Emergence of three-dimensional order and structure in growing biofilms

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Surface-attached bacterial biofilms are self-replicating active liquid crystals and the dominant form of bacterial life on Earth1–4. In conventional liquid crystals and solid-state materials, the interaction potentials between the molecules that comprise the system determine the material properties. However, for growth-active biofilms it is unclear whether potential-based descriptions can account for the experimentally observed morphologies, and which potentials would be relevant. Here, we have overcome previous limitations of single-cell imaging techniques5–6 to reconstruct and track all individual cells inside growing three-dimensional biofilms with up to 10,000 individuals. Based on these data, we identify, constrain and provide a microscopic basis for an effective cell–cell interaction potential, which captures and predicts the growth dynamics, emergent architecture and local liquid-crystalline order of Vibrio cholerae biofilms. Furthermore, we show how external fluid flows control the microscopic structure and three-dimensional morphology of biofilms. Our analysis implies that local cellular order and global biofilm architecture in these active bacterial communities can arise from mechanical cell–cell interactions, which cells can modulate by regulating the production of particular matrix components. These results establish an experimentally validated foundation for improved continuum theories of active matter and thereby contribute to solving the important problem of controlling biofilm growth.

Vibrio cholerae cells can swim through liquids as isolated individuals, but are more commonly attached to surfaces, where they grow into clonal colonies termed biofilms, with reproducible spatial organization, global morphology and cellular arrangements3–9. Biofilm architectures often display striking local nematic order analogs to molecular ordering in abiotic liquid crystals, yet biofilms differ fundamentally in that they are active systems, driven by cell growth and metabolism10–14. As these active nematic systems operate far from thermodynamic equilibrium1, there are no relevant fundamental conservation laws known that could be used to characterize the biofilm developmental dynamics. To achieve a detailed qualitative and quantitative understanding of such biologically ubiquitous yet physically exotic bacterial communities, we developed new experimental imaging and image analysis techniques for obtaining high spatiotemporal-resolution data of the biofilm developmental process up to 10⁹ cells, representing mid-sized biofilm microcolonies that have already established the architectural state of macroscopic V. cholerae biofilms1. By using automated confocal microscopy, with an adaptive live feedback between image acquisition, feature recognition and microscope control, followed by a ground-truth-calibrated, novel three-dimensional (3D) image-segmentation technique (see Methods and Supplementary Information) we were able to observe complete 3D biofilm development at cellular resolution with minimal phototoxicity (Fig. 1a,b) and minimal segmentation error (see Supplementary Information). The high temporal resolution (Δt = 5–10 min) allows for cell lineage reconstruction, measurements of local growth rates, and the identification of all cells in a field of view that are not related to the original biofilm founder cell (Fig. 1b–d and Supplementary Movie 1).

When investigating whether the non-equilibrium dynamics of biofilm development and the emergence of local order can be captured quantitatively through effective cell–cell interaction potentials, it is important to account for the essential biophysical processes—cell growth, cell division, cell–surface interactions and cell–cell interactions15–17. Whereas growth and division are driven by nutrient availability and metabolism, cell–surface and cell–cell attractions are typically mediated by secreted or membrane-associated polysaccharides and proteins18–21. For V. cholerae biofilms, the molecular basis for cell–cell interactions has been intensively investigated: cells are embedded in a self-secreted extracellular matrix composed of the Vibrio polysaccharide (VPS), extracellular DNA and proteins22–24. The osmotic pressure resulting from a high concentration of matrix components in the intercellular space, as well as steric cell–cell interactions, are both expected to contribute to cell–cell repulsion. Cell–cell attraction is primarily mediated by the protein RbmA, which localizes throughout the biofilm (Fig. 1c)25,26 and links cells to each other21–23; its expression levels are inversely related to cell–cell spacing (Fig. 2a). VPS also weakly binds cells together, yet elevated levels of VPS production do not cause stronger cell–cell attraction or decreased cell–cell spacing (Supplementary Fig. 11). Based on these cell–cell interaction processes, we hypothesized that biofilm architectures are primarily determined by the relative strength of the effective mechanical cell–cell attraction and repulsion forces.

To determine the impact of cell–cell attraction, we quantitatively compared the 3D biofilm architecture dynamics of a rogueous wild-type strain with straight cell shape (WT*) with that of a mutant strain (∆rbmA) with significantly weakened intercellular adhesion (see Methods). Biofilms grown in a low-shear environment approximately display hemispherical symmetry (Fig. 1d), which allows us to characterize the biofilm architectures (Fig. 1c) as a function of the distance to the biofilm centre in the

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Basal plane, $d_{\text{centre}}$, using the cell number in the biofilm, $N_{\text{cells}}$, as a quantification of the developmental state. Our measurements reveal strong structural differences between the outer biofilm layer and its central part, as well as several distinct architectural phases of the biofilm during growth (Fig. 1c,f). Interestingly, the cellular growth rate remains homogeneous in space during WT* biofilm development in our conditions and for our biofilm sizes (Fig. 1e and Supplementary Fig. 5), in contrast to theories assuming steep nutrient gradients inside biofilms. The nematic order, cell–cell spacing and cellular orientations with respect to the vertical ($z$) and radial ($r$) directions differ significantly between WT* and ΔrbmA mutants (Fig. 1e,f and Supplementary Figs. 5–8), revealing the strong effect of cell–cell adhesion on biofilm architecture dynamics.

Based on the high-resolution spatiotemporal data of biofilm development of different bacterial strains, we investigated the
hypothesis that the biofilm internal structure and external shape originate from mechanical interactions between cells. Focusing on a minimal model, we describe the effective mechanical interactions in terms of an effective potential that depends on the distance $r_{\alpha\beta}$ between neighboring cells $\alpha$ and $\beta$, and their orientations $\hat{n}_\alpha$ and $\hat{n}_\beta$. We made the simplifying assumption that the potential is independent of the biofilm developmental state or nutrient levels. As shown below, this simplification suffices to capture the main features of the small to medium-sized biofilms studied here but is expected to become inaccurate at the later stages of biofilm development, when spatiotemporal heterogeneities become relevant. Given the molecular components of the cell–cell interaction and their qualitative effects on attraction and repulsion outlined above, we assume the pair potential

$$U = e_\sigma f_1 \left( \frac{r_{\alpha\beta}^2}{\ell^2} + \frac{\nu}{1 + e^{\frac{r_{\alpha\beta}}{\phi}}} \right)$$

where $\rho = r_{\alpha\beta}/\sigma$ is the shape-normalized cell–cell distance. The range parameter $\sigma(\hat{r}_{\alpha\beta}, \hat{n}_\alpha, \hat{n}_\beta)$ depends on the instantaneous cell lengths, the orientation of the cells relative to each other and the...
individual cell orientations, and it maps the potential onto non-identical ellipsoidal cells (Supplementary equation (20)). The amplitude is set by the repulsion strength \( e_{\theta} \) and instantaneous cell lengths and cell orientations through the strength parameter \( \epsilon_{\theta} (\hat{n}_a, \hat{n}_b) \) (Supplementary equation (19)). The first term of the interaction potential describes the combined effects of hard steric and osmotic repulsion with range \( \lambda_3 \) (Fig. 2b). The second term corresponds to cell–cell attraction and adds an attractive part of relative depth \( \nu \), width \( \lambda_2 \) and position \( \rho_2 \) (Fig. 2c). Each contribution and parameter in the potential \( U \) thus has a well-defined physical meaning (schematic diagram in Supplementary Fig. 15 and Supplementary Table 3).

We assume here that the interaction parameters are taken to be constant for a given bacterial strain, a simplification that could be relaxed in future models. With these simplifying assumptions, initial estimates of the potential parameters prior to systematic scans can be obtained from basic physical considerations (see Methods).

This potential was then implemented in a particle-based model of biofilm development, in which individual cells were modelled as growing and dividing ellipsoids without self-propulsion (see Supplementary Information), whose interactions are described by \( U \). Bypassing previous limitations of individual-based biofilm models, all the parameters of our model (cell aspect ratio, division time distribution; Supplementary Table 3) were determined from single-cell properties of experimental biofilms, and the dynamics were solved with a massively parallel computation approach using graphics processing units to evaluate all pairwise interactions (see Supplementary Information). To obtain the key potential parameters \( e_{\theta}, \lambda_2, \nu, \lambda_3 \) and \( \rho_2 \) for \( V. cholerae \) biofilms, we assumed that the attractive term in \( U \) can be attributed primarily to RbmA levels, with the VPS acting as a Woods–Saxon background potential (see Methods) akin to the mean-field potential in nucleon models. This assumption is motivated by the experimental findings that increased VPS levels do not increase the cell–cell attraction (Supplementary Fig. 11), yet biofilms that lack RbmA display a small residual mechanical cohesion (Fig. 3e), indicating that VPS does contribute weakly to cell–cell binding. To first obtain the parameters \( e_{\theta} \) and \( \lambda_2 \), we fitted the repulsive part of the potential \( U \) by comparing experimental \( \Delta \text{rbmA} \) biofilms, which lack the attractive potential term (\( \nu = 0 \)), with simulated biofilms, using the mean squared difference (MSD) of a feature vector as a metric. The feature vector contains 14 different architectural properties and their temporal variation up to 300 cells (Supplementary Fig. 14), allowing a comprehensive comparison of biofilm architecture and development between simulations and experiment at the same time. Note that even at small sizes, the \( V. cholerae \) biofilms used in this study produce RbmA and VPS (Supplementary Fig. 10). For \( \Delta \text{rbmA} \) biofilms we found a broad minimum in the \((e_{\theta}, \lambda_3)\) space as shown in the MSD heatmap (Supplementary Fig. 16), resulting in best-fit simulations that show high similarity to experiments (Supplementary Fig. 17). The effective translational and rotational interaction forces acting on two neighbouring \( \Delta \text{rbmA} \) cells for the best-fit potential are illustrated in Fig. 2b and Supplementary Fig. 18 for different cellular orientations. The interaction range for two aligned cells is very close to the experimentally observed average cell–cell spacing of the \( \Delta \text{rbmA} \) mutant (dashed cell).

Because the attraction parameters \((\nu, \lambda_2, \rho_2)\) in potential \( U \) depend on the concentration of RbmA, we genetically modified \( V. cholerae \) so that we could tune the production of RbmA (and therefore tune the strength of the attraction), by adding different concentrations of a compound that induces the \( \text{rbmA} \)-expression construct homogeneously inside the biofilm: arabinose (see Methods and Supplementary Fig. 13). Experimentally, we observed that increasing arabinose concentrations resulted in decreased cell–cell spacing (Fig. 2a), consistent with the assumption that RbmA mediates cell–cell attraction. By fixing the repulsive component \((e_{\theta}, \lambda_3)\) based on the \( \Delta \text{rbmA} \) biofilms, we then fitted the attractive potential component \((\nu, \lambda_2, \rho_2)\) for a range of different arabinose concentrations (Fig. 2c,d). The MSD isosurfaces in \((\nu, \lambda_2, \rho_2)\) space and corresponding 3D renderings for simulated and experimental biofilms grown at 0.5% (wt/vol) of arabinose reveal tight fits (Supplementary Figs. 21–23), and the resulting best-fit interaction force displays an attractive region (red) at the average experimental cell–cell distance (Fig. 2c).

With the calibrated simulation, we then inferred an effective arabinose concentration for the WT* of \( c = 0.68 \pm 0.19\% \) (wt/vol), by locating the WT* biofilm architecture in the \((\nu, \lambda_2, \rho_2)\) space along the curve of different arabinose concentrations (Fig. 2d). Extracting an effective arabinose concentration and RbmA level for the WT* is based on the simplifying assumption that all cells in the biofilm express the same levels of the key matrix components, which represents a minimal model that is in quantitative agreement with the experimental data, as the best-fit \((\nu, \lambda_2, \rho_2)\) values for the WT* are close to the effective \((\nu, \lambda_2, \rho_2)\) values for WT* on the curve of different arabinose concentrations (Fig. 2d). The simulations based on the WT* parameters for biofilms up to 300 cells show good quantitative agreement with experiments (Fig. 2f). Remarkably, these simulations also show architectural properties that were not included in the feature vector used for MSD minimization, such as local density variations and the occurrence of patches of highly aligned cells inside the biofilm (red cells in Fig. 2c, characterized by high local ordering), which are characteristic for biofilms with high concentrations of RbmA. Predictions of the architectural development for larger biofilms \((N_{\text{cells}} > 300)\) show high quantitative and qualitative agreement with experimental data, for both the WT* (Fig. 2g,h and Supplementary Movie 5) and \( \Delta \text{rbmA} \) (Supplementary Fig. 24b and Supplementary Movie 5) biofilms up to 100 cells. To achieve accurate simulation results for very large biofilms (>1000 cells), spatiotemporal heterogeneity in gene expression, matrix composition and growth rates probably have to be included in future simulations. Our combined experimental and theoretical analysis therefore suggests that mechanical interactions between cells suffice to account for the internal cellular order and architecture up to mid-sized \( V. cholerae \) biofilms.

To determine how external fields can affect the orientational order and morphology of 3D biofilms, we perturbed biofilm growth by applying external fluid fields of varying strength, corresponding to shear rates of \( \dot{\gamma} = 2-2000 \text{s}^{-1} \), typically encountered by bacteria in natural and man-made environments. At high shear rates \((>600 \text{s}^{-1})\), corresponding to average flow speeds \(>10 \text{mm} \text{s}^{-1}\) through the growth chamber), the WT* cells formed smaller, more compact biofilm colonies with droplet-like shapes, compared with low shear environments (Fig. 3a,b, Supplementary Movies 1 and 3). To understand the mechanisms underlying these architectural changes, we investigated both local and global effects of increased shear on biofilms, and changes in matrix production. Exposure to higher shear resulted in a significantly decreased cell–cell spacing and lower growth rate in WT* biofilms (Fig. 3c), but the height-to-width aspect ratio was unaffected when comparing biofilms with similar \( N_{\text{cells}} \) (Fig. 3e) despite the increased levels of shear stress applied to the top (Fig. 3d). We therefore hypothesized that cells in WT* biofilms at higher shear secrete increased levels of RbmA, allowing increased cell–cell attraction forces to balance shear forces, but leading to a strong reduction in overall growth rate owing to the metabolic cost of increased RbmA production. Using a fluorescent transcriptional reporter for \( \text{rbmA} \) expression, we confirmed that high shear increases RbmA levels (Supplementary Fig. 12), indicating that cells actively modulate the mechanical cell–cell interactions via gene expression.

To explain the observed droplet-like shapes of biofilms grown at high shear rate, we investigated cellular alignment with flow and analysed biovolume flux inside the biofilm using the optical flow method (Fig. 3a–f; see Supplementary Information). We determined that cell alignment with flow increases with increasing shear rate (Fig. 3c–g), and an anisotropic biomass shift downstream occurs at \( \dot{\gamma} > 60 \text{s}^{-1} \) (Fig. 3f), indicating that the observed biofilm development is driven by anisotropic shear flows that are amplified by cell–cell interactions.
Fig. 3 | Biofilm architecture is shaped by external shear flow. a, WT* biofilms grown under strong shear ($\gamma = 2,000$ s$^{-1}$) display droplet-like shapes. Inset, Biovolume flux field inside the biofilm (see Supplementary Information). b, WT* biofilms under high shear ($\gamma = 2,000$ s$^{-1}$) display strong alignment with flow throughout growth, yet biofilms grown in flow with low shear ($\gamma = 2-200$ s$^{-1}$) do not show strong architectural modifications. c, Quantification of the effect of shear on biofilm architecture: measurements of cellular alignment with flow, cell–cell distance and cell growth rate at the bottom and top of biofilms with $N_{\text{mbb}} > 800$ cells show that WT* biofilms in high shear are smaller, more compact and display stronger flow alignment. Statistical significance: *$P < 0.05$; **$P < 0.01$ (t-test); error bars indicate standard error ($n = 4$ biofilms). d, Simulated shear stress distribution for a WT* biofilm, demonstrating that the region of highest shear is at the top of the biofilm. The streamlines indicate the profile of the external flow. e, Biofilm aspect ratio (height/width) increases in time for WT* (red) biofilms, but decreases for $\Delta rbmA$ mutant biofilms (blue) in high flow owing to shear-induced erosion ($n = 4$, error bars indicate standard deviations). f, Biomass shift is defined as the fraction of the average total biomass flux through planes parallel (I) or perpendicular (L) to flow (see Supplementary Fig. 2 for details). Positive biomass shift along the flow direction at higher shear rates indicates anisotropic biofilm expansion towards the downstream direction of the external flow. Zero biomass shift perpendicular to the flow indicates no directional bias ($n \geq 3$, error bars indicate standard errors). g, The tensorial nematic order parameter (Q tensor, see Supplementary Information) and cellular alignment with the flow direction were measured at equally spaced points inside biofilms at low and high shear rates, indicating the regions in which cells are predominantly aligned with the flow and each other. h, Biofilm volumetric growth for $\Delta rbmA$ mutant biofilms is captured by a continuum model (see Supplementary Information) with varying ratios of shear-induced erosion and cell–cell adhesion (experiment: $n = 4$, error bars indicate standard deviations).
shapes are caused by anisotropic expansion of cells aligned with the flow as a result of growth and viscoelastic deformation. Our above measurements regarding increased RbmA levels in WT* biofilms at high shear predict that if RbmA levels are in fact primarily responsible for cell–cell attraction, then most effects of shear on ΔrbmA-mutant biofilms should be explained by shear-induced cell erosion. Indeed, these biofilms showed a reduction in upward growth with higher flow (Fig. 3e), indicating that shear forces are larger than cell–cell attraction forces. This was confirmed by simulations of shear-dependent erosion using a continuum model (see Supplementary Information), which captured the decreased volumetric growth of ΔrbmA-mutant biofilms owing to cell erosion (Fig. 3h and Supplementary Movie 4). Fluid flow therefore strongly affects biofilm architectural development through the effect of shear on growth rate, matrix composition, alignment with flow, biomass shift and shear-induced erosion. These results demonstrate that mechanical interactions at the cellular scale remain important in sculpting biofilm architecture when an external field is applied.

In conclusion, our combined experimental and theoretical analysis shows that the emergence of local nematic order in growing V. cholerae biofilms can be captured by an experimentally constrained minimal effective cell–cell interaction potential that translates molecular mechanisms into force parameters. Given the immense complexity of the molecular interactions, metabolism and signalling that occur between cells, the availability of an experimentally validated potential-based description of biofilm development presents a significant conceptual advance that can provide a microscopic basis for constructing predictive macroscopic continuum theories, by building on course-graining techniques recently developed for other classes of active matter. At the same time, a refined model will be needed to account for the spatial heterogeneities and time dependencies that become relevant at the later stages of biofilm development. Such progress is essential for identifying new strategies towards understanding, controlling and inhibiting biofilm growth under realistic physiological conditions, which remains one of the foremost challenges in biomolecular and biophysical research.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41567-018-0356-9.

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References
1. Zhou, S., Sokolov, A., Lavrentovich, O. D. & Aranson, I. S. Living liquid crystals. Proc. Natl Acad. Sci. USA 111, 1265–1270 (2014).
2. Hagan, M. F. & Baskaran, A. Emergent self-organization in active materials. Curr. Opin. Cell Biol. 38, 74–80 (2016).
3. Doostmohammadi, A., Adamer, M. F., Thampi, S. P. & Yeomans, J. M. Stabilization of active matter by flow-vortex lattices and defect ordering. Nat. Commun. 7, 10557 (2016).
4. Volfson, D., Cookson, S., Hasty, J. & Tsimring, L. S. Biomechanical ordering of dense cell populations. Proc. Natl Acad. Sci. USA 105, 15346–15351 (2008).
5. Drescher, K. et al. Architectural transitions in Vibrio cholerae biofilms at single-cell resolution. Proc. Natl Acad. Sci. USA 113, E2066–E2072 (2016).
6. Yan, J., Sharo, A. G., Stone, H. A., Wingreen, N. S. & Bassler, B. L. Vibrio cholerae biofilm growth program and architecture revealed by single-cell live imaging. Proc. Natl Acad. Sci. USA 113, E5337–E5343 (2016).
7. Kragh, K. N. et al. Role of multicellular aggregates in biofilm formation. mBio 7, e00237 (2016).
8. Flemming, H.-C. et al. Biofilms: an emergent form of bacterial life. Nat. Rev. Microbiol. 14, 563–575 (2016).
9. Marchetti, M. C. et al. Hydrodynamics of soft active matter. Rev. Mod. Phys. 85, 1143–1189 (2013).
10. Persat, A. et al. The mechanical world of bacteria. Cell 161, 988–997 (2015).
11. Liu, J. et al. Coupling between distant biofilms and emergence of nutrient-time-sharing. Science 356, 638–642 (2017).
12. Rodensky, C. A. et al. Mechanosensing of shear by Pseudomonas aeruginosa leads to increased levels of the cyclic-di-GMP signal initiating biofilm development. Proc. Natl Acad. Sci. USA 114, 5906–5911 (2017).
13. Grant, M. A., Paez, R. L., Allen, R. J. & Cicuta, P. The role of mechanical forces in the planar-to-bulk transition in growing Escherichia coli microcolonies. J. R. Soc. Interface 11, 20140400 (2014).
14. You, Z., Pearce, D. J. G., Sengupta, A. & Giomi, L. Geometry and mechanics of microdomains in growing bacterial colonies. Phys. Rev. X 8, 031065 (2018).
15. Delarue, M. et al. Self-driven jamming in growing microbial populations. Nat. Phys. 12, 762–776 (2016).
16. Seminara, A. et al. Osmotic spreading of Bacillus subtilis biofilms driven by an extracellular matrix. Proc. Natl Acad. Sci. USA 109, 1116–1121 (2012).
17. Trejo, M. et al. Elasticity and wrinkled morphology of Bacillus subtilis biofilms. Proc. Natl Acad. Sci. USA 110, 2011–2015 (2013).
18. Maier, B. & Wong, G. C. L. How bacteria use type IV pili machinery on surfaces. Trends Microbiol. 23, 775–788 (2015).
19. Teschner, J. K. et al. Living in the matrix: assembly and control of Vibrio cholerae biofilms. Nat. Rev. Microbiol. 13, 255–268 (2015).
20. Berk, V. et al. Molecular architecture and assembly principles of Vibrio cholerae biofilms. Science 337, 236–239 (2012).
21. Feng, J. C. et al. Structural dynamics of RbmA governs plasticity of Vibrio cholerae biofilms. Elife 6, e26163 (2017).
22. Maestre-Reyna, M., Wu, W.-J. & Wang, A.-J.-H. Structural insights into RbmA, a biofilm scaffolding protein of V. cholerae. PLoS ONE 8, e82458 (2013).
23. Feng, J. C. N., Karpus, K., Schoolnik, G. K. & Yildiz, F. H. Identification and characterization of RbmA, a novel protein required for the development of rugose colony morphology and biofilm structure in Vibrio cholerae. J. Bacteriol. 188, 1049–1059 (2006).
24. Hellweg, C. L., Chegg, R. J., Clark, J. R., Plugge, C. M. & Kreft, J. U. Advancing molecular science by individual-based modelling. Nat. Rev. Microbiol. 14, 461–471 (2016).
25. Lardon, L. A. et al. iDynoMiCS: next-generation individual-based modelling of biofilms. Environ. Microbiol. 13, 2416–2434 (2011).
26. Marcos, P., Chen, D. & Yeomans, J. M. Entropic rheotaxis. Proc. Natl Acad. Sci. USA 109, 4780–4785 (2012).
27. Mitchell, W. H. & Spagnolie, S. E. A generalized traction integral equation for Stokes flow, with applications to near-wall particle mobility and viscous erosion. J. Comput. Phys. 333, 462–482 (2017).
28. Cates, M. E. & Tjhung, E. Theories of binary fluid mixtures: from phase-separation kinetics to active emulsions. J. Fluid. Mech. 836, 1–68 (2018).
29. Singh, P. K. et al. Vibrio cholerae combines individual and collective sensing to trigger biofilm dispersal. Curr. Biol. 27, 3359–3366 (2017).
30. Vidakovic, L., Singh, P. K., Hartmann, R., Nadell, C. D. & Drescher, K. Dynamic biofilm architecture confers individual and collective mechanisms of viral protection. Nat. Microbiol. 3, 26–31 (2017).
31. Smith, W. P. et al. Cell morphology drives spatial patterning in microbial communities. Proc. Natl Acad. Sci. USA 114, E280–E286 (2017).

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Author contributions
K.D. and J.D. designed and supervised the study. R.H. and P.K.S. performed experiments. P.K.S. and E.D.P. created bacterial strains. R.H. developed experimental and analysis software. P.P. developed continuum simulations. R.M. developed cell-based simulation framework. R.M., R.H. and B.S. performed cell-based simulations. R.M., R.H., P.P. and B.S. developed cell–cell potentials. R.H., with the help of P.P., J.D. and K.D., analysed the data. R.H., P.P. and K.D. wrote the manuscript, with the help of all authors.

Competing interests
The authors declare no competing interests.

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Methods

Media and cloning approaches. All strains were grown in lysogeny broth (LB) medium supplemented with appropriate antibiotics at 37 °C for normal growth and during cloning. Biofilm experiments with V. cholerae were performed in M9 minimal medium, supplemented with 2 mM MgSO4, 100 mM CaCl2, and 1 M9 medium mimicking essential medium (MEM) vitamins, 0.5% glucose and 15 mM triethanolamine (pH 7.1). Standard molecular biology techniques were applied to construct plasmids and strains. Restriction enzymes and DNA polymerase enzymes were purchased from New England Biolabs. Oligonucleotides were commercially synthesized by Eurofins. All V. cholerae strains used in this study are derivatives of the rugose variant of the wild-type V. cholerae O1 biovar El Tor strain N16961 (termed strain KDV148). V. cholerae deletion mutations were engineered using the pKAS32 suicide vector harbouring in Escherichia coli in the presence of λpir S17-1 Apir (ref. 1). Complementation constructs were inserted at the lacI site with the help of the suicide plasmid pKAS32. The plasmid pNUT542, containing gene coding for the supF older green fluorescent protein (mGFP) expressed under the control of the Psp promoter, was conjugated into all V. cholerae strains except for the complementation strain KDV1082. Plasmid clones were first constructed in the E. coli strain Top10 and then mated into V. cholerae with the help of an additional E. coli strain harbouring the conjugation plasmid pRK600. Arabinose was used as an inducer to control the expression of rmbA from the arabinose-regulated promoter Psp. Details of the strains, plasmids and oligonucleotides are listed in the Supplementary Information.

Strain construction. The rugose variant of the V. cholerae strain N16961 (strain KDV148) displays strong surface attachment and biofilm formation as a consequence of high c-di-GMP production. V. cholerae cells are usually characterized by a slightly curved cell shape. To allow V. cholerae cells to be modelled by ellipsoids in theory and simulations, we generated a mutant with a straight cell shape (that is, the common bacterial rod shape) by deleting the gene ΔcrvA according to the method of ref. 1. In detail, the 1 kb flanking regions of gene crvA (VCA0175) were amplified with the oligonucleotides kdo1182/kdo1183 and kdo1183/kdo1184, and the fused PCR product was amplified using kdo1182/kdo1185. The final PCR product was ligated into plasmid pNUT144 (a derivative of pKAS32). The resulting plasmid pNUT961 was conjugated into strain KDV148, to generate the ΔcrvA deletion mutant, following the selection protocol described in ref. 1. Finally, cells containing the correct mutation were screened by PCR. Plasmid pNUT542 was conjugated into KDV611 strain to construct strain KDV613 containing the ΔcrvA deletion (referred to as WT*). The ΔrmbA deletion strain (KDV698) was constructed by conjugating plasmid pNUT336 into strain KDV611. The mutant screening was performed by PCR.

Tuning cell–cell interaction by inducing rmbA expression. To control the timing and rate of RmbA production, an inducible strain (KDV1082) was generated by conjugating plasmid pNUT1519 into the ΔrmbA strain KDV698. Plasmid pNUT1519 was created by cloning a Ptac-construct into plasmid pNUT1268. Plasmid pNUT1268 is a derivative of plasmid pNUT542 in which Ptac was replaced with a constitutive promoter Psp, used as an inducer to control the expression of rmbA from the arabinose-regulated promoter Psp. Details of the strains, plasmids and oligonucleotides are listed in the Supplementary Information.

Visualization of secreted RbmA. To visualize RbmA during biofilm growth, the wild-type copy of rmbA was exchanged by a FLAG-tagged rmbA (with the octapeptide DYKDDDDK) by matting the plasmid pNUT462 into strain KDV148, resulting in V. cholerae strain KDV829. Successful FLAG-tagging of RbmA was confirmed by PCR and sequencing. The final strain KDV835 was generated by conjugating the fluorescence protein expression plasmid pNUT542 into strain KDV829. For RbmA visualization in flow chambers, biofilms were grown in M9 minimal medium containing 1% of the cell–cell interaction by inducing rmbA expression. To control the timing and rate of RmbA production, an inducible strain (KDV1082) was generated by conjugating plasmid pNUT1519 into the ΔrmbA strain KDV698. Plasmid pNUT1519 was created by cloning a Ptac-construct into plasmid pNUT1268. Plasmid pNUT1268 is a derivative of plasmid pNUT542 in which Ptac was replaced with a constitutive promoter Psp, used as an inducer to control the expression of rmbA from the arabinose-regulated promoter Psp. Details of the strains, plasmids and oligonucleotides are listed in the Supplementary Information.

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cell–cell repulsion dominates. The attraction shift $\rho_1$ was estimated via the average cell–cell distance at the edge of the biofilms, where attraction dominates. The attraction width $\lambda_0$ was determined by considering the standard deviation of experimental cell–cell distances at the edge of the biofilms.

**Background potential.** Cell–cell adhesion mediated by the VPS matrix component was modelled by a mean-field background Woods–Saxon potential, and was assumed to provide the weak cell–cell binding that prevents the disintegration of biofilms owing to fluid shear acting on $\Delta rbmA$ mutant biofilms (which lack the major cell–cell attraction, mediated by RbmA). The mean-field VPS-mediated binding strength was estimated to be approximately equal to the Stokes drag felt by a cell at the edge of the biofilm at low flow rate ($0.1 \mu l min^{-1}$), because significant numbers of cells in the $\Delta rbmA$ background were sheared off at higher flow rate ($100 \mu l min^{-1}$). However, WT* biofilms were found to be robust to this increased fluid shear, suggesting that the increased expression of $rbmA$ at higher flow rate (Supplementary Information) increases the RbmA-mediated cell–cell attraction strength by approximately two orders of magnitude above the value predicted at low flow rate. In simulations performed at zero shear, the VPS contribution to cell–cell attraction can be neglected as the Woods–Saxon potential is approximately constant in the bulk.

**Comparing simulations with experimental data.** The dynamic biofilm architecture was summarized in a feature vector representing key phenotypic and structural properties temporally. The similarity between a vector characterizing a simulation and a real biofilm was assessed in terms of the MSD between them. For details, see Supplementary Information.

**Continuum model.** The mathematical continuum model of growing biofilms in shear flow is described in the Supplementary Information.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**References**

32. Sambrook, J., Fritsch, E. F. & Maniatis, T. *Molecular Cloning: A Laboratory Manual*. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989).
33. Skorupski, K. & Taylor, R. K. Positive selection vectors for allelic exchange. *Gene* 169, 47–52 (1996).
34. Beyhan, S. & Yildiz, F. H. Smooth to rugose phase variation in *Vibrio cholerae* can be mediated by a single nucleotide change that targets c-di-GMP signalling pathway. *Mol. Microbiol.* 63, 995–1007 (2007).
35. Bartlett, T. M. et al. A periplasmic polymer curves *Vibrio cholerae* and promotes pathogenesis. *Cell* 168, 172–185 (2017).
36. Nadell, C. D., Drescher, K., Wingreen, N. S. & Bassler, B. L. Extracellular matrix structure governs invasion resistance in bacterial biofilms. *ISME J.* 9, 1700–1709 (2015).
37. Edelstein, A. D. et al. Advanced methods of microscope control using $\mu$ Manager software. *J. Biol. Methods* 1, e10 (2014).
38. Nyland, L., Harris, M. & Prins, J. Fast N-body simulation with CUDA. *Simulation* 3, 677–696 (2007).
39. Woods, R. D. & Saxon, D. S. Diffuse surface optical model for nucleon–nuclei scattering. *Phys. Rev.* 95, 577–578 (1944).
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1. **Sample size**
   
   Describe how sample size was determined.

   Each of the n replicates of each experiment is the average of hundreds to thousands of bacterial cells as a sample size. Each experiment was replicated independently at least 3 times, but usually n>3. The n for each experiment is indicated in each figure.

2. **Data exclusions**
   
   Describe any data exclusions.

   No data were excluded.

3. **Replication**
   
   Describe the measures taken to verify the reproducibility of the experimental findings.

   Each experiment was replicated n times (and n is given in each figure for each experiment). Although the exact quantitative results differ between replicates, the qualitative results were the same, so that it is reasonable to state that the "replication was successful".

4. **Randomization**
   
   Describe how samples/organisms/participants were allocated into experimental groups.

   There were many bacterial cells within each of the n replicates. Because of the large sample size for each replicate, a representative number of samples were collected for each replicate. There was no allocation of samples into experimental groups, beyond conducting independent biological replicates.

5. **Blinding**
   
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Blinding of group allocation is irrelevant to our data analysis, because there was no allocation to experimental groups, beyond collecting n replicates, all of which were analyzed by software equally.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. **Statistical parameters**
   
   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   | n/a | Confirmed |
   |-----|-----------|
   | ☒    | ☐         |
   | ☐    | ☒         |
   | ☐    | ☒         |
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   | ☐    | ☒         |
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   | ☒    | ☐         |

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- Test values indicating whether an effect is present
- Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.
7. Software

Describe the software used to analyze the data in this study.

Matlab (version R2016b) was used to analyze data. A detailed description of the analysis is provided in the Materials and Methods section.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

All materials that were used in this study are available from standard commercial suppliers or from the corresponding author.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

All antibodies that were used are listed in the Materials and Methods section, including part numbers and supplier information. Validation: Biofilms grown from strains that do not contain the FLAG modification to RbmA did not show any antibody signal.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

b. Describe the method of cell line authentication used.

c. Report whether the cell lines were tested for mycoplasma contamination.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No eukaryotic cell lines were used.

No eukaryotic cell lines were used.

No eukaryotic cell lines were used.

No commonly misidentified cell lines were used.

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

No animals were used.

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study did not involve human research participants.