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Supporting Material

Absolute quantitation of bacterial biofilm adhesion and viscoelasticity by microbead force spectroscopy

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SUPPLEMENTARY MATERIAL

TABLES

TABLE S1 Examples of quantitative bacterial adhesion studies by AFM in the literature

Subjects of Study	Probing Method	Adhesive Forces measured	Authors (Year)
Sulfate-reducing bacteria silicon nitride	Native tip on cells	3.9-4.3 nN (cell surface) 5.1-5.9 nN (cell-substratum boundary) 6.5-6.8 nN (cell-cell boundary)	Fang *et al.* (2000)
Bacillus mycoides spores glass	Cell probe on surfaces	7.4 ± 3.7 nN (hydrophilic glass) 49.4 ± 14.4 nN (hydrophobic-coated glass)	Bowen *et al.* (2002)
Pseudomonas aeruginosa *Candida parapsilosis*	Cell probe on surfaces	2. 0 ± 0.4 nN (bacterium-yeast interaction)	Emerson *et al.* (2004)
Escherichia coli silicones	Cell probe on surfaces	7.35 ± 2.31 nN (silicone) 22.75 ± 3.19 nN (fluoroalkylsilane coated silicone)	Cao *et al.* (2006)
Pseudomonas aeruginosa mica	Cell probe on surfaces	95 nN (pilus-mica rupture force)	Touhami *et al.* (2006)
Pseudomonas sp. metals	Cell probe on surfaces	5.6 ± 0.8 nN (aluminum) 2.2 ± 0.5 nN (stainless steel) 0.5 ± 0.2 nN (copper)	Sheng *et al.* (2007)
Mycobacterium bovis CH_3 surfaces	Modified tip on cells	3.032 ± 0.102 nN (CH_3)	Alsteens *et al.* (2007)
Streptococcus mutans laminin films	Modified tip on cells	5.0 nN (parent strain, pH 5.8) 4.9 nN (parent strain, pH 6.8) 1.5 nN (mutant strain, pH 5.8) 2.1 nN (mutant strain, pH 6.8)	Busscher *et al.* (2007)
Pseudomonas aeruginosa glass and organics	Colloid probe on cells	1.4 nN (wild-type to glass) 3.9 nN (B-band LPS mutant to glass) 1.7-3.5 nN (wild-type to organics) 1-2.5 nN (B-band LPS mutant to organics)	Abu-Lail *et al.* (2007)

TABLE S2 Examples of quantitative viscoelastic studies of intact biofilms in the literature

Subjects of Study	Stressing Method	Viscoelastic Properties measured	Authors (Year)
Mixed culture and *Pseudomonas aeruginosa*	Flow cell and light microscopy (shear)	Shear modulus = 27 Pa Apparent elastic modulus = 17-40 Pa Yield strength = 5.09-10.11 Pa Viscosity = $7.9 \times 10^4 - 1.9 \times 10^5$ Pa·s	Stoodley *et al.* (1999)
Pseudomonas aeruginosa	Film rheometry (compression)	Apparent elastic modulus = 6500 ± 500 Pa Yield strength = 990 ± 90 Pa	Korstgens *et al.* (2001)
Pseudomonas aeruginosa	Flow cell and light microscopy (shear)	Shear modulus = 64.67 ± 21.03Pa Viscosity = 8.0×10^3 Pa·s	Klapper *et al.* (2002)
Pseudomonas aeruginosa	Flow cell and light microscopy (shear)	Shear modulus = 1-280 Pa Viscosity = $3.6 \times 10^5 \pm 2.6 \times 10^5$ Pa·s	Stoodley *et al.* (2002)
Desulfovibrio sp.	Flow cell and light microscopy (shear)	Elastic modulus = 2.5-5 Pa Yield point ~ 0.27 m/s	Dunsmore *et al.* (2002)
Mixed culture	Rotating disk rheometry (shear)	Shear modulus = 0.2-24 Pa Viscous coefficient = 10-3000 Pa	Towler *et al.* (2003)
Streptococcus mutans	Parallel plate rheometry (shear)	Effective shear modulus = $1.9 \pm 3.8 \times 10^2$ Pa Effective viscosity = $2.8 \pm 6.4 \times 10^5$ Pa·s	Vinogradov *et al.* (2004)
Streptococcus mutans *Pseudomonas aeruginosa* Mixed species photosynthetic mats	Parallel plate rheometry (shear)	Effective shear modulus = 10^{-2}-10^6 Pa Effective viscosity = 10-10^8 Pa·s	Shaw *et al.* (2004)
Staphylococcus aureus	Flow cell and light microscopy (shear)	Shear modulus = 4.9 ± 3.7 Pa Viscosity = 3500 ± 2900 Pa·s	Rupp *et al.* (2005)
Streptococcus mutans	Microindentation Confocal microscopy (compression)	Storage modulus = 0.73-8.56 kPa Loss modulus = 5.03-10.4 kPa Viscosity = 256-2140 Pa·s	Cense *et al.* (2006)
Proteus mirabilis	Viscometry Rotational and oscillatory rheometry (shear)	Storage modulus = 900-1000 Pa Loss modulus = 125-130 Pa Viscosity = 50000 Pa·s	Lahaye *et al.* (2007b)
Nano-filtration membrane biofilm	Rotational and oscillatory rheometry (shear)	Elasticity: 3000-3500 Pa Viscosity: 800-1200 Pa	Houari *et al.* (2008)

FIGURES

FIGURE S1 Combined histogram showing frequency distribution of *Pseudomonas aeruginosa* adhesive forces to glass under standard conditions. Frequency distribution of adhesive forces is shown from six independent experiments per early biofilm sample and four independent experiments per mature biofilm sample. Each experiment consists of 10 replicate force plots at standard loading force, contact time and ramp velocity (SFTV).

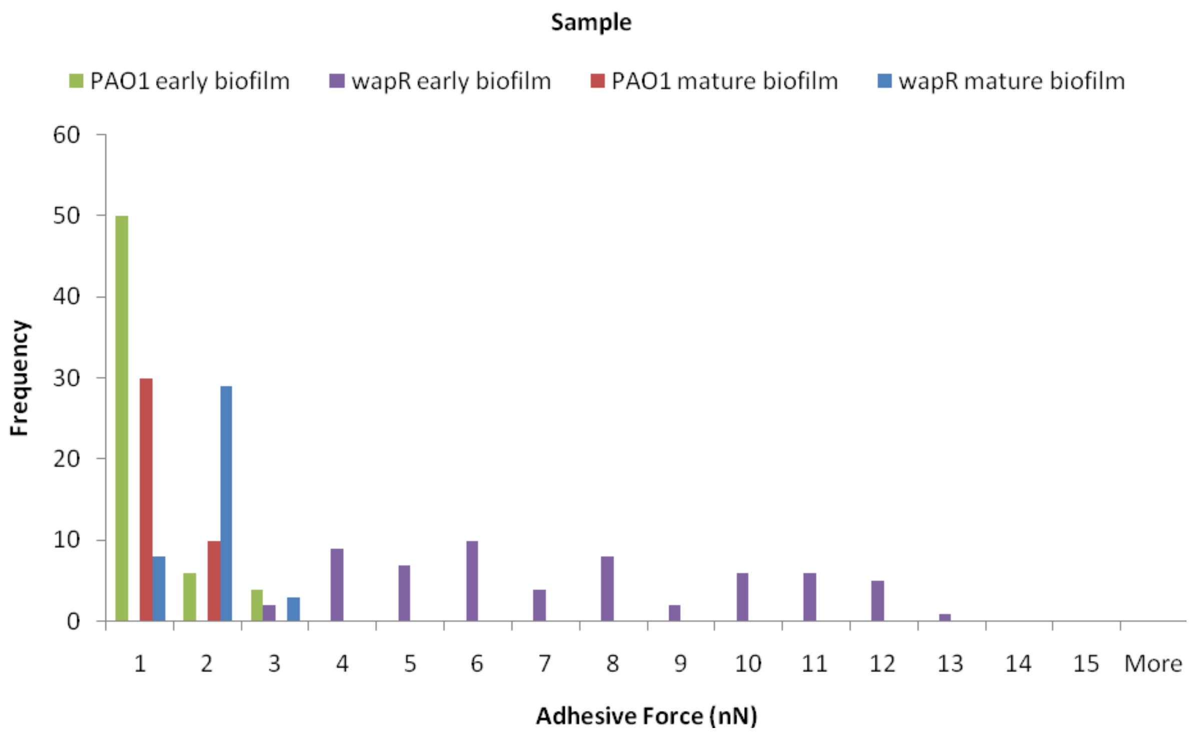
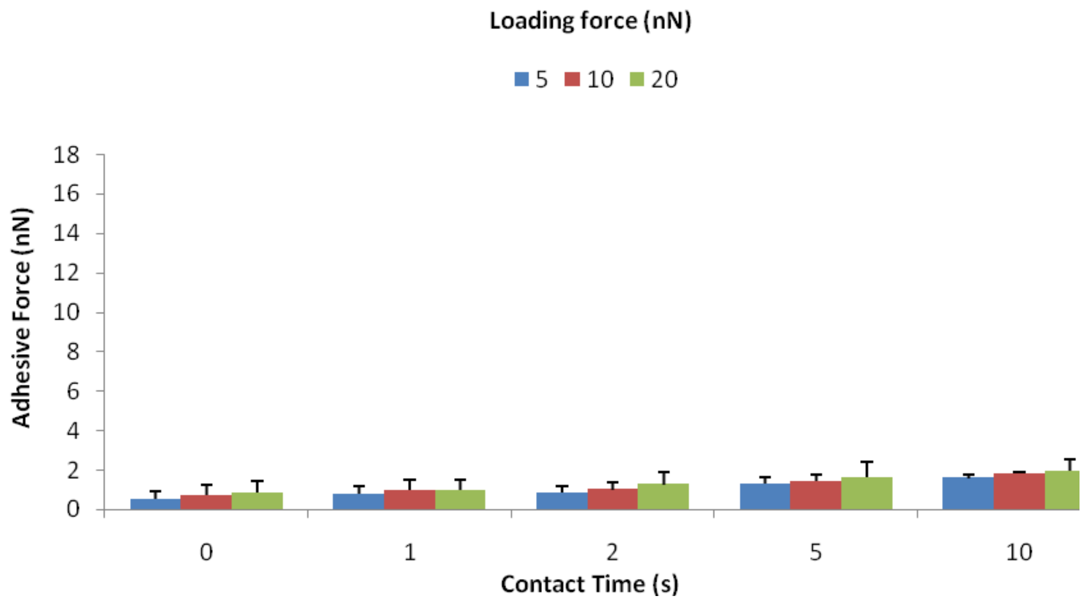


FIGURE S2 Loading force and contact time dependence of *Pseudomonas aeruginosa* adhesive force to glass. (*A*) PAO1 early biofilm. (*B*) wapR early biofilm. (*C*) PAO1 mature biofilm. (*D*) wapR mature biofilm. Means and standard errors of data are shown from three independent experiments per early biofilm sample and two independent experiments per mature biofilm sample. Each experiment consists of 10 replicate force plots for each combination of loading force and contact time.

A



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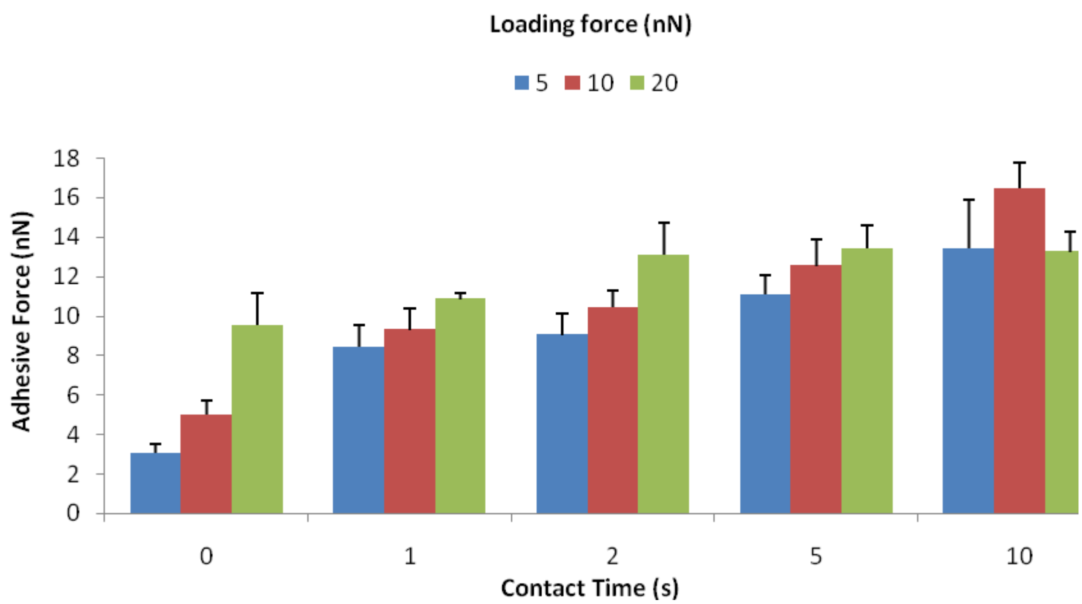

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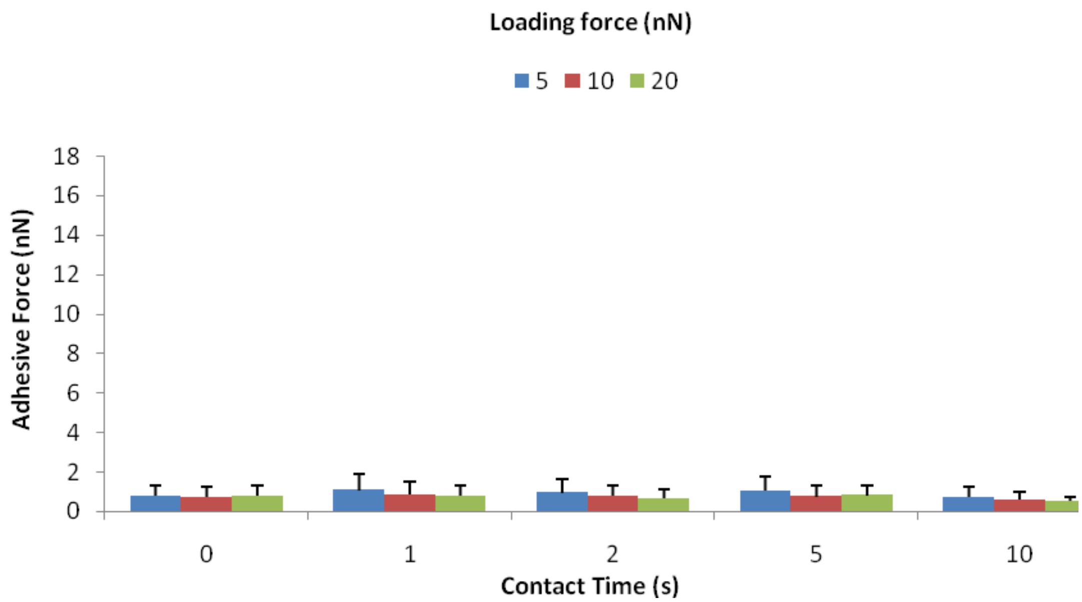

Loading force (nN)
5
10
20
Adhesive Force (nN)
18
16
14
12
10
8
6
4
2
0
0
1
2
5
10
Contact Time (s)

D

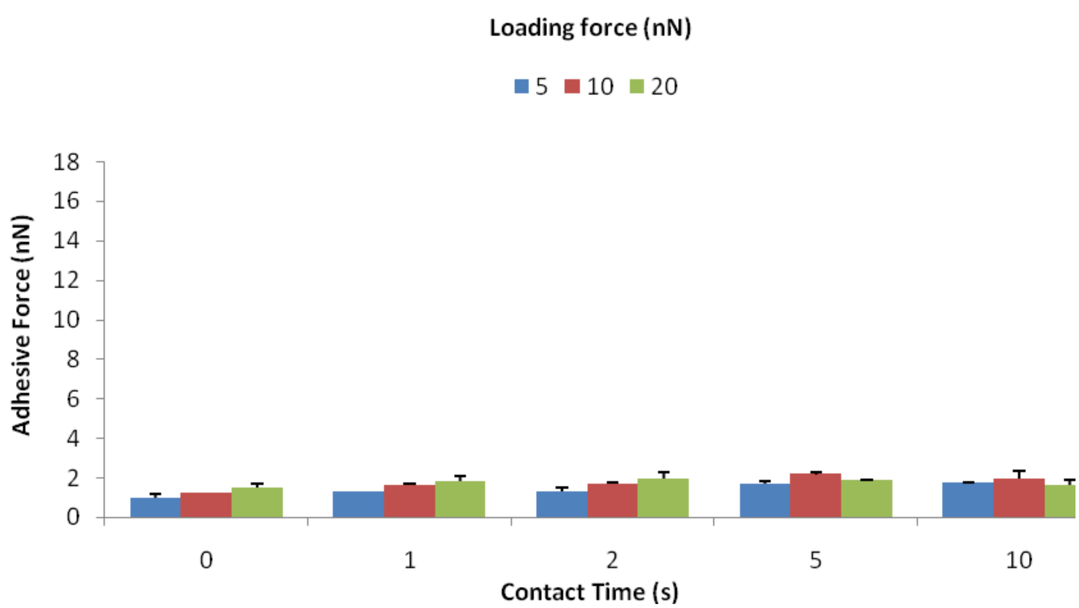
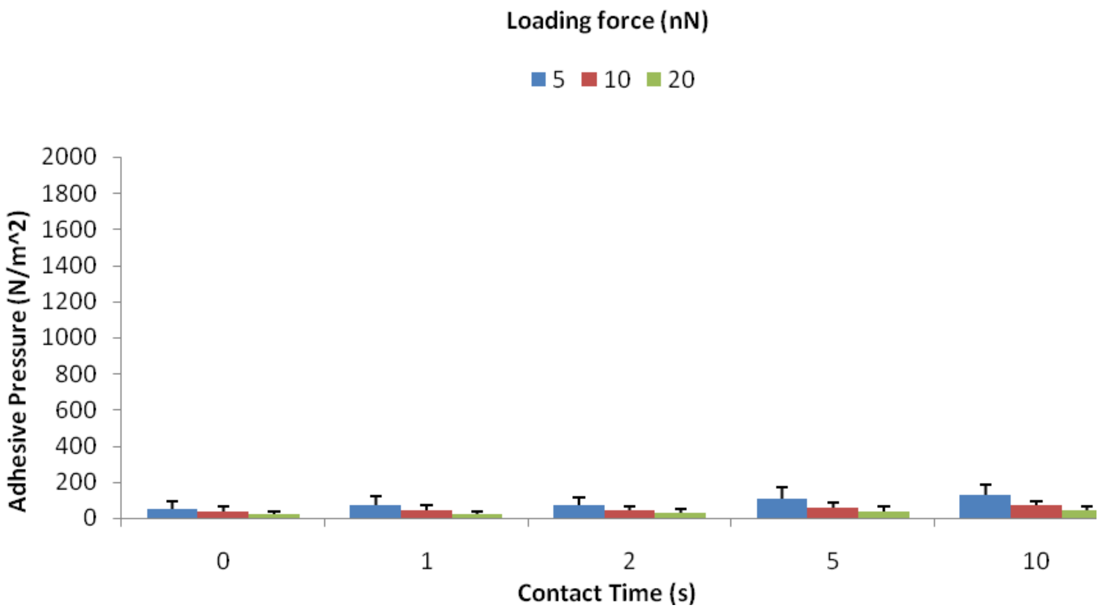
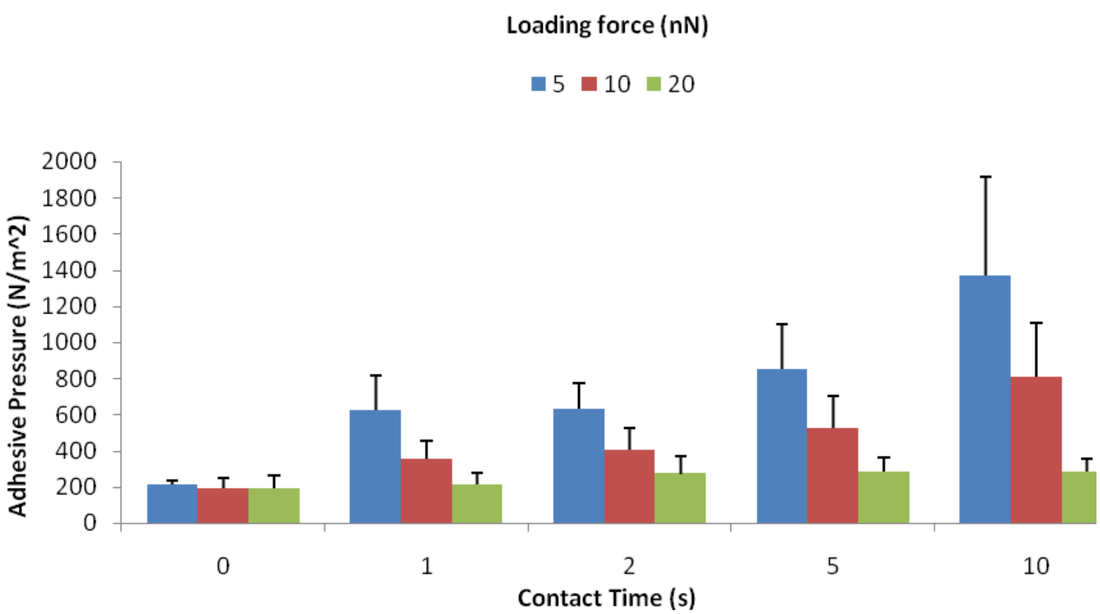

Loading force (nN)
5
10
20
Adhesive Force (nN)
18
16
14
12
10
8
6
4
2
0
0
1
2
5
10
Contact Time (s)

FIGURE S3 Loading force and contact time dependence of *Pseudomonas aeruginosa* adhesive pressure to glass. (*A*) PAO1 early biofilm. (*B*) wapR early biofilm. (*C*) PAO1 mature biofilm. (*D*) wapR mature biofilm. Means and standard errors of data are shown from three independent experiments per early biofilm sample and two independent experiments per mature biofilm sample. Each experiment consists of 10 replicate force plots for each combination of loading force and contact time.

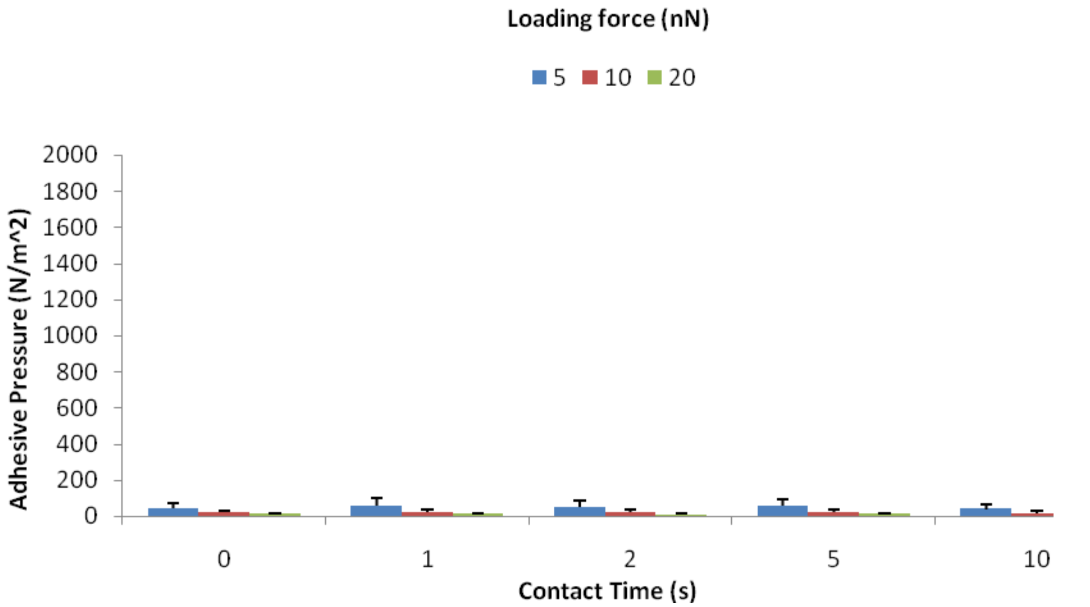
A



B



C


Loading force (nN)
5
10
20
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Adhesive Pressure (N/m^2)
0
1
2
5
10
Contact Time (s)

D

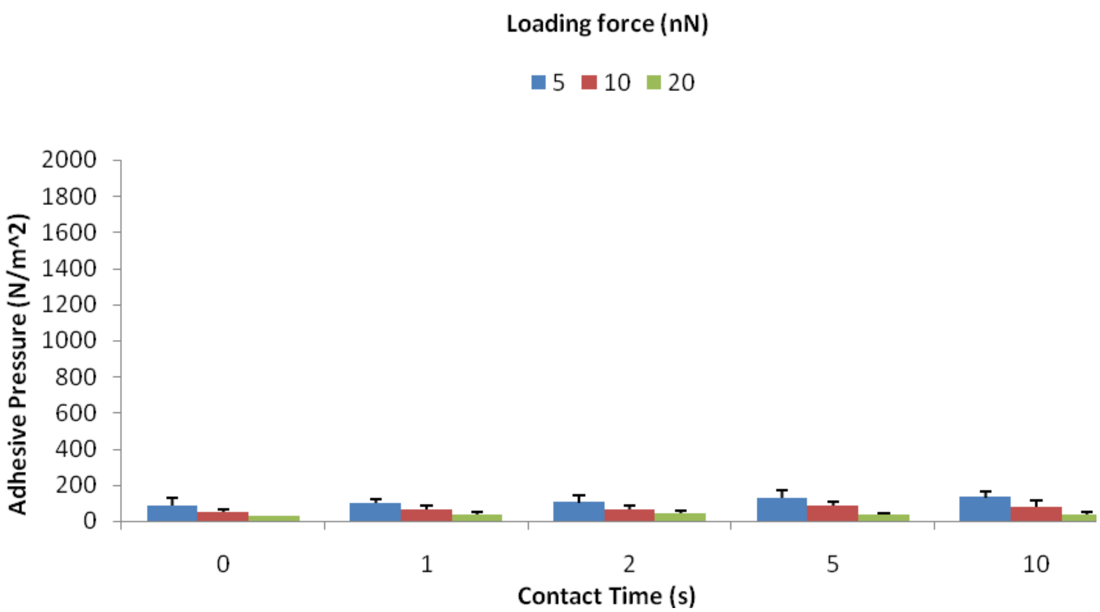
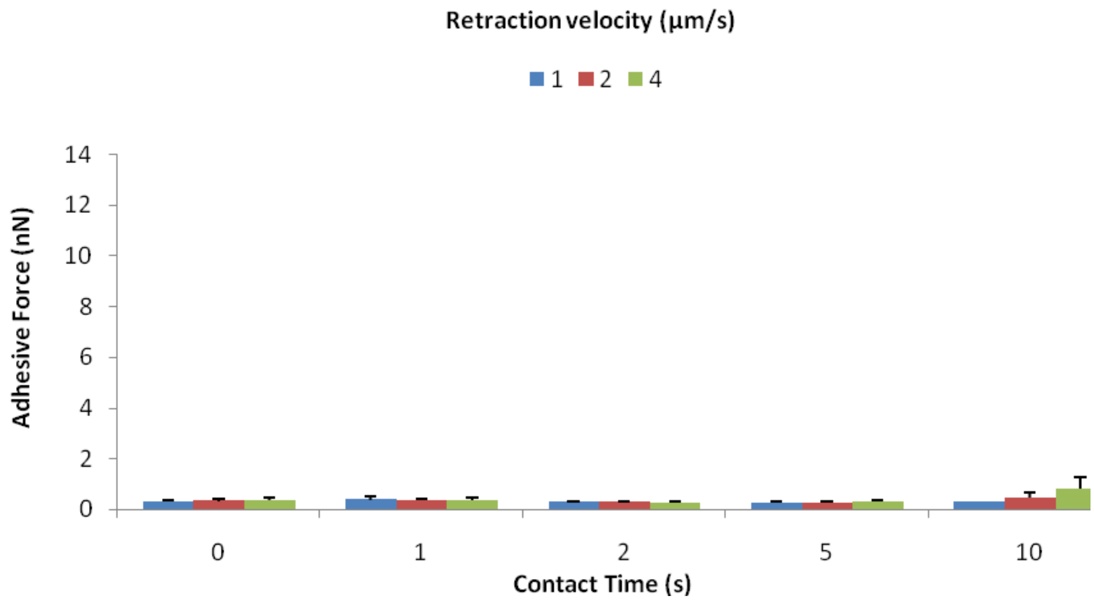

Loading force (nN)
5
10
20
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Adhesive Pressure (N/m^2)
0
1
2
5
10
Contact Time (s)

FIGURE S4 Retraction velocity and contact time dependence of *Pseudomonas aeruginosa* adhesive force to glass. (*A*) PAO1 early biofilm. (*B*) wapR early biofilm. (*C*) PAO1 mature biofilm. (*D*) wapR mature biofilm. Means and standard errors of data are shown from three independent experiments per early biofilm sample and two independent experiments per mature biofilm sample. Each experiment consists of 10 replicate force plots for each combination of retraction velocity and contact time.

A



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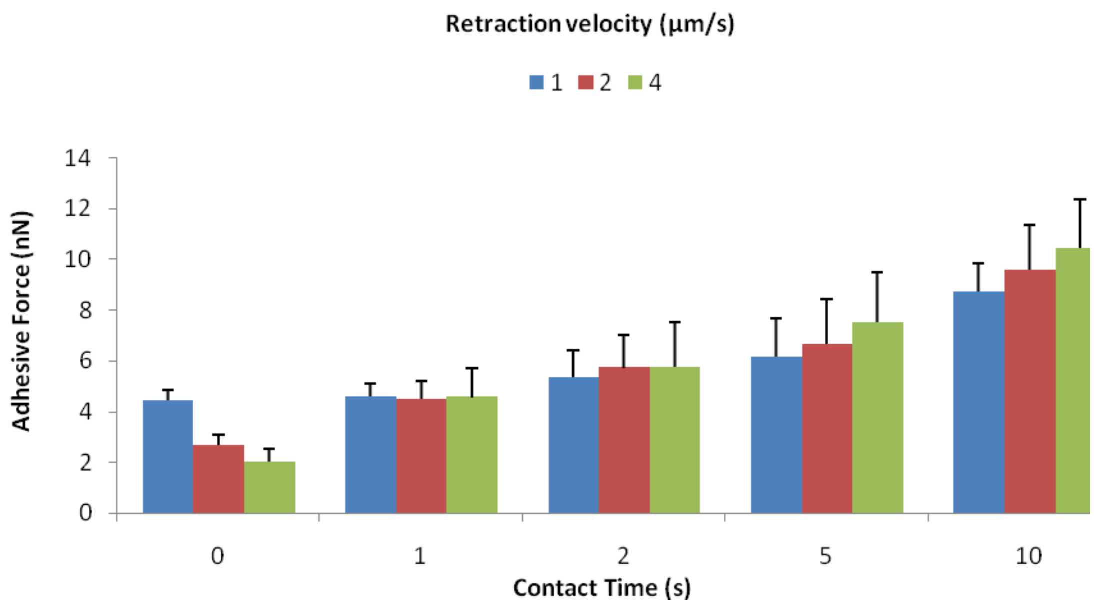
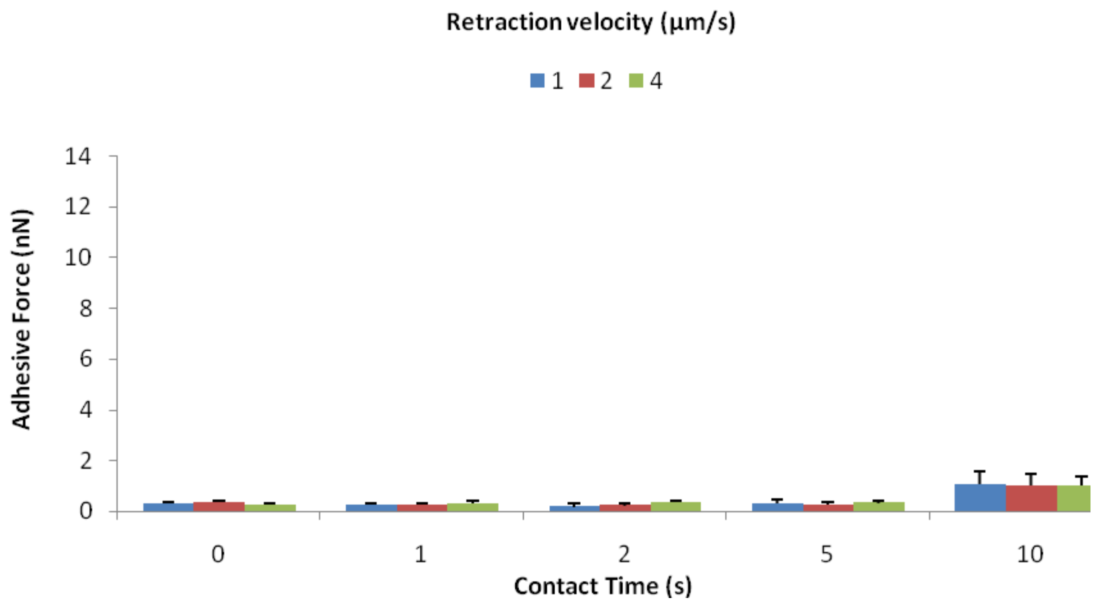


C


Retraction velocity (μm/s)
1
2
4
Adhesive Force (nN)
14
12
10
8
6
4
2
0
0
1
2
5
10
Contact Time (s)

D

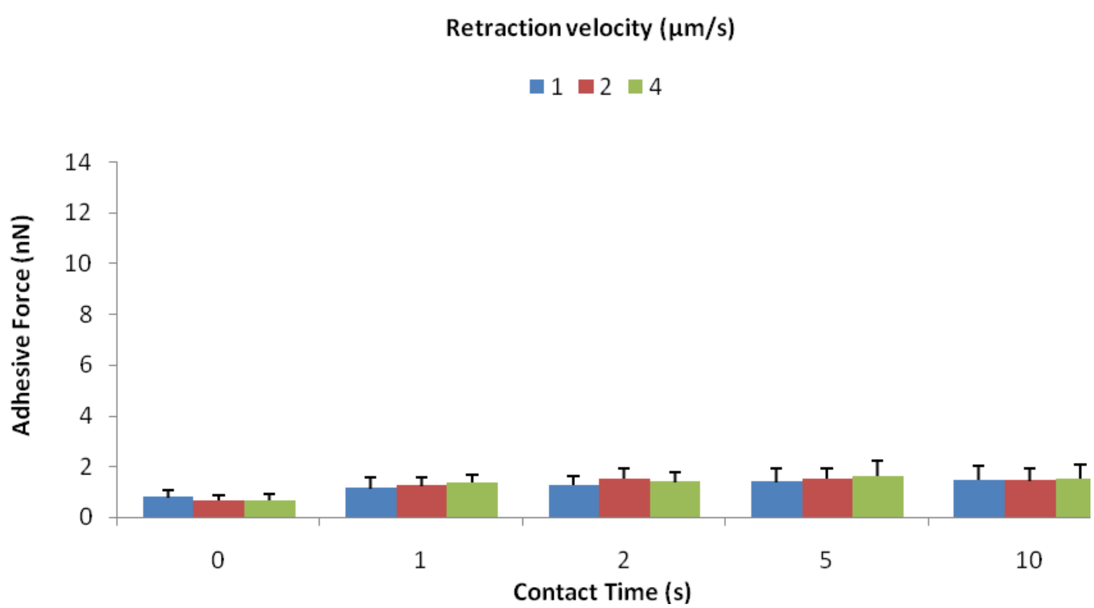
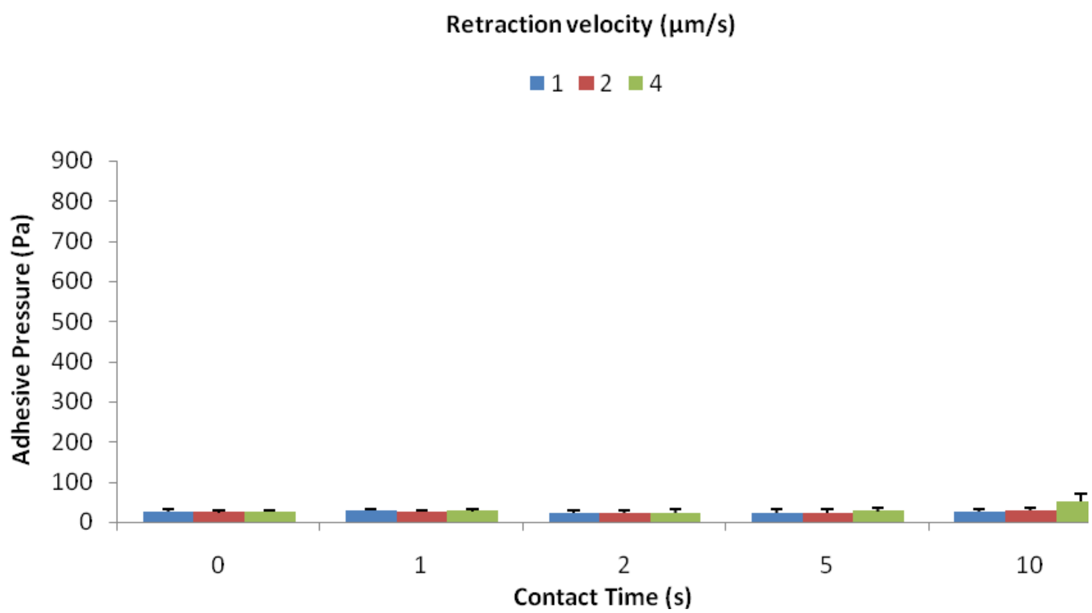
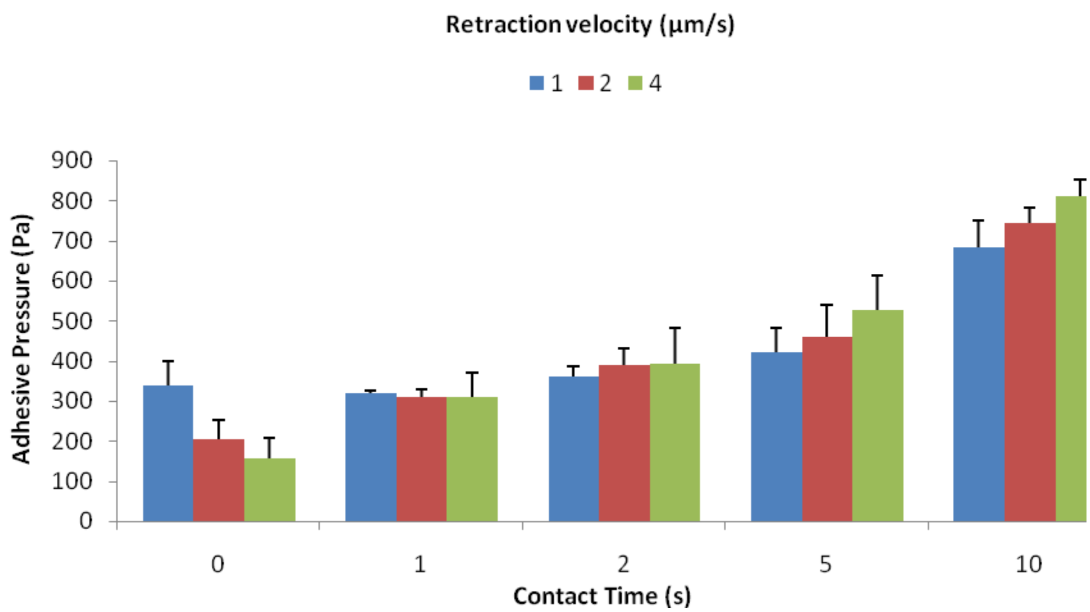

Retraction velocity (μm/s)
1
2
4
Adhesive Force (nN)
14
12
10
8
6
4
2
0
0
1
2
5
10
Contact Time (s)

FIGURE S5 Retraction velocity and contact time dependence of *Pseudomonas aeruginosa* adhesive pressure to glass. (*A*) PAO1 early biofilm. (*B*) wapR early biofilm. (*C*) PAO1 mature biofilm. (*D*) wapR mature biofilm. Means and standard errors of data are shown from three independent experiments per early biofilm sample and two independent experiments per mature biofilm sample. Each experiment consists of 10 replicate force plots for each combination of retraction velocity and contact time.

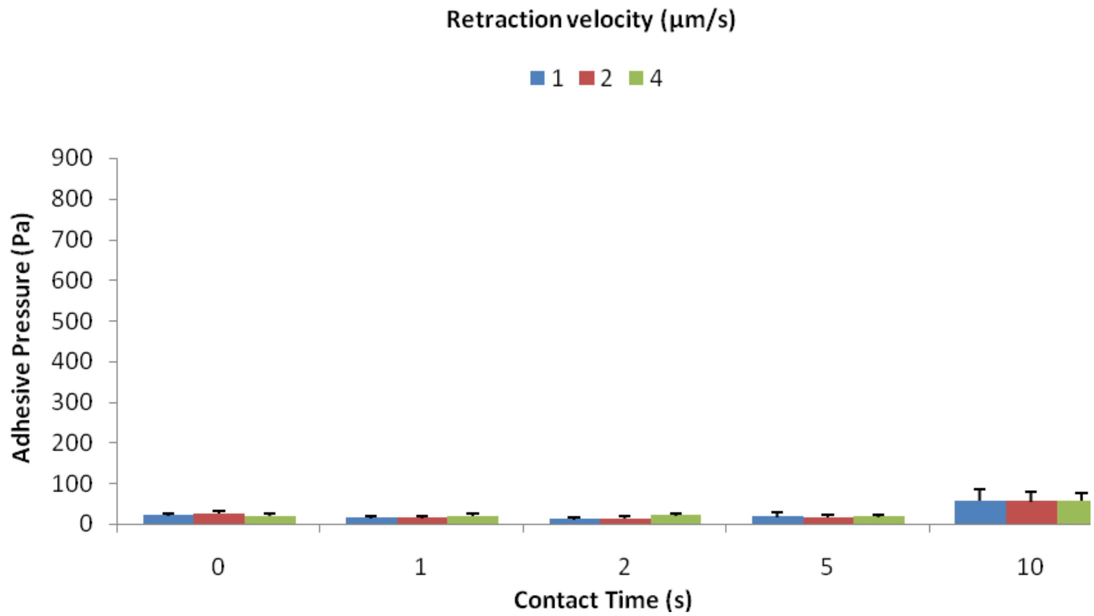
A



B



C


Retraction velocity (µm/s)
1
2
4
900
800
700
600
500
400
300
200
100
0
Adhesive Pressure (Pa)
0
1
2
5
10
Contact Time (s)

D

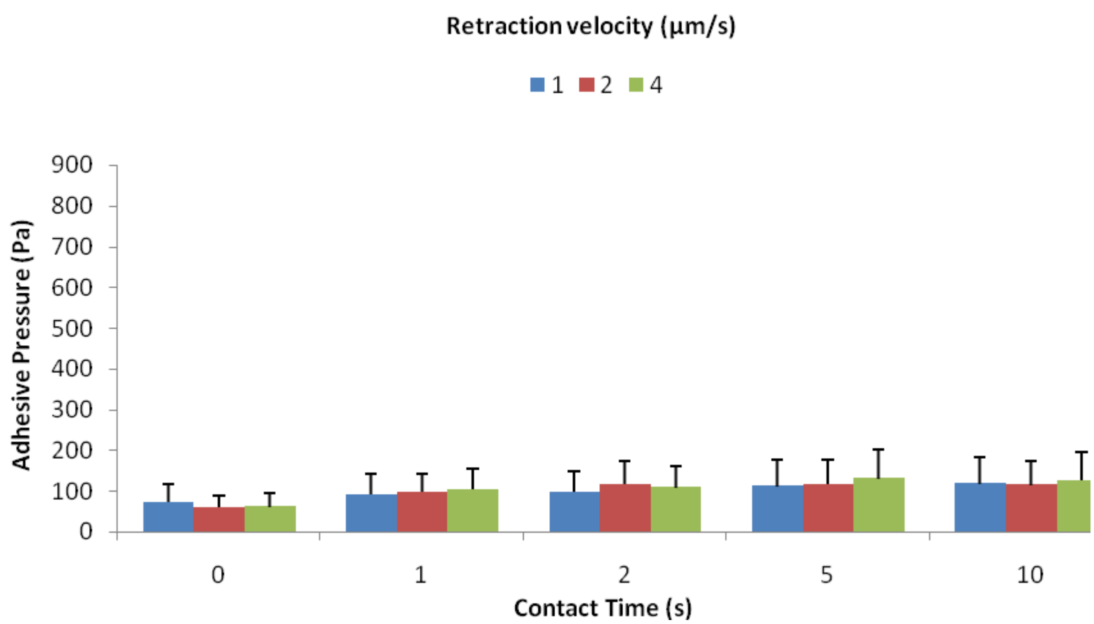
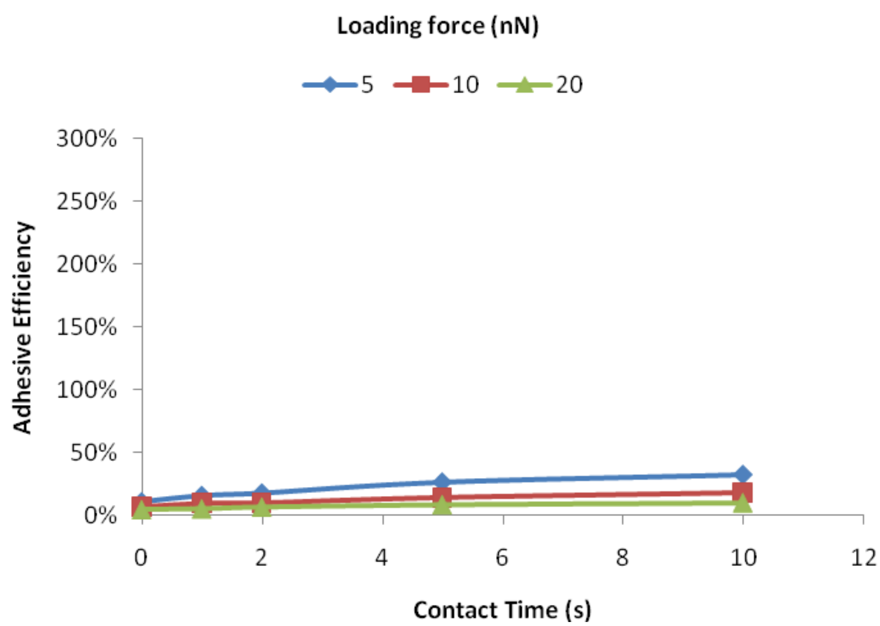
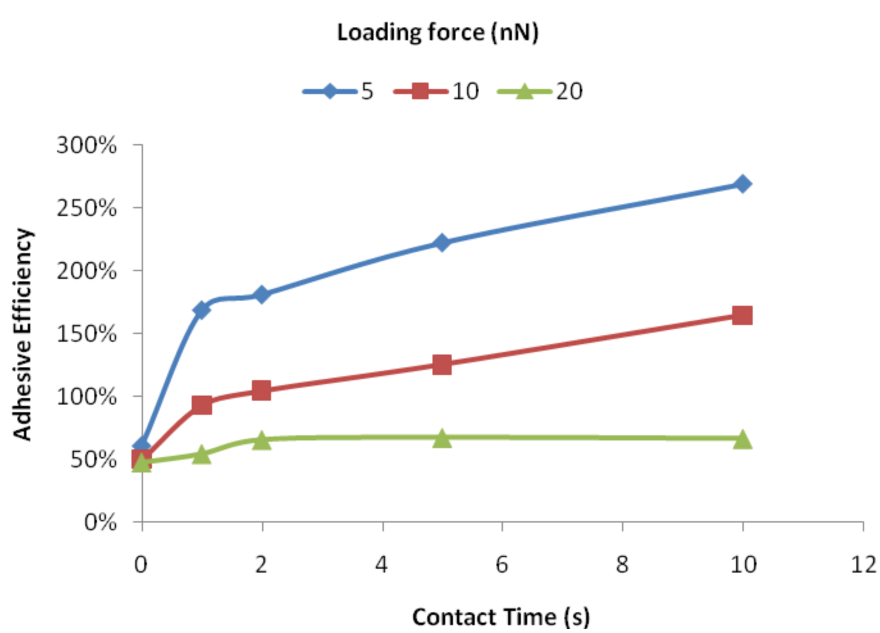

Retraction velocity (µm/s)
1
2
4
900
800
700
600
500
400
300
200
100
0
Adhesive Pressure (Pa)
0
1
2
5
10
Contact Time (s)

FIGURE S6 Loading force and contact time dependence of *Pseudomonas aeruginosa* adhesive efficiency to glass. (*A*) PAO1 early biofilm. (*B*) wapR early biofilm. (*C*) PAO1 mature biofilm. (*D*) wapR mature biofilm. Means of data are shown from three independent experiments per early biofilm sample and two independent experiments per mature biofilm sample. Each experiment consists of 10 replicate force plots for each combination of loading force and contact time.

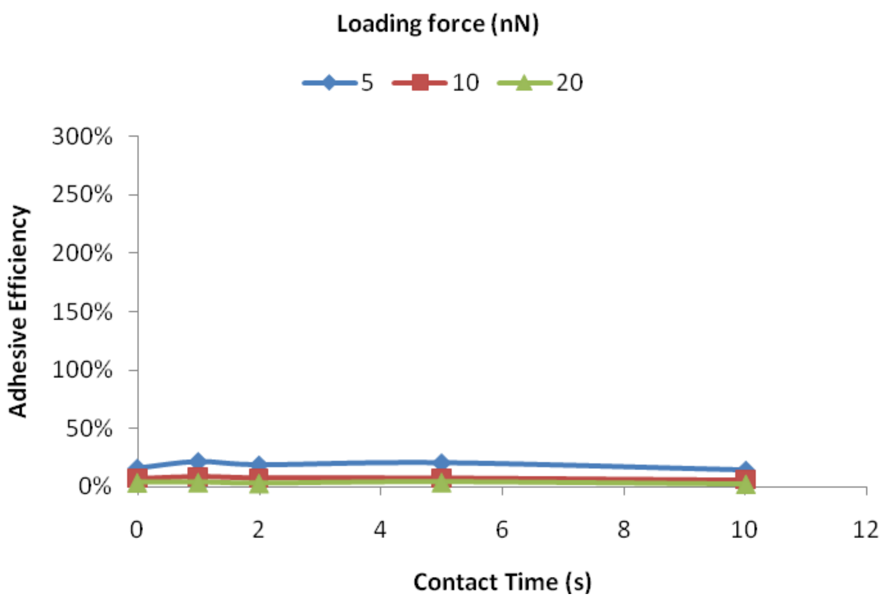
A



B



C


Loading force (nN)
5
10
20
300%
250%
200%
150%
100%
50%
0%
Adhesive Efficiency
0
2
4
6
8
10
12
Contact Time (s)

D

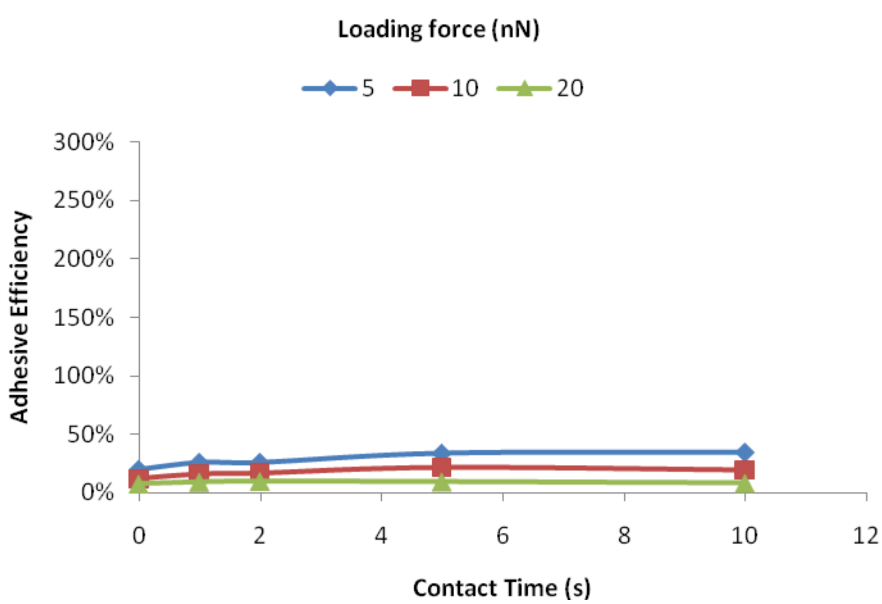

Loading force (nN)
5
10
20
300%
250%
200%
150%
100%
50%
0%
Adhesive Efficiency
0
2
4
6
8
10
12
Contact Time (s)

FIGURE S7 Comparison of instantaneous indentations of *Pseudomonas aeruginosa* biofilms at SFTV. Means and standard errors of data are shown from two independent experiments per sample. Each experiment consists of ten replicate force plots under standard load force (10 nN), contact time (1 s) and ramp velocity (2 μm/s). Indentation data were extracted from the resulting creep curves. PAO1-ebf: PAO1 early biofilm; wapR-ebf: wapR early biofilm; PAO1-mbf: PAO1 mature biofilm; wapR-mbf:wapR mature biofilm.

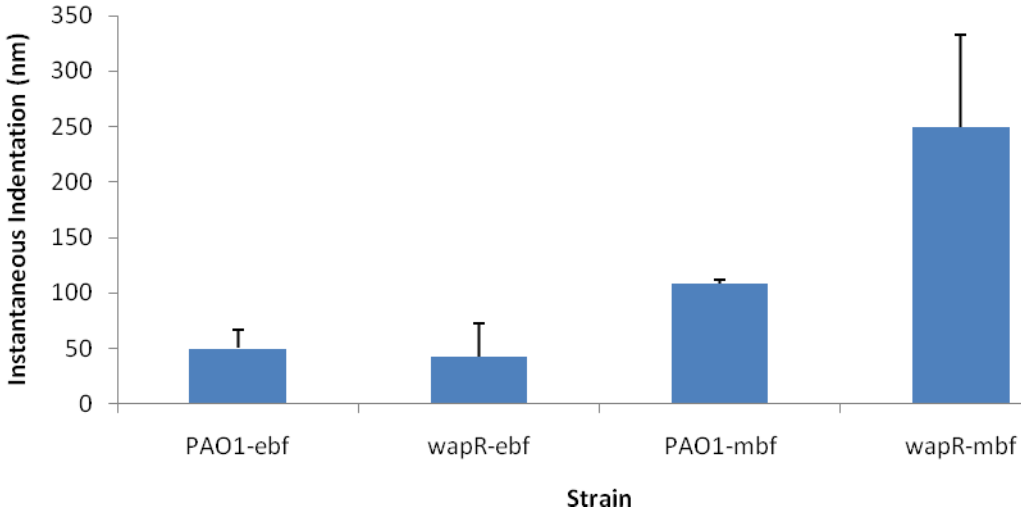


FIGURE S8 Comparison of creep indentations of *Pseudomonas aeruginosa* biofilms at SFTV. Means and standard errors of data are shown from two independent experiments per sample. Each experiment consists of ten replicate force plots under standard load force (10 nN), contact time (1 s) and ramp velocity (2 µm/s). Indentation data were extracted from the resulting creep curves. PAO1-ebf: PAO1 early biofilm; wapR-ebf: wapR early biofilm; PAO1-mbf: PAO1 mature biofilm; wapR-mbf: wapR mature biofilm.