Supporting Information

Bacterial Biofilm Material Properties Enable Removal and Transfer by Capillary Peeling

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Table S1. Summary of measured rheological properties of *V. cholerae* biofilms.

V. cholerae Strain	Agar Conc.[a)]	Parameters			Comments
		G'_P/kPa	ε_Y	σ_Y/kPa	
WT	0.6%	1.11 ± 0.16	0.13 ± 0.05	0.10 ± 0.03	With dual networks
	0.8%	1.16 ± 0.30	0.15 ± 0.00	0.12 ± 0.03	
	1.0%	1.12 ± 0.18	0.15 ± 0.07	0.14 ± 0.07	
	1.5%	1.38 ± 0.07	0.17 ± 0.03	0.17 ± 0.03	
Δ*rbmA*	0.6%	0.59 ± 0.12	0.18 ± 0.02	0.08 ± 0.02	With crosslinked polymer network only
	0.8%	0.54 ± 0.10	0.20 ± 0.02	0.09 ± 0.01	
	1.0%	0.42 ± 0.11	0.27 ± 0.04	0.09 ± 0.01	
	1.5%	0.70 ± 0.04	0.27 ± 0.03	0.15 ± 0.01	
Δ*bap1* Δ*rbmC*	0.6%	0.76 ± 0.15	0.10 ± 0.01	0.05 ± 0.02	With cellular network only
	0.8%	1.14 ± 0.05	0.09 ± 0.02	0.08 ± 0.01	
	1.0%	1.30 ± 0.12	0.13 ± 0.01	0.13 ± 0.01	
	1.5%	1.63 ± 0.12	0.18 ± 0.02	0.21 ± 0.02	
Δ*rbmA* Δ*bap1* Δ*rbmC*	0.6%	0.11 ± 0.02	0.38 ± 0.01	0.03 ± 0.00	With non-crosslinked polymer network only
	0.8%	0.20 ± 0.02	0.36 ± 0.01	0.05 ± 0.00	
	1.0%	0.31 ± 0.04	0.35 ± 0.02	0.07 ± 0.00	
	1.5%	0.69 ± 0.05	0.28 ± 0.01	0.14 ± 0.01	
Δ*vpsL*	0.6%	0.23 ± 0.10	0.15 ± 0.02	0.02 ± 0.01	No polymer network, accessory matrix proteins nonfunctional
	0.8%	0.43 ± 0.12	0.13 ± 0.00	0.04 ± 0.01	
	1.0%	0.67 ± 0.10	0.13 ± 0.00	0.06 ± 0.01	
	1.5%	1.13 ± 0.25	0.15 ± 0.02	0.12 ± 0.04	
Δ*rbmA* Δ*vpsL*	0.6%	0.14 ± 0.05	0.12 ± 0.02	0.01 ± 0.00	No polymer network, accessory matrix proteins nonfunctional
	0.8%	0.29 ± 0.01	0.11 ±0.02	0.02 ± 0.00	
	1.0%	0.45 ± 0.08	0.12 ± 0.01	0.03 ± 0.01	
	1.5%	1.04 ± 0.13	0.13 ± 0.00	0.10 ± 0.04	
Δ*rbmA* Δ*bap1* Δ*rbmC* Δ*vpsL*	0.6%	0.12 ± 0.06	0.13 ± 0.05	0.01 ± 0.00	No polymer network, no accessory matrix proteins
	0.8%	0.23 ± 0.06	0.11 ± 0.02	0.02 ± 0.00	
	1.0%	0.36 ± 0.09	0.12 ± 0.02	0.04 ± 0.01	
	1.5%	0.90 ± 0.24	0.14 ± 0.03	0.11 ± 0.01	

a) Abbreviation for agar concentration in the substrate.

Table S2. Summary of measured contact angles for surface energy calculations.

V. cholerae Strain	Agar Conc.[d)]			Liquid		
		H_2O	CH_2I_2	1-Br-NP[e)]	1-Me-NP[f)]	CH_2Br_2
WT	0.6%	109 ± 4°	54 ± 2°	35 ± 4°	27 ± 2°	16 ± 2°
WT	0.8%	105 ± 3°	57 ± 1°	35 ± 1°	28 ± 1°	14 ± 3°
WT	1.0%	111 ± 10°	56 ± 3°	35 ± 4°	26 ± 1°	18 ± 2°
WT	1.5%	105 ± 6°	59 ± 4°	42 ± 3°	28 ± 1°	17 ± 3°
Δ*bap1*[a)]	1.5%	31 ± 4°	76 ± 4°	N/A	N/A	N/A
Δ*bap1*Δ*rbmC*[b)]	1.5%	11 ± 1°	70 ± 4°	N/A	N/A	N/A
Δ*vpsL*[c)]	1.5%	21 ± 1°	68 ± 2°	N/A	N/A	N/A

a) Strain lacking a key surface-active matrix protein.

b) Strain lacking two key surface-active matrix proteins.

c) Strain lacking the key matrix polysacharride.

d) Abbreviation for agar concentration in the substrate.

e) Abbreviation for 1-bromonaphthalene.

f) Abbreviation for 1-methylnaphthalene.

Table S3. Bacterial strains and plasmids used in this study.

Strains/plasmids	Relevant Features	Reference
E. coli		
S17 λ-*pir*	Wild Type	[1]
DH5α	F⁻ *endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20* φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*) U169, hsdR17($r_K^-m_K^+$), λ⁻	Laboratory stock
SM10λ*pir*	*thi thr leu tonA lacY supE recA*::RP4-2-Tc::Mu	Laboratory stock
V. cholerae		
C6706*str*2	El Tor Wild Type	[2]
JY283	*vpvC*^{W240R} Δ*pomA* (denoted WT)	[3]
JY284	*vpvC*^{W240R} Δ*pomA*Δ*rbmA*	[3]
JY285	*vpvC*^{W240R} Δ*pomA*Δ*bap1*Δ*rbmC*	[3]
JY286	*vpvC*^{W240R} Δ*pomA*Δ*rbmA*Δ*bap1*Δ*rbmC*	[3]
JY287	*vpvC*^{W240R} Δ*pomA*Δ*vpsL*	[3]
JY288	*vpvC*^{W240R} Δ*pomA*Δ*rbmA*Δ*vpsL*	This study
JY290	*vpvC*^{W240R} Δ*pomA*Δ*rbmA*Δ*bap1*Δ*rbmC*Δ*vpsL*	This study
JY370	*vpvC*^{W240R} Δ*pomA lacZ*:P_{tac}-*mKate2*:*lacZ*	[3]
JY393	*vpvC*^{W240R} Δ*pomA*Δ*rbmC*	[3]
JY400	*vpvC*^{W240R} Δ*pomA*Δ*bap1*	[3]
P. aeruginosa		
UCBPP-PA14	Wild Type	Laboratory stock
SM404	Δ*pelA*	[4]
SM1141	ΔP_{pelA}::$P_{hyspank}$-*pelABCDEFG*	This study
Plasmid		
pKAS32	Suicide vector, Amp^R Sm^S	[5]
pNUT144	Suicide vector, Amp^R Kan^R Sm^S	[6]
pNUT157	pNUT144 *vpvC*^{W240R}	[6]
pCMW112	pKAS32 Δ*vpsL*	[7]
pCN004	pKAS32 *lacZ*:P_{tac}-*mKate2*:*lacZ*	[8]
pCN007	pKAS32 Δ*rbmA*	[9]
pCN008	pKAS32 Δ*rbmC*	[9]
pCN009	pKAS32 Δ*bap1*	[3]
pCDN010	pKAS32 Δ*pomA*	[9]
pEXG2	Allelic exchange vector with pBR origin, gentamicin resistance, *sacB*	[10]

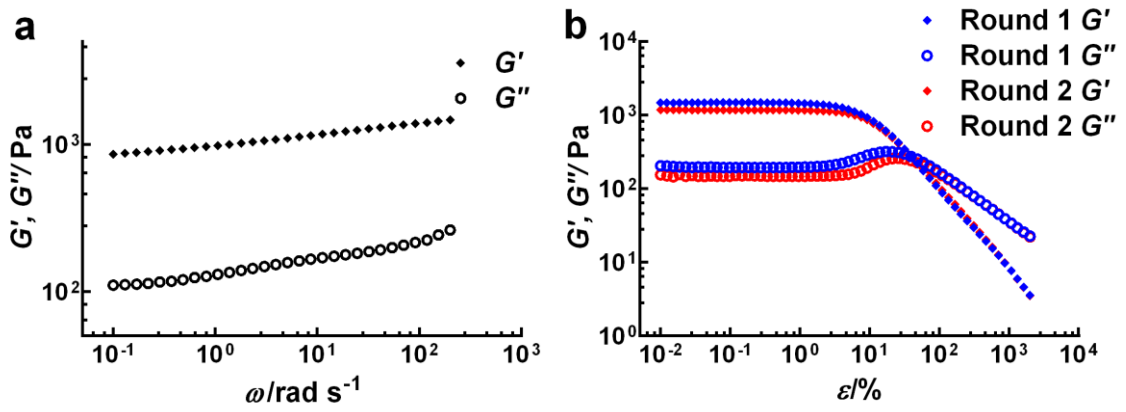

Figure S1. *V. cholerae* biofilms behave as hydrogels. a) Storage modulus G' (filled diamonds) and loss modulus G'' (open circles) of WT *V. cholerae* biofilms grown for two days on plates with 0.6% agar, as a function of frequency ω, measured in a parallel-plate geometry. b) Storage modulus G' and loss modulus G'' of the same *V. cholerae* biofilm samples as a function of the amplitude of oscillatory shear strain ε. The red curves were measured immediately after the blue curves. Irreversible structural changes during yielding cause a modest decrease in both G' and G'' in the second round of measurements.

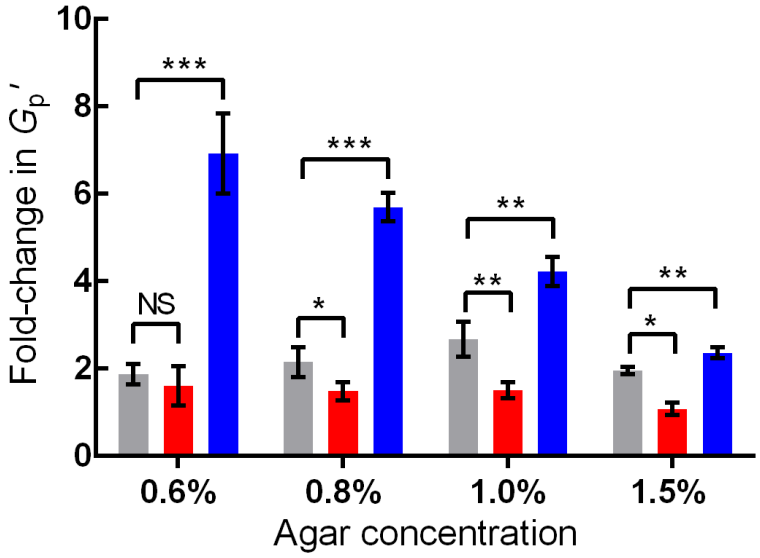

Figure S2. RbmA-mediated cell-cell connections strengthen biofilms in a VPS and RbmC/Bap1-dependent manner. Plotted are fold-changes in G'_p in biofilms made of cells possessing *rbmA* compared to those lacking *rbmA* that are otherwise WT (gray), Δ*vpsL* (red), and Δ*bap1*Δ*rbmC* (blue). The bacterial strains were grown on plates with the designated agar concentrations. NS stands for not significant; * denotes $P < 0.05$, ** denotes $P < 0.01$, *** denotes $P < 0.001$. Error bars correspond to standard deviations with $n = 3$. In the absence of VPS, RbmA cannot mediate cell-cell connections to increase G'_p (i.e. red bars are not significantly different from a value = 1). This result is consistent with previous microscopy results showing that the retention of RbmA in a *V. cholerae* biofilm requires VPS.[11] On the other hand, in the absence of RbmC/Bap1, the strengthening effect of RbmA-mediated cell-cell connections is amplified compared to its strengthening effect in the WT (compare blue to gray bars).

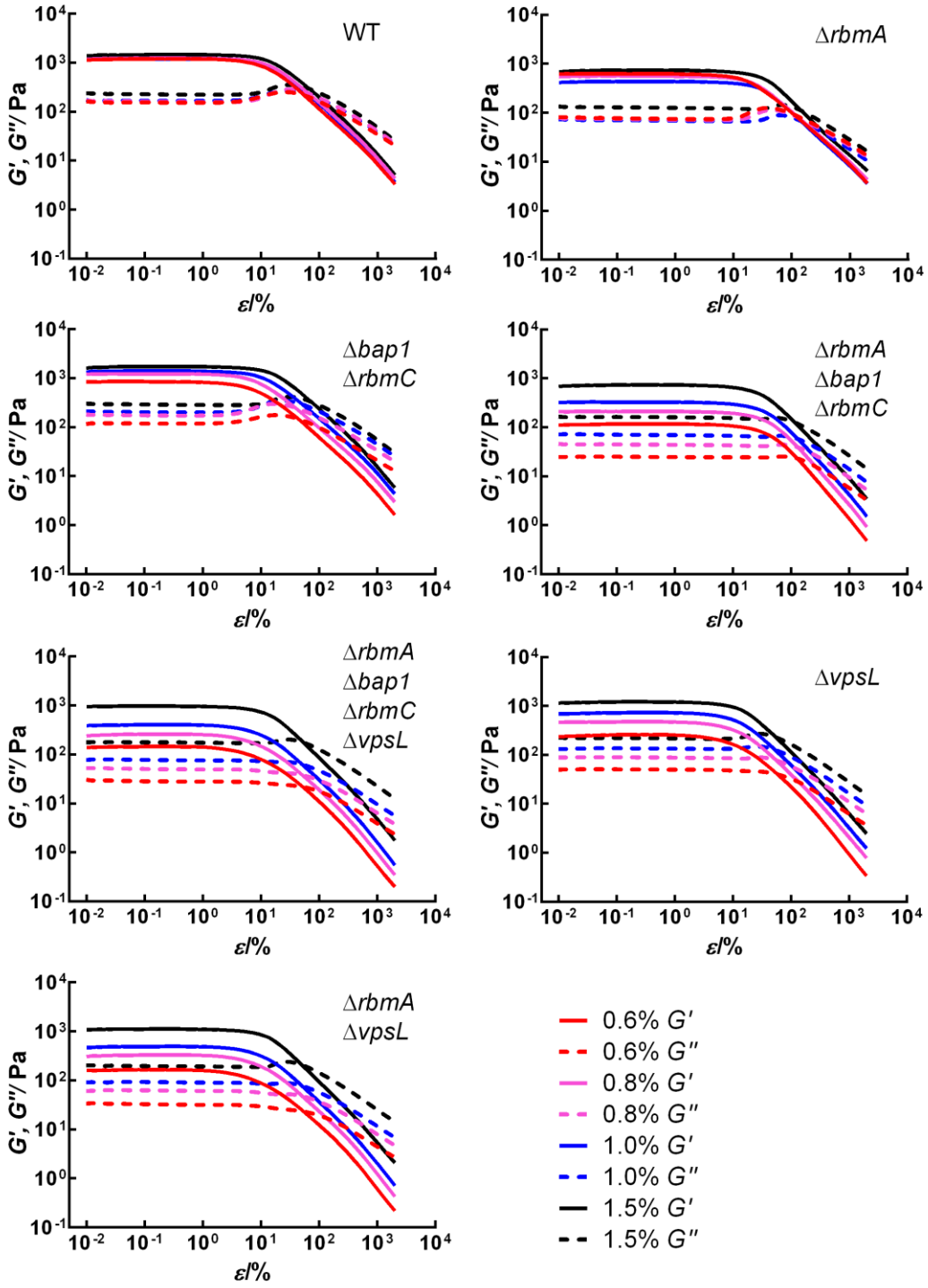

Figure S3. Complete rheological data for main Figures 1-2, Figure S2, and Table S1. Shown are the storage modulus G' (solid curves) and loss modulus G'' (dashed curves) as a function of the amplitude of oscillatory strain ε for the *V. cholerae* strains indicated on the plots.

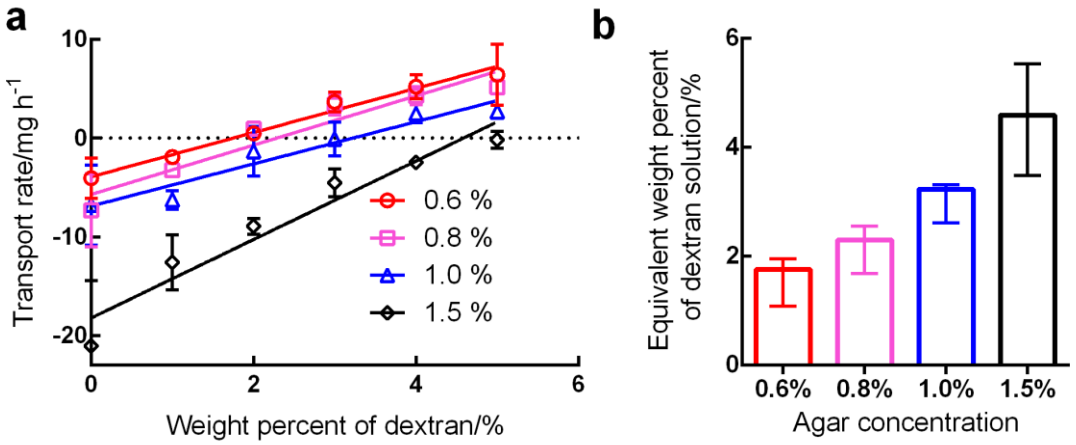

Figure S4. Measurement of osmotic pressures of agar substrates. a) A droplet of LB medium containing dextran was placed on a semi-permeable membrane on top of different concentration agar substrates (0.6-1.5%). Depending on the dextran concentration in the droplet and the agar concentration in the substrate, an osmotic contrast is established across the semi-permeable membrane. Thus, the liquid droplet either takes up water from the agar or loses water to the agar. Using linear interpolation, we identified the concentration of dextran at which there is zero net flow across the membrane. We used these values as proxies for the osmotic pressures of the agar at each concentration, shown in (b). Importantly, we find that the osmotic pressure of agar plates is equivalent to polymer concentrations between 1-5%, which is on the order of the matrix polysaccharide concentration in the biofilm.[3] Specifically, in our earlier contribution, we found that the volume fraction of the vibrio polysaccharide matrix is ~ 1-4% of the extracellular biofilm space.[3] Therefore, depending on the agar concentration, the biofilm matrix will either take up or lose water,[3, 12] similar to the dextran droplet in the above experiments. Error bars correspond to standard deviations in (a) and 95% confidence intervals in (b).

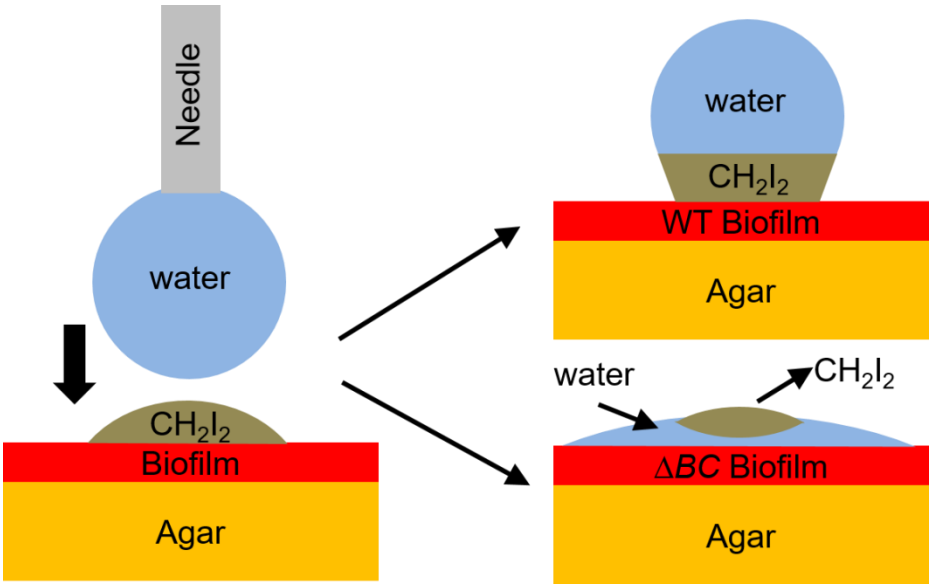

Figure S5. Schematic representation of the experiment in main Figure 3c. Not drawn to scale. Depending on the polarity of the biofilm, either water or CH_2I_2 is in preferential contact with the biofilm.

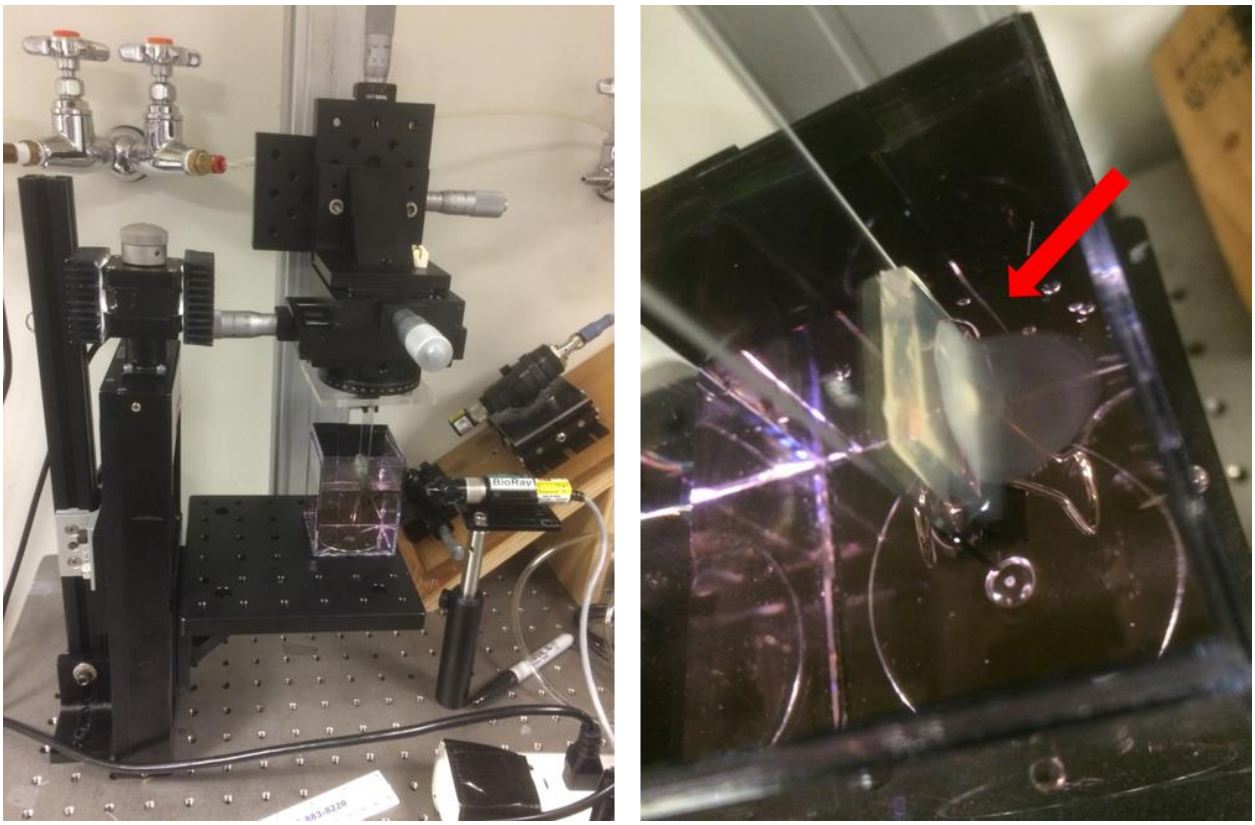

Figure S6. *Left*: Image of the experimental setup for capillary peeling with controlled peeling velocity. *Right*: Close-up view of the biofilm. The red arrow indicates the position of the *V. cholerae* biofilm. Half of the biofilm has been peeled off and floats on the water while the other half remains adhered to the agar substrate.

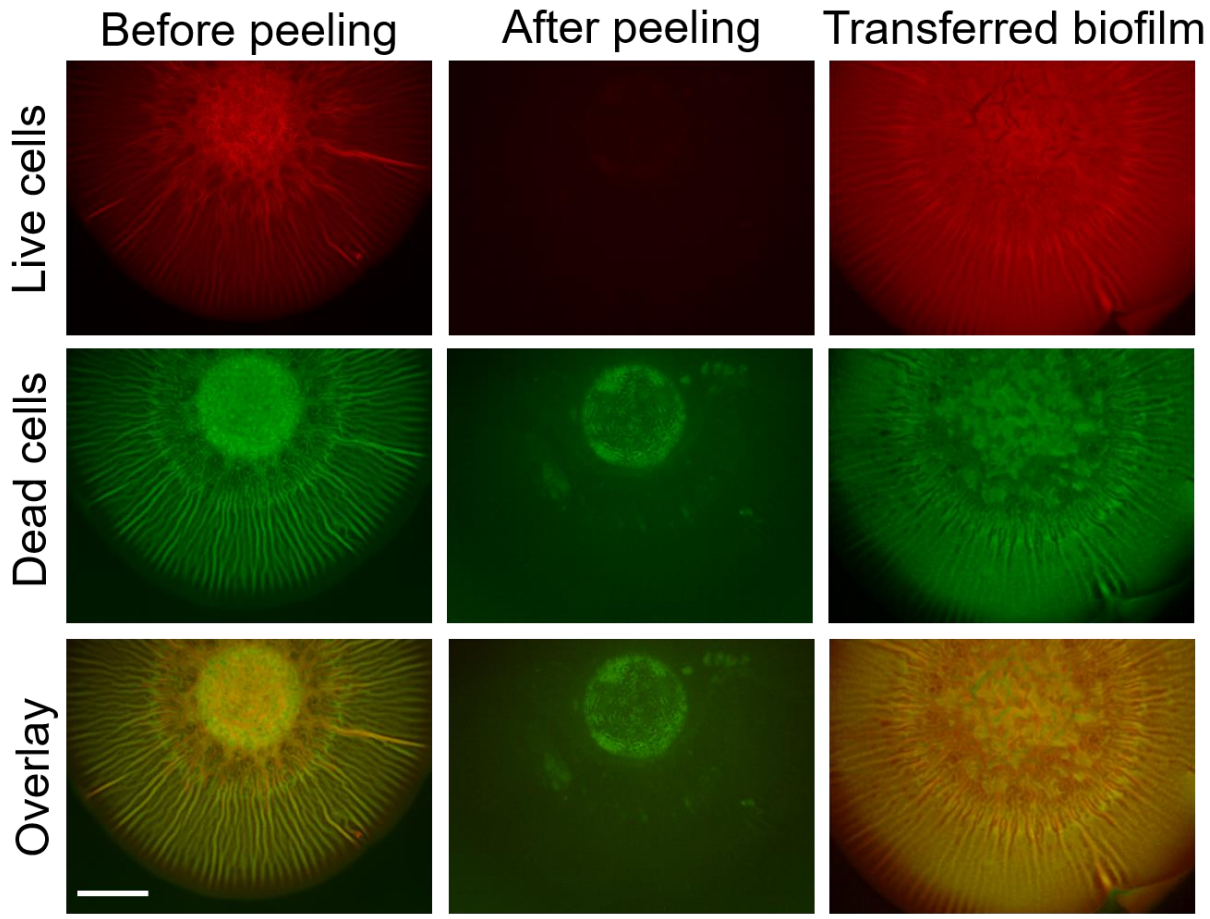

Figure S7. Capillary peeling as a biofilm removal and transfer technique. Shown are fluorescence images of the agar substrate (0.6%) before (*left*) and after (*middle*) capillary removal of a WT *V. cholerae* biofilm, as well as the images of the transferred biofilm (*right*). Red (*top*) is fluorescence from mKate2 in live cells. Green (*middle*) corresponds to SytoX DNA staining of dead cells. *Bottom* row shows images by overlaying the red and green channels. Scale bar: 3 mm.

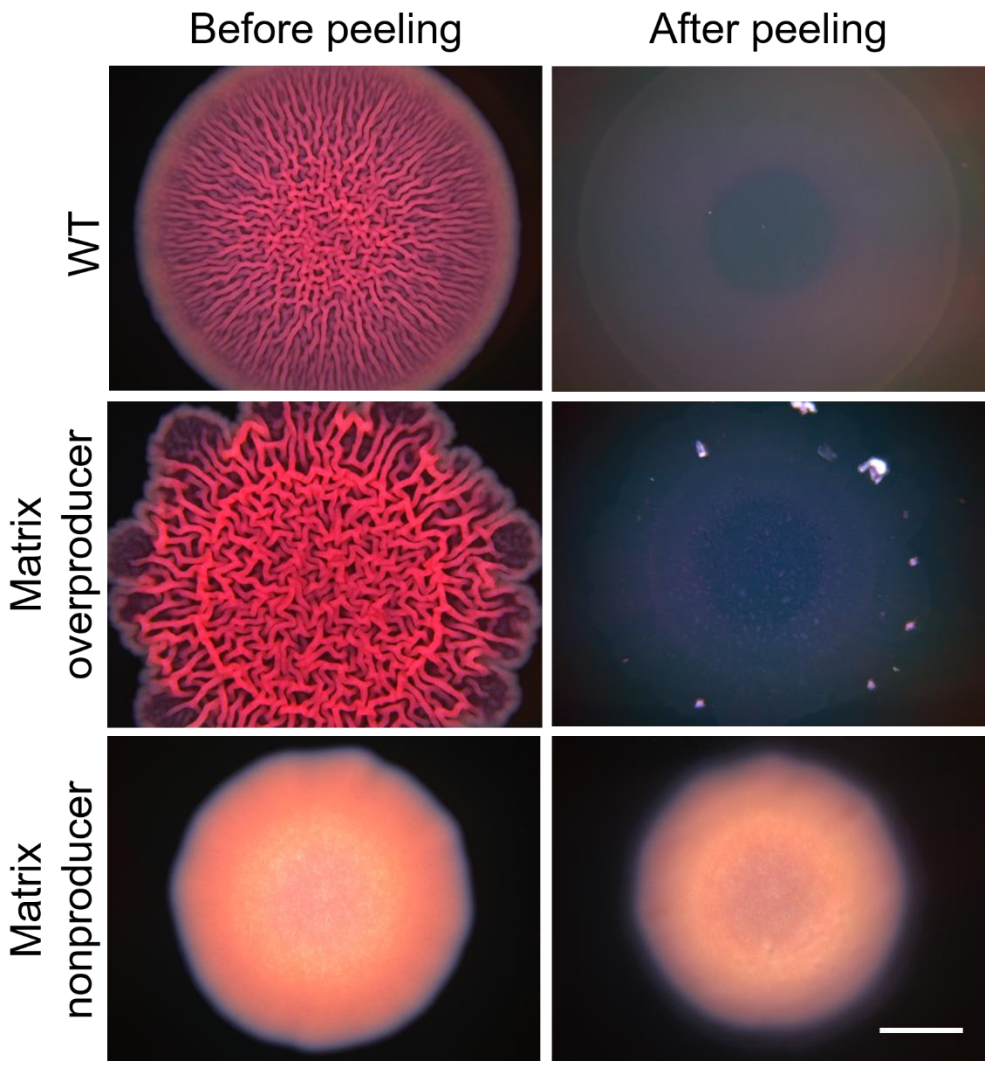

Figure S8. Capillary peeling of *Pseudomonas aeruginosa* biofilms grown on 1.0% agar substrates containing congo red dye. Images of the biofilms taken before peeling are shown on the *left* and images of the agar substrates after peeling are shown on the *right*. From *top* to *bottom* are biofilms of WT *P. aeruginosa* PA14, a *P. aeruginosa* PA14 strain that overproduces the Pel matrix polysaccharide, and a *P. aeruginosa* PA14 strain that lacks Pel (See Table S3 for details). *P. aeruginosa* biofilms can only be peeled off an agar substrate if the strain produces the matrix polysacharide. Scale bar: 3 mm.

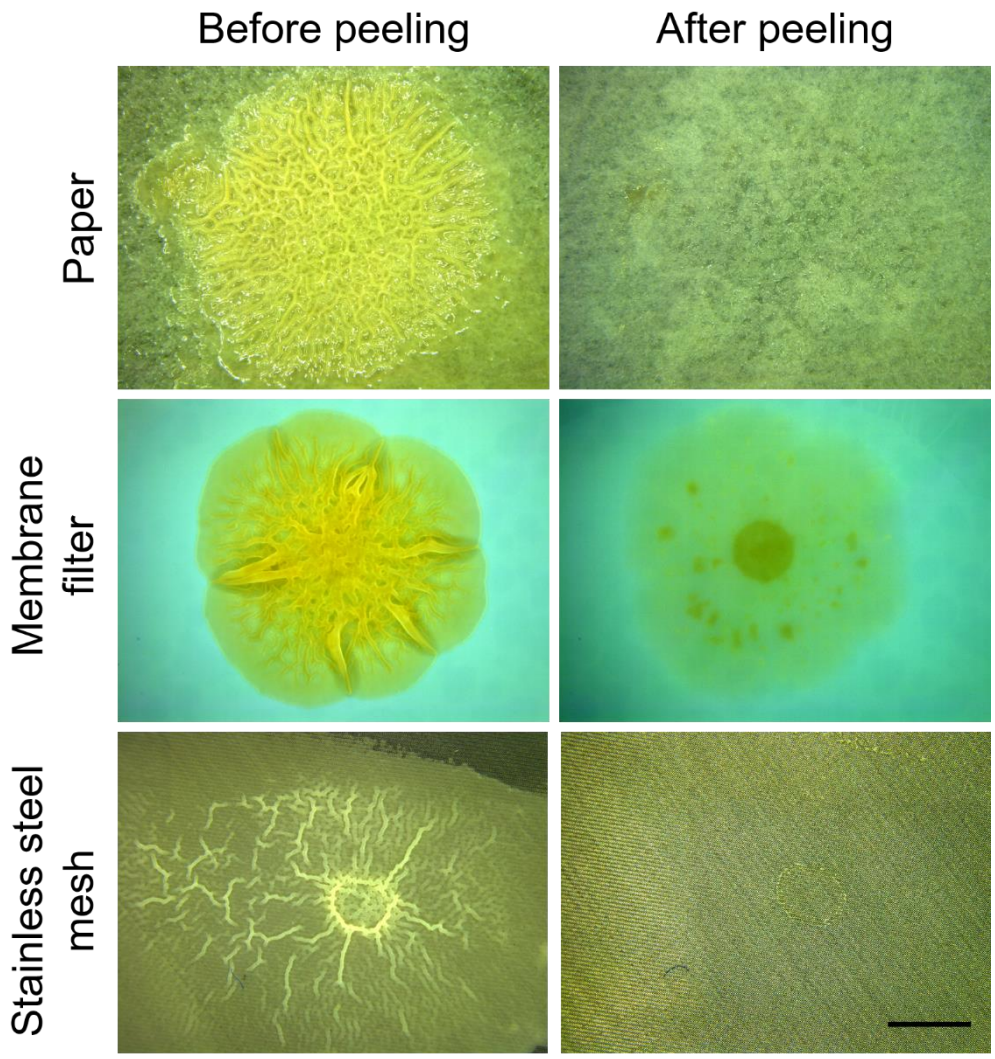

Figure S9. Application of the capillary peeling method to *V. cholerae* biofilms grown on different substrates (See Methods for growth conditions). Biofilm images before peeling are shown on the *left* and surface images after peeling are shown on the *right*. Scale bar: 3 mm.

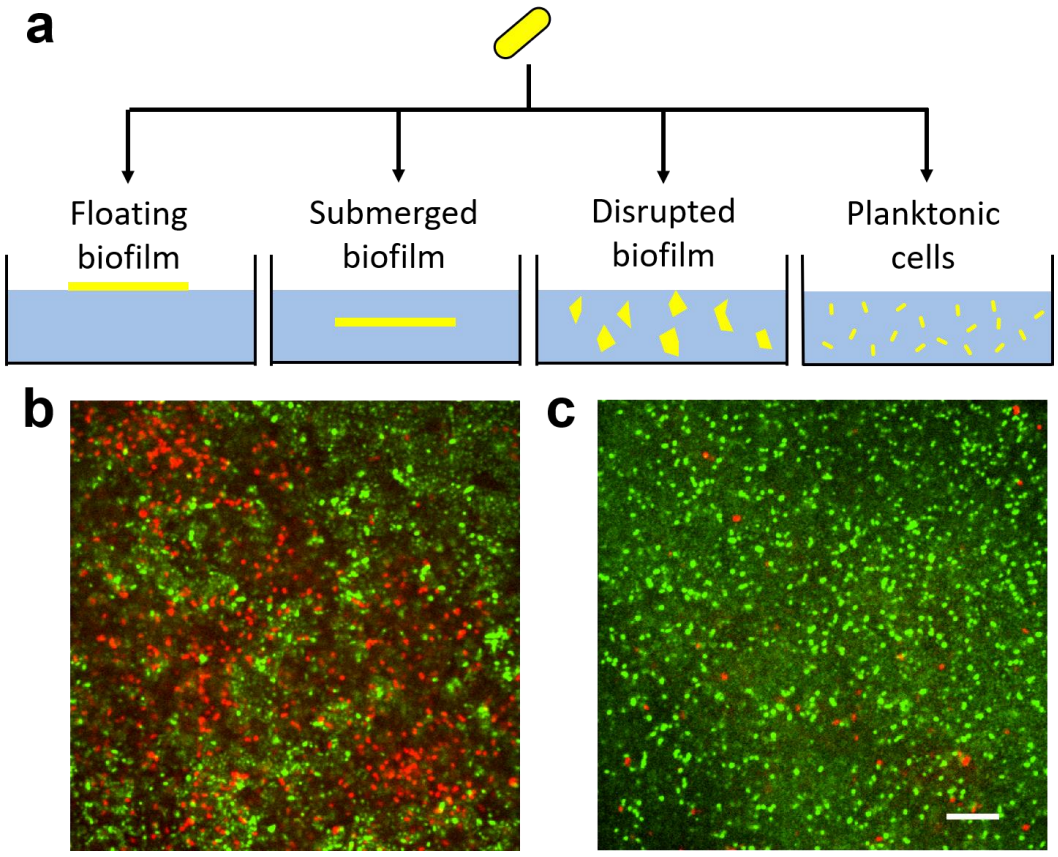

Figure S10. Application of the biofilm transfer technique. a) Schematic representation of the experiment in main Figure 5c. Yellow denotes *V. cholerae* cells. Blue denotes the liquid LB medium containing the antibiotic (tetracycline at 50 μg mL^{-1}). Not drawn to scale. b,c) Confocal miscroscopy images of live-dead staining of biofilm-dwelling cells. Green and red signals correspond to live cells and dead cells, respectively. For panels b and c, a WT *V. cholerae* biofilm was grown for two days on a plate containing 0.6% agar. Subsequently, the biofilm was peeled off the agar substrate via the capillary peeling method (left-most schematic in panel a), and floated on LB medium containing tetracycline. After 1 h of antibiotic treatment, the floating biofilm was transferred to a #1.5 glass coverslip with the original base of the biofilm attached to the coverslip (via the *right* configuration in main text Figure 5a). The biofilm was imaged from the bottom through the coverslip. Panel b shows the base of the biofilm and panel c shows an image taken 10 μm above the biofilm base. Scale bar: 10 μm.

Supplementary Methods: Principles of surface energy measurements

The calculation of surface energy follows the original manuscript by Owens and Wendt,[13] which is briefly summarized here. The surface energy of a biofilm γ_f can be decomposed into the nonpolar, dispersion force component γ_f^d and the polar component due to hydrogen bonding and/or dipole-dipole forces γ_f^p,

$$\gamma_f = \gamma_f^d + \gamma_f^p \tag{1}$$

The interfacial energy γ_{fl} between a biofilm and a liquid (l) located on top of the biofilm follows:

$$\gamma_{fl} = (\sqrt{\gamma_f^d} - \sqrt{\gamma_l^d})^2 + (\sqrt{\gamma_f^p} - \sqrt{\gamma_l^p})^2 \tag{2}$$

in which γ_l^d and γ_l^p are the dispersion and polar components of the surface energy of the liquid, respectively. Neglecting the vapor pressure effect, the Young equation for a liquid droplet located on top of a biofilm is:

$$\gamma_l \cos\theta = \gamma_f - \gamma_{fl} \tag{3}$$

where θ is the contact angle. Combining equations (2) and (3), one arrives at the expression:

$$1 + \cos\theta = 2\sqrt{\gamma_f^d}\left(\frac{\sqrt{\gamma_f^d}}{\gamma_l}\right) + 2\sqrt{\gamma_f^p}\left(\frac{\sqrt{\gamma_f^P}}{\gamma_l}\right) \tag{4}$$

Therefore, by measuring the contact angle θ of two liquids with known γ_l^d, γ_l^p, and γ_l, against a biofilm, simultaneous equations are obtained which can be used to solve for γ_f^d and γ_f^p. We chose water and CH_2I_2 as the test liquids and used the surface energy values reported in the original manuscript by Owens and Wendt.[13]

SI Movie Caption

Video S1: Capillary peeling of a WT *V. cholerae* biofilm grown on a 0.6% agar substrate for two days and subsequent pick-up with a glass substrate. The movie is played in real time.

Supplementary Reference

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