minimum of three times.

Table 1
Bath composition and operating conditions for electroless Ni–P and Ni–P–PTFE coatings

Composition	Ni–P	Ni–P–PTFE
$NiSO_4 \cdot 6H_2O$ (g/l)	25	25
$Na_3C_6H_5O_7 \cdot 2H_2O$ (g/l)	18	18
$NaH_2PO_2 \cdot H_2O$ (g/l)	30	30
CH_3COONa (g/l)	18	18
PTFE (60%) (ml/l)		0–20
Surfactant (g/l)		0–0.8
(CH2)CS (mg/l)		1
Temperature (°C)	80–95	80–95
pH	3.6–5.6	3.6–5.6

2.3. Contact angle measurements

Contact angles were obtained using a sessile drop method with a Dataphysics OCA-20 contact angle analyser. This instrument consists of a CCD video camera with a resolution of 768 × 576 pixel and up to 50 images per second, multiple dosing/micro-syringe units and a temperature controlled environmental chamber. The drop image was processed by an image analysis system, which calculated both the left and right contact angles from the shape of the drop with an accuracy of ± 0.1°. Three test liquids were used as a probe for surface free energy calculations: distilled water, diiodomethane (Sigma) and ethylene glycol (Sigma). The data for surface tension components of the test liquids are given in Table 2 [14,15]. All measurements were made at 25 °C. Untreated samples were ultrasonically cleaned in acetone, ethanol and deionized water in sequence for 5 min before contact angle measurement. Five replicate measurements were made for the contact angle experiments.

The contact angle of bacterial cells was measured on the lawns of bacteria deposited on membrane filters with pore diameter of 0.45 μm. Prior to contact angle measurement, the bacterial lawns were dried in the air to a certain state, indicated by stable water contact angles. Usually this state of drying of a microbial lawn lasts 30–60 min and indicated that only bound water is present on the surface. Five replicate measurements were made for the contact angle experiments.

2.4. Surface energy

The theory of the contact angle of pure liquids on a solid was developed nearly 200 years ago in terms of the Young equation [16]:

$$\gamma_L \cos\theta = \gamma_S - \gamma_{SL} \quad (1)$$

where γ_L is the known surface tension of the liquid, θ is the contact angle, γ_S is the surface energy of the solid and γ_{SL} is the solid/liquid interfacial energy. In order to obtain the solid surface free energy γ_S an estimate of γ_{SL} has to be obtained.

van Oss et al. [17] and van Oss and Good [18] developed an acid–base theory for surface energy calculation. The

Table 2
Test liquids and their surface tension components [14,15]

Surface tension data (mJ/m^2)	γ_L	γ_L^{LW}	γ_L^{AB}	γ_L^+	γ_L^-
Water (W), H_2O	72.8	21.8	51.0	25.5	25.5
Diiodomethane (D), CH_2I_2	50.8	50.8	0	0	0
Ethylene glycol (E), $C_2H_6O_2$	48.0	29.0	19.0	1.92	47.0

surface energy is seen as the sum of a Lifshitz-van der Waals apolar component γ_i^{LW} and a Lewis acid–base polar component γ_i^{AB}:

$$\gamma_i = \gamma_i^{LW} + \gamma_i^{AB}. \quad (2)$$

The acid–base polar component γ_i^{AB} can be further subdivided by using specific terms for an electron donor (γ_i^-) and an electron acceptor (γ_i^+) subcomponent:

$$\gamma_i^{AB} = 2\sqrt{\gamma_i^+ \gamma_i^-}. \quad (3)$$

The solid/liquid interfacial energy is then given by:

$$\gamma_{SL} = \gamma_S + \gamma_L - 2\left(\sqrt{\gamma_S^{LW} \cdot \gamma_L^{LW}} + \sqrt{\gamma_S^+ \cdot \gamma_L^-} + \sqrt{\gamma_S^- \cdot \gamma_L^+}\right). \quad (4)$$

Combining this with the Young Eq. (1), a relation between the measured contact angle and the solid and liquid surface free energy terms can be obtained:

$$\gamma_L \cdot (1 + \cos\theta) = 2\left(\sqrt{\gamma_S^{LW} \cdot \gamma_L^{LW}} + \sqrt{\gamma_S^+ \cdot \gamma_L^-} + \sqrt{\gamma_S^- \cdot \gamma_L^+}\right). \quad (5)$$

In order to determine the three surface free energy components (γ_S^{LW}, γ_S^+ and γ_S^-) of a solid, three equations are required. Therefore the contact angle values of three liquids with known surface tension components (γ_L^{LW}, γ_L^+, γ_L^-) have to be determined.

3. Experimental results

3.1. Surface energy

The element compositions of electroless Ni–P and Ni–P–PTFE coatings were measured using EDX. PTFE content in the coatings was calculated based on F element concentration. The PTFE content in the coatings increased with increasing PTFE concentration in the plating solution. Table 3 shows the element components of the coatings.

Table 3
Element components of coatings

Coatings	Chemical composition (wt.%)			
	Ni	P	PTFE	C
Ni–P–PTFE	81.4	10.7	6.7	1.2
Ni–P–PTFE	78.1	9.3	11.5	1.1
Ni–P	88.7	11.3	0	0

Table 4
Contact angles and surface energy components of Ni–P coating, Ni–P–PTFE coatings, stainless steel 304, titanium and *E. coli* XA90

Surfaces/bacteria	Contact angle θ (°)				Surface energy components (mJ/m^2)			
	θ^W	θ^{Di}	θ^{EG}	γ^{LW}	γ^+	γ^-	γ^{AB}	γ^{TOT}
Ni–P	81.3	51.3	58.1	33.55	0.07	7.21	1.42	34.97
Ni–P–PTFE (PTFE: 6.7 wt.%)	108.0	64.3	80.6	26.11	0.00	0.00	0.00	26.11
Ni–P–PTFE (PTFE: 11.5 wt.%)	117.8	71.2	87.0	21.04	0.00	0.00	0.00	21.04
SS 304	65.8	38.1	51.5	39.62	0.00	18.43	0.00	39.62
Titanium	42.0	36.5	29.4	41.32	0.04	41.14	2.57	43.89
E. coli XA90	16.9	47.9	22.7	35.37	0.16	67.33	6.56	41.93

Contact angles of the test liquids, distilled water (θ^W), diiodomethane (θ^D) and ethylene glycol (θ^{EG}) on Ni–P coating, Ni–P–PTFE coatings with various PTFE contents,

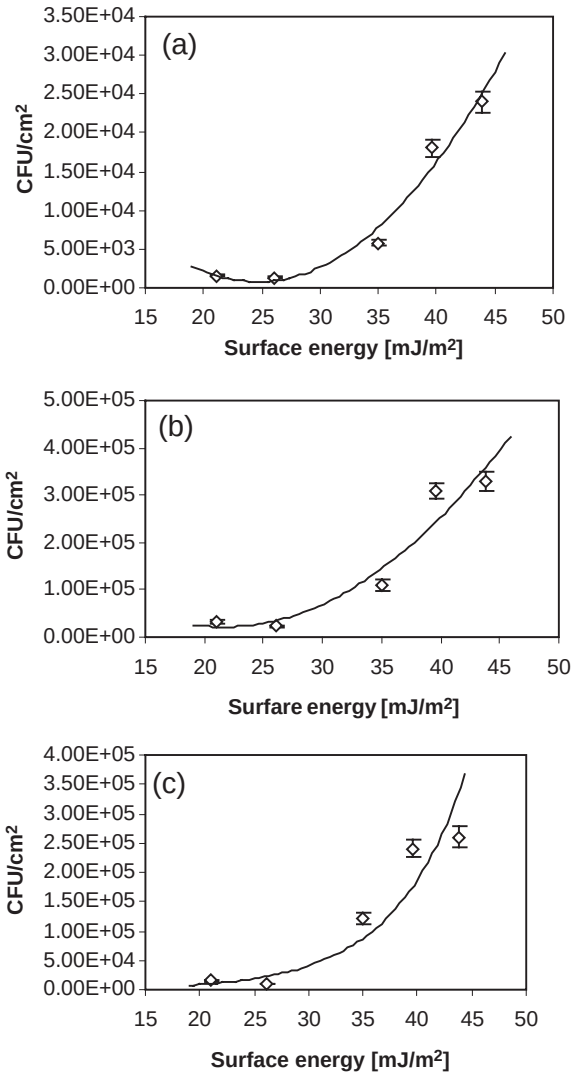

Fig. 1. Effect of surface energy on *E. coli* XA90 attachment at various contact times: (a) 30 min, (b) 5 h, (c) 18 h.

stainless steel 304, titanium, *E. coli* XA90 are given in Table 4. According to the contact angle values, the surface energies of the samples and their dispersive and polar components were calculated using van Oss acid–base approach. The results are also given in Table 4. Table 4 showed that the surface energy of Ni–P–PTFE coatings decreases significantly with increasing PTFE content in the coatings.

3.2. Bacterial adhesion

Fig. 1 shows the comparison of the number of cell colonies attached to Ni–P–PTFE coated surface (PTFE 11.5 wt.%; γ_S=21.04 mJ/m^2), Ni–P–PTFE coated surface (PTFE 6.7 wt.%; γ_S=26.11 mJ/m^2), Ni–P coated surface (γ_S=34.97 mJ/m^2), stainless steel 304 sheet (γ_S=39.62 mJ/m^2) and titanium sheet (γ_S=43.89 mJ/m^2) at various contact time. The number of cell colonies attached to the surfaces decreased with decreasing surface energy and reached minimum when the surface energy of the coatings was in the range 21–26 mJ/m^2. The Ni–P–PTFE coated surfaces performed best and reduced *E. coli* attachment by over 95%, compared with stainless steel 304. Fig. 1 also shows that the contact time has an influence on the bacterial adhesion. With increasing contact time the number of cell colonies reached to a maximum value and then slightly decreased. Bacterial adhesion mechanism is complex and many factors affect cell adhesion [19,20]. In this study the effect of surface energy on bacterial adhesion was investigated using the extended DLVO theory.

4. Discussion—bacterial adhesion mechanism

The first theory used to explain bacterial adhesion onto solid surfaces was the DLVO theory, named after four scientists, Derjaguin, Landau, Verwey and Overbeek [21,22]. van Oss proposed an extension of DLVO theory, generally known as extended DLVO theory [23]. The principle interaction forces determining hetero-coagulation by the extended DLVO theory include a Lifshitz-van der Waals (LW) attractive interaction component, an electrostatic double-layer repulsive component (EL), a Lewis acid–base component (AB), and a Brownian motion component (Br) [23]. The total interaction energy ΔE^{TOT} between a particle and a solid surface can be written as the sum of these corresponding interaction terms:

$$\Delta E^{TOT} = \Delta E^{LW} + \Delta E^{EL} + \Delta E^{AB} + \Delta E^{Br}. \quad (6)$$

Recently, Azeredo et al. [24] and Oliveira [25] suggested that the balance between all possible interactions determine whether or not the particle (or bacterium) attach on the surface: adhesion will take place when ΔE^{TOT} is negative (i.e. total interaction force is attractive).

4.1. Lifshitz-van der Waals interaction

For the interaction energy between a sphere of radius R and a flat surface, the Lifshitz-van der Waals interaction energy can be calculated using the following equation:

$$\Delta E^{LW} = -\frac{A\cdot R}{6H} \quad (7)$$

where H is the distance of separation and A is the Hamaker constant related to the properties of the interacting materials. van Oss [23] presented a very simple method for the calculation of Hamaker constant based on the surface energy of the interaction materials:

$$A_{ii} = 24\pi H_0^2 \cdot \gamma_i^{LW} \quad (8)$$

where γ_i^{LW} is the Lifshitz-van der Waals apolar component of the surface energy and H_0 is the minimum equilibrium distance between the two interacting bodies, which has been found for a large range of materials to be equal to 0.157 nm. γ_i^{LW} values of the solid surfaces and bacteria used in this study were measured and are given in Table 4. The Hamaker constant for interaction between bacteria 1 and a solid surface 2 in water 3 is given by:

$$A_{132} = \left(\sqrt{A_{11}} - \sqrt{A_{33}}\right)\left(\sqrt{A_{22}} - \sqrt{A_{33}}\right). \quad (9)$$

Combining Eq. (8) with Eq. (9), the Hamaker constant becomes:

$$A_{132} = 24\pi H_0^2\left(\sqrt{\gamma_1^{LW}} - \sqrt{\gamma_3^{LW}}\right)\left(\sqrt{\gamma_2^{LW}} - \sqrt{\gamma_3^{LW}}\right). \quad (10)$$

Combining Eq. (7) with Eq. (10), the LW interaction energy can be expressed as:

$$\Delta E^{LW} = -\frac{24\pi H_0^2\left(\sqrt{\gamma_1^{LW}} - \sqrt{\gamma_3^{LW}}\right)\left(\sqrt{\gamma_2^{LW}} - \sqrt{\gamma_3^{LW}}\right)\cdot R}{6H} \quad (11)$$

4.2. Electrostatic double-layer interaction

The electrostatic double-layer interaction term ΔE^{EL} between sphere (bacteria) 1 and flat surface 2 is given by Eq. (12) when the interaction takes place at constant surface potential φ [23–25].

$$\Delta E^{EL} = \varepsilon\pi R\Big\{(\varphi_1+\varphi_2)^2\ln[1+\exp(-\kappa H)] + (\varphi_1-\varphi_2)^2\ln[1-\exp(-\kappa H)]\Big\} \quad (12)$$

where φ_1 and φ_2 are the electrical surface potential of the spherical particle (e.g. bacteria) and the flat surface (e.g. coated surface); R is the sphere radius; ε is the electrical permittivity of the medium or solution and κ is Debye-Hûckel parameter ($1/\kappa$=1.1 nm). Since the surface potential

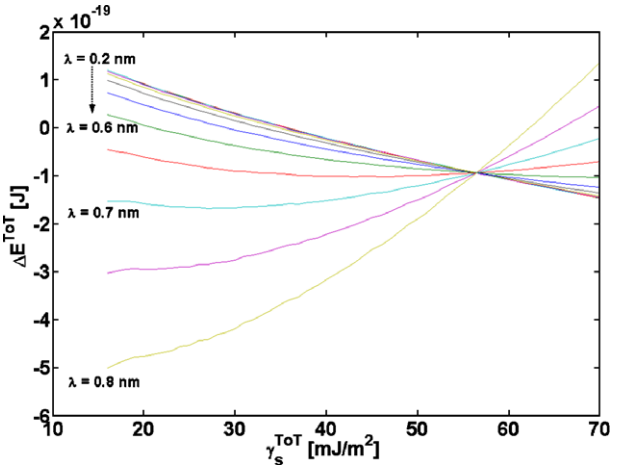

Fig. 2. Surface energy of substrates vs. interaction energy between bacteria and substrates.

φ cannot be determined experimentally, it is usually replaced by zeta potential.

4.3. Lewis acid–base interaction

van Oss [23] extended the DLVO theory by including the Lewis acid–base interaction. The contribution of ΔE^{AB} to the overall interaction energy ΔE^{TOT} between sphere (e.g. bacteria) 1 and flat surface (e.g. coated surface) 2 in liquid medium (e.g. water) 3 is given by:

$$\Delta E^{AB} = 2\pi R\lambda \Delta E_{132}^{AB} \exp\left(\frac{H_0 - H}{\lambda}\right) \quad (13)$$

where λ is the decay-length pertaining to water molecules, approximately equal to 0.2 nm for pure water [25], H_0 is the equilibrium distance and H is the distance. ΔE_{132}^{AB} is a function of the electrodonor (γ^-) and electroacceptor (γ^+) parameters of the polar component (γ^{AB}) of the surface tension of interacting bodies. It can be expressed as:

$$\Delta E_{132}^{AB} = 2\left[\sqrt{\gamma_3^+}\cdot\left(\sqrt{\gamma_1^-} + \sqrt{\gamma_2^-} - \sqrt{\gamma_3^-}\right) + \sqrt{\gamma_3^-}\cdot\left(\sqrt{\gamma_1^+} + \sqrt{\gamma_2^+} - \sqrt{\gamma_3^+}\right) - \sqrt{\gamma_1^+\cdot\gamma_2^-} - \sqrt{\gamma_1^-\gamma_2^+}\right]. \quad (14)$$

For water $\gamma_3^+ = \gamma_3^- = 25.5$ mJ/m^2.

4.4. Brownian motion

Particles (e.g. bacteria) adhering to a surface have two instead of three degrees of freedom, as one perpendicular to the surface has been blocked by bonding. Since Brownian motion comprise $(1/2)kT$ per degree of freedom, the corresponding free energy term ΔE^{Br} of a particle adhering to a surface equals $1kT = 0.414 \times 10^{-20}$ J.

$$\Delta E^{Br} = 0.414 \times 10^{-20} \text{J}. \quad (15)$$

The effect of surface energy of substrates on the total interaction energy between bacteria and the substrates in water was calculated using the above equations. The radius and zeta potential of *E. coli* were assumed to be 0.65 μm [26] and −15.4 mV [27], respectively. The distance of H and Zeta potential of the solid materials were taken as 4 nm and −25 mV in the calculation. λ is the correlation length of the molecules of the liquid medium, depending on the liquid properties. In the calculation it was in the range 0.2 nm–0.8 nm. The average surface energy of 147 types of bacteria including *E. coli* was used in the calculation ($\gamma_1^{LW} = 35.57$ mJ/m^2, $\gamma_1^+ = 1.93$ mJ/m^2, $\gamma_1^- = 37.89$ mJ/m^2) [28]. Fig. 2 shows typical calculation results on the effect of surface energy of substrates on the total interaction energy. According to the extended DLVO theory, bacterial adhesion

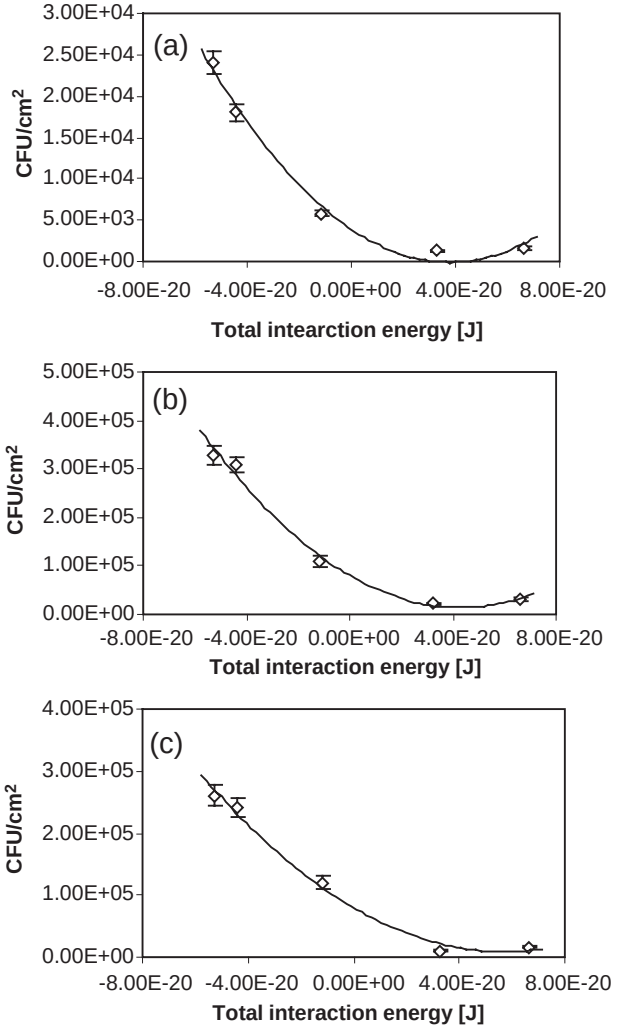

Fig. 3. Effect of total interaction energy ΔE^{TOT} on *E. coli* XA90 attachment at various contact times: (a) 30 min, (b) 5 h, (c) 18 h.

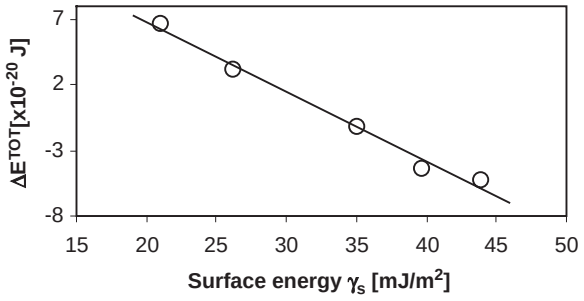

Fig. 4. Total interaction energy ΔE^{TOT} vs. surface energy γ_S.

decreases with increasing total interaction energy. Fig. 2 shows that when λ is in the range 0.2 nm–0.6 nm, total interaction energy increases with decreasing surface energy of substrates. When λ is in the range 0.6 nm–0.8 nm, total interaction energy increases with increasing surface energy of substrates. When $\lambda = 0.6$ nm, the surface energy of substrates has no significant effect on total interaction energy (or bacterial adhesion). Therefore λ (or solution characteristic) plays an important role on the effect of surface energy of substrates on interaction energy (or bacterial adhesion behaviour). For pure water, $\lambda = 0.2$ nm [25]. When solution includes higher ionic strength, λ could increase up to 13 nm [29].

The total interaction energy ΔE^{TOT} between the *E. coli* XA90 and the solid surfaces with various surface energies in water was also calculated using the above equations. In the calculation, the surface energy and the components of *E. coli* XA90 in Table 4 were used. Fig. 3 shows the effect of the total interaction energy ΔE^{TOT} on the cells' adhesion at contact time 30 min, 5 h and 18 h, respectively. The number of cell colonies attached to the surfaces decreases with increasing total interaction energy ΔE^{TOT}, which is in agreement with the extended DLVO theory. The higher the total interaction energy ΔE^{TOT}, the more repulsive the coatings to bacteria. Fig. 4 shows that the total interaction energy ΔE^{TOT} increases linearly with decreasing surface energies γ_S of the 5 surfaces and coatings which were 21.04; 26.11; 34.97; 39.62 and 43.89 mJ/m^2, respectively. According to the extended DLVO theory, the bacterial adhesion decreases with increasing total interaction energy ΔE^{TOT}. Fig. 4 further explains why the numbers of CFU decrease with decreasing surface energy γ_S. In Figs. 3 and 4, $\lambda = 0.2$ nm was used for the predictions of ΔE^{TOT} [25].

5. Conclusions

Bacterial adhesion may decrease or increase with increasing surface energy of substrates, depending on the physical and chemical properties of bacteria, substrates and water solutions. There is a correlation between the surface energy of substrates and the interaction energy of bacteria to the substrates. In this investigation the number of cell colonies attached to the surfaces decreased with decreasing surface energy or with increasing total interaction energy ΔE^{TOT}. The Ni–P–PTFE composite coatings with surface energy in the range 21–26 mJ/m^2 showed the excellent anti-microbial properties and reduced *E. coli* XA90 adhesion by 95%, compared with stainless steel 304.

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