Role of the surface nanoscale roughness of stainless steel on bacterial adhesion and microcolonies formation

Songmei Wu,[1,+,*] Stefanie Altenried,[2,+] Andi Zogg,[3] Flavia Zuber,[2] Katharina Maniura-Weber,[2] and Qun Ren[2,*]

[1] School of Science, Beijing Jiaotong University, No.3 Shangyuancun, Haidian District, Beijing 100044, P. R. China

[2] Laboratory for Biointerfaces, Empa, Swiss Federal Laboratories for Materials Science and Technology, Lerchenfeldstrasse 5, 9014 St. Gallen, Switzerland

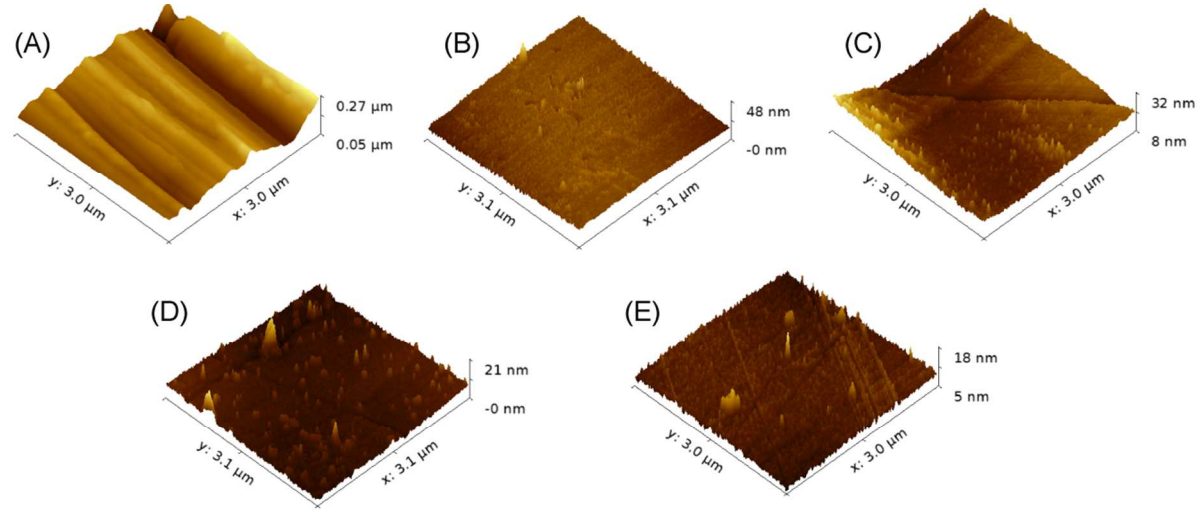
[3] HESS Medizintechnik AG, Grabenstrasse 14, 8865 Bilten, Switzerland

[+]: equal contribution

* Correspondence: smwu@bjtu.edu.cn, qun.ren@empa.ch

S1. AFM images and topographical profiles for different stainless steel surfaces at the scale of 3 μm.

(a)



(b)

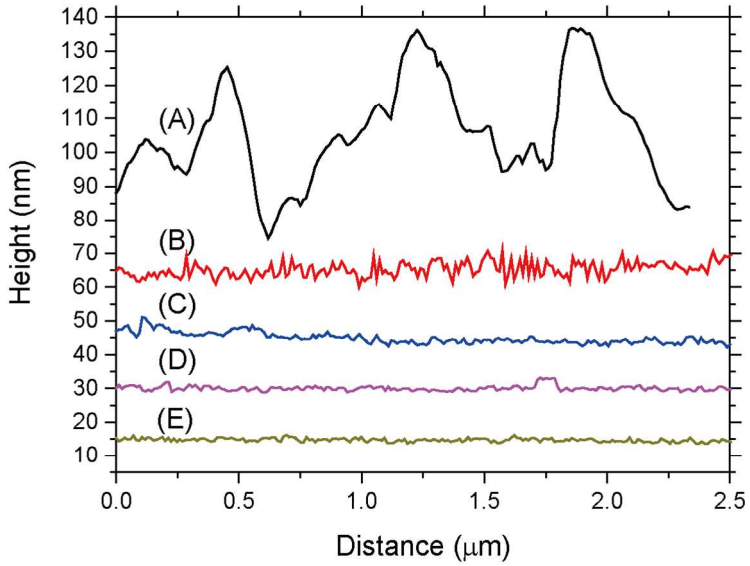


Figure S1. (a): AFM images and (b): Topographical profiles of stainless steel surfaces at the scale of 3 μm. (A) Untreated surface; (B) Electropolished surface for 20 s; (C) Electropolished surface for 60 s; (D) Electropolished surface for 120 s; (E) Electropolished surface for 240 s.

Table S1. XPS spectra of the stainless steel surfaces.

	O	C	Cr	Fe	Ni	N	Na	P	Ca	SUMME
non_polished	29.8	60.8	4.4	2.5	-	2.5	-	-	-	100.0
20sec	46.6	41.1	3.9	2.4	-	1.8	1.3	3.0	-	100.1
60sec	47.6	37.8	8.1	4.3	-	0.9	0.8	-	0.4	99.9
120sec	47.4	38.6	6.2	3.4	-	0.9	1.7	1.6	0.3	100.1
240sec	49.1	37.2	8.0	4.0	-	0.4	0.5	1.0	-	100.2

S2. Live/dead staining of bacterial cells on stainless steel showing very few dead cells.

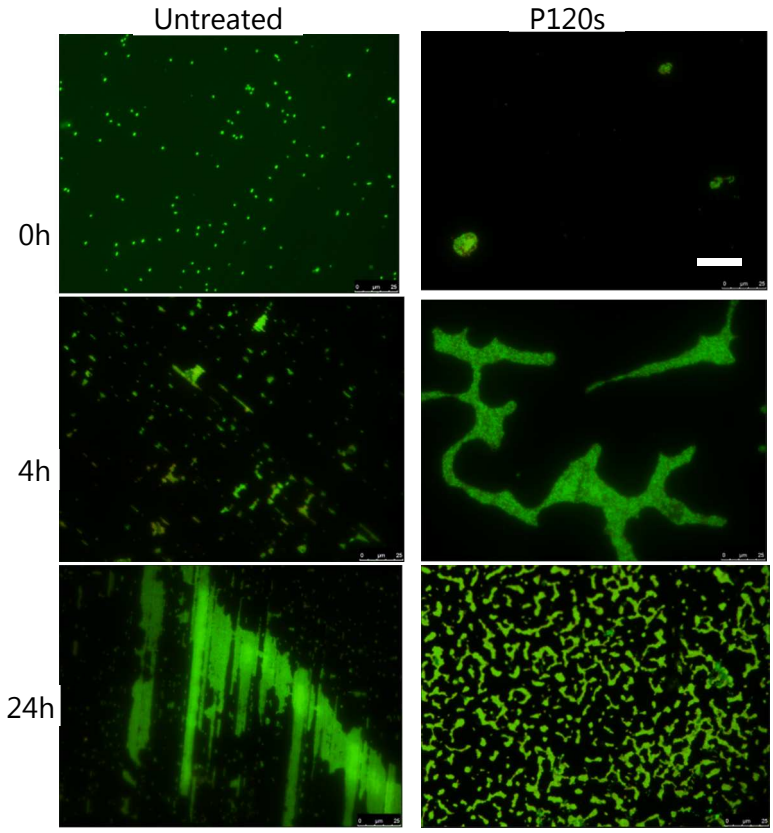


Figure S2. Representative images of *P. aeruginosa* on stainless steel surfaces (untreated and electropolised for 120s) of after 0, 4 h and 24 h incubation time and staining with Syto9 and PI. Scale bar: 25 µm.

S3. DLVO/XDLVO theory

The classical DLVO theory and extended DLVO (XDLVO) theory are recently used to estimate the total free energy of interaction between a bacterium and a flat material surface immersed in aqueous medium. The total free energy is the sum of the attractive Lifshitz van der Waals energy, the repulsive electrostatic double layer interaction energy and the Lewis acid-base interaction energy. The total interaction energy E as a function of the separation distance h can be calculated by the sphere-plate model using Derjaguin approximation.

$$E^{DLVO}(h) = E^{LW}(h) + E^{EL}(h)$$

$$E^{XDLVO}(h) = E^{LW}(h) + E^{EL}(h) + E^{AB}(h)$$

The non-retarded Lifshitz van der Waals (LW) is:

$$E^{LW}(h) = -\frac{A_H \cdot a}{6h} \quad \text{(S1)}$$

Where A_H is the effective Hamaker constant of the interaction system and given by:

$$A_H = -12\pi h_0^2 2\left(\sqrt{\gamma_L^{LW}} - \sqrt{\gamma_S^{LW}}\right)\cdot\left(\sqrt{\gamma_B^{LW}} - \sqrt{\gamma_L^{LW}}\right) \quad \text{(S2)}$$

h_0 (=0.158 nm) is the minimum separation distance between bacterium and surface due to Born repulsion, γ_L^{LW} (= 21.8 mJ/m²), γ_B^{LW}, γ_S^{LW} are the Lifshitz van der Waals (LW) surface tension component of the surrounding aqueous solution (L), bacterium (B), and surface (S), respectively. For the constant surface potential electrostatic double layer (EL) interaction energy follows:

$$E^{EL}(h) = \pi\varepsilon_r\varepsilon_0 \cdot a\left[2\Psi_B\Psi_S ln\left(\frac{1+e^{-\kappa h}}{1-e^{-\kappa h}}\right) + (\Psi_B^2 + \Psi_S^2) ln(1 - e^{-2kh})\right] \quad \text{(S3)}$$

ε_0 (= 8.854x10^{-12} F/m) is the vacuum permittivity, ε_r (= 80.1) the relative permittivity of the surrounding liquid, κ (= 1.27x10^9 m^{-1}) is the inverse Debye length (for phosphate buffer saline), ψ_B the bacterium surface charge and ψ_S the biomaterial surface charge respectively.

The Lewis acid-base (AB) interaction energy is defined as:

$$E^{AB}(h) = 2\pi a\lambda\Delta G_{h_0}^{AB} exp\left(\frac{h_0-h}{\lambda}\right) \quad \text{(S4)}$$

where λ (= 0.2 nm) is the characteristic decay length of the Lewis acid-base interaction, $\Delta G_{h_0}^{AB}$ is the Lewis acid-base component and is given by:

$$\Delta G_{h_0}^{AB} = 2\left[\sqrt{\gamma_L^+}\left(\sqrt{\gamma_B^-}+\sqrt{\gamma_S^-}-\sqrt{\gamma_L^-}\right)+\sqrt{\gamma_L^-}\left(\sqrt{\gamma_B^+}+\sqrt{\gamma_S^+}-\sqrt{\gamma_L^+}\right)-\sqrt{\gamma_B^+\gamma_S^-}-\sqrt{\gamma_B^-\gamma_S^+}\right] \quad \text{(S5)}$$

γ_L^+ (= 25.5 mJ/m²), γ_B^+, γ_S^+ are the electron-acceptor parameters of the Lewis acid-base surface tension component of the surrounding aqueous solution (L), bacterium (B) and surface (S) respectively, γ_L^- (= 25.5 mJ/m²) , γ_B^-, γ_S^- are the corresponding electron-donor components.

The parameters for a Gram-positive bacterium *S. epidermidis* are taken from literature [1]. The parameters for stainless steel are measured by zeta potential and contact angles of three liquids (deionized water, diiodomethane and glycerol) are shown below:

	Ψ (mV)	γ^{LW}	γ^+	γ^- (mJ/m^2)	a (nm)
Stainless steel	– 42.9	35.4	0.33	5.74	
S. epidermidis	– 10.0	24.8	0.01	40.0	500

[1] Siegismund, D.;Undisz, A.;Germerodt, S.;Schuster, S.; Rettenmayr, M. Quantification of the interaction between biomaterial surfaces and bacteria by 3-D modeling. *Acta Biomater* **2014**, *10*, 267-275.