A Physical Insight of Biofilms

3

Sarangam Majumdar and Sukla Pal

Contents

Abstract

Microbial communities are the assemblies of microorganisms which live collectively on a surface being encapsulated in a matrix of extracellular polymeric substances (EPS). This particular form of the lifestyle which is collective in nature and shares the common living space is known as biofilm—the most familiar form of bacterial growth. In the last few decades, a considerable number of researches have been dedicated towards the search for mechanisms behind the biofilm growth. In this chapter, we provide a description of the collective and social microbial behavior of biofilm with underlying mechanism for better understanding of the fundamental mechanisms behind the biofilm formation. Our aim is to describe the collective behavior of the microbial community with specific emphasis on the physical process of the biofilm development on the basis of statistical theory and hydrodynamics. The physical process of biofilm

S. Majumdar (✉)
Dipartimento di Ingegneria Scienze Informatiche e Matematica, Università degli Studi di L'Aquila, L'Aquila, Italy

S. Pal
Department of Physics, Centre for Quantum Science, Dodd-Walls Centre for Photonic and Quantum Technologies, University of Otago, Dunedin, New Zealand

B. Siddhardha et al. (eds.), *Model Organisms for Microbial Pathogenesis, Biofilm Formation and Antimicrobial Drug Discovery*,
https://doi.org/10.1007/978-981-15-1695-5_3

formation often encounters the phenomena of pattern formation and is influenced by the external parameters such as environmental stress conditions and nutrient limitation. Finally, we accumulate some recent significant observations on biofilms.

Keywords
Bacteria · Biofilms · Environmental stress · Pattern formation

3.1 Introduction

Bacteria are the oldest living microorganisms in the entire world, which cause different microbial infection diseases in the body such as infections of gastrointestinal tract, eye, dental implants, urogenital tract, lung tissue, and many more (Majumdar and Roy 2018b). Bacteria as a single cell is not harmful, but when behaves collectively, it becomes pathogenic to humans. We find many pathogenic bacteria (i.e., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*) in the different parts of the human body. The bacterial coordinated collective behavior (or bacterial communication process) is known as quorum sensing. It is a density-dependent complex biochemical phenomenon and is mediated by autoinducers (small chemical diffusible signaling molecules). Bacteria emit and receive autoinducers in order to mediate gene expression which governs the communication process. When the concentration of the autoinducers reaches (at high bacterial cell density) a threshold level, a qualitative change in the bacterial communication process occurs. The single cell bacterial behavior at low cell density switches to multicellular network when the cell density reaches the threshold level (see Fig. 3.1b). It activates the genes expression (Gray et al. 1994; Fuqua et al. 1996; Shapiro 1998; Williams et al. 2007; Majumdar and Mondal 2016; Majumdar and Pal 2016; Majumdar and Pal 2017b; Majumdar et al. 2017; Majumdar and Roy 2018a). Various types of quorum sensing circuits such as ComD/ComE, AgrC/AgrA, ComP/ComA, LuxI/LuxR, TraI/TraR, LasI/LasR–RhlI/RhlR, ExpI/ExpR–CarI/CarR, AhyI/AhyR, CepI/CepR, EsaI/EsaR, EagI/EagR, YenI/YenR, and YtbI/YtbR system have been observed in gram-positive and gram-negative bacteria (Miller and Bassler 2001). Besides intraspecies communication, bacteria can communicate with other bacterium using autoinducers-2. The production of autoinducers-2 is controlled by *lux*S gene (interspecies communication) (Majumdar et al. 2012; Majumdar and Pal 2017a). This cell-to-cell communication mechanism also regulates several other biological phenomena, which includes biofilm formation, antibiotic production, virulence, motility, sporulation, symbiosis, competence, and conjugation (Miller and Bassler 2001; Majumdar and Pal 2018).

"Biofilms are aggregates of microbial cells, which are embedded in extracellular polymeric substances (EPS) that are adherent to each other and a surface" (Vert et al. 2012). This bacterial lifestyle is completely different from free living bacteria

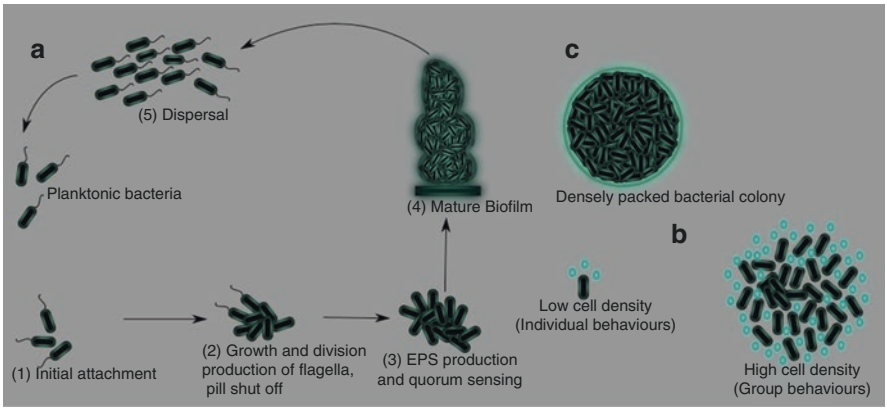

Fig. 3.1 (**a**) Schematic visualization of bacterial lifestyle and a multistage process of bacterial biofilm formation, (**b**) In cell to-cell communication process, a single bacterial cell secretes autoinducers, but we can't see any quorum sensing at low cell density. On the other hand, bacteria emit autoinducers, which are received by surrounding bacterial cells at high cell density (cell-to-cell communication occurs), (**c**) illustration of densely packed bacterial cells, which can be considered as coarse-grained system (Adapted from Majumdar and Roy 2019)

while having the emergent properties as well (see Fig. 3.1a). Fundamental emergent properties of the biofilm include social interaction, novel structures, patterns, and physical interaction (biofilm formation) (Flemming et al. 2016). Biofilms are allied with many human diseases, contamination of several medical devices, plant infections, animal infections, wastewater treatment, food technology, and many more. Biofilm consists of high cell number density (10^8–10^{11} cells per gram wet weight) and undergoes differentiation.

3.2 Single Particle Tracking Method

One can use microfluidic devices (Hohne et al. 2009), standard rheometers (Pavlovsky et al. 2013), atomic force microscopy (Aggarwal and Hozalski 2010), and combination of all to understand the physical properties of the biofilms. It is very difficult to understand several details of three-dimensional biofilm architecture in a different biofilm stage. We can study properties of reconstituted EPS (Cheong et al. 2009), motion of non-flagellated and flagellated bacteria (Rogers et al. 2008), and the effects of environment (Galy et al. 2012) using the single particle tracking. Charge interaction plays a significant role in mediating mobility inside the biofilms. We were observed that *E. coli* biofilms show height dependent charge density over time by microrheological concept and single particle tracking. We find some novel insight of the interconnecting micron scale channels (related to nutrient transfer) by the statistical analysis of bead trajectory. Moreover, this method provides a significant evidence of the biofilm structure (i.e., permeability) and its properties over time (Birjiniuk et al. 2014).

3.3 Relevance of Physical Properties in Biofilms Formation

Bacteria live inside the biofilm, which is considered as a bacterial response to external stress (osmolarity, shear, pH, starvation). Inside the biofilms, bacteria are densely packed and resistant to the hostile environment (i.e., antibiotics). Biofilm formation begins with collective cells and inert EPS matrix (as a response of external stress) (Branda et al. 2005; Monds and O'Toole 2009). This bacterial lifestyle is very stressful and heterogeneous because of nutrient limitation and limited metabolites diffusion in the matrix. In the initial stage of biofilm formation, bacteria conversation takes place, which is mediated by autoinducers (quorum sensing mechanism). This cell-to-cell communication process induces cell aggregation as well as biofilm formation. We study the chemoattraction (neglecting cell-to-cell communication) by the following set of equations:

$$\frac{\partial \rho_b}{\partial t} = -D_b \nabla^2 \rho_b + k \nabla \rho_f \tag{3.1}$$

$$\frac{\partial \rho_f}{\partial t} = -D_f \nabla^2 \rho_f - C_{\rho b \rho f} \tag{3.2}$$

where ρ_b is the local bacterial density, ρ_f is the local food density, k represents chemoattractive sensitivity, C represents bacterial consumption rate of food, D_b and D_f represent the effective diffusion coefficient of bacteria at a coarse-grained level (see Fig. 3.1c) and nutrients, respectively. The behavior of the solutions of the set of Eqs. (3.1) and (3.2) is thus highly influenced by the external environmental conditions. We notice that bacteria diffuse over time in the absence of chemotaxis (or consumption) and follow the food gradient (in presence of food). Thus, it is failed to describe the cell aggregation (Lambert et al. 2014).

Now, we consider the cell-to-cell communication process and c_t represents signaling field (quorum sensing) for chemotaxis-based bacterial movement within each micro-habitat patch (MHP) and follow the equation below:

$$T = -D.\nabla \psi_t + \chi.\psi_t.\nabla c_t \tag{3.3}$$

$$\partial_t \psi_t = \mathcal{G} + \nabla T \tag{3.4}$$

where ψ_t represents bacterial density, $\mathcal{G}$ is the local growth, and $\nabla \mathcal{T}$ is the chemotactic spatial coupling. The first term of the above Eq. (3.3) describes the balance between dispersive forces and other term shows chemotaxis-based aggregation (depend on density), at local scale (Lambert et al. 2014).

Later, Keller–Segel equations (for similar situation) are described as follows:

$$\frac{\partial \rho}{\partial t} = D_b \nabla^2 \rho - \nabla.\left[k \rho \nabla c\right] + \alpha \rho \tag{3.5}$$

$$\frac{\partial c}{\partial t} = D_c \nabla^2 c + \beta f \rho \tag{3.6}$$

$$\frac{\partial f}{\partial t} = D_f \nabla^2 f - \gamma \rho \tag{3.7}$$

where bacteria consume food at a rate γ, signaling molecule is produced by the food at a rate β, net growth rate is α, f is the concentration of the food, and c is the concentration of the chemoattractant molecule. We find instability in the bacterial population by perturbative analysis of the Keller–Segel equations. This happens at the early stage of the biofilm formation (Lambert et al. 2014).

The Keller–Segel equations give an instability at the very beginning of the biofilm formation and trigger bacterial population to crowd into small volume. We can investigate the whole developmental process from the single species of bacteria to biofilm inside micro-habitat (see detail in Lambert et al. 2014). Moreover, we find the viscoelastic properties of non-adherent biofilms, competition dynamics, and horizontal gene transfer between isolated biofilm populations (Lambert et al. 2014).

3.4 Branching Patterns

Branching patterns are common in nature, which is fascinating for both a mathematician and a biologist. We observe branching patterns in different cases, which includes circulatory system, lungs in vertebrates (Metzger et al. 2008), networks of slime molds (Tero et al. 2010), the branched colonies of corals (Helmuth et al. 1997), and roots of trees (de Smet and Jürgens 2007). Swarming is considered as population dispersal process. Bacterial colonies migrate over surface (agar plate) due to swarming. Bacterium (i.e., *P. aeruginosa*) swarming colonies can form a branching pattern (see Fig. 3.2). The dispersal ability is growing and hyperswarming has been noticed, which destroy the patterns. This experimental evidence reconstructs by computer simulation of SIMSWARM and ecological model of colony dispersal (Deng et al. 2014).

Now, we describe the mathematical formalism of branching patterns. Let us assume $L(d)$ be a spatial kernel which represents the colonization of patch at a distance d to the focal point. Using integro-difference technique, we can find out the dynamics of colonization of focal patch, N as

$$N_{t+1} = \int_{-\infty}^{+\infty} L\left(d_{x,y}\right) N_t\left(x,y\right) dxdy \tag{3.8}$$

The niche is located in coordinate (x, y) and $d_{x,y}$ represents the distance between focal niche. Shape and variance are separated by the below exponential equation for the kernel.

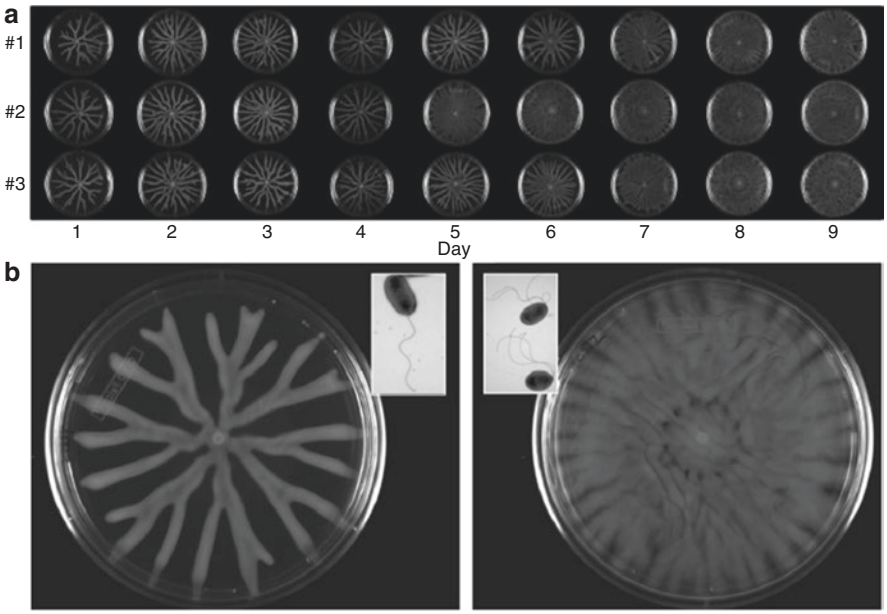

Fig. 3.2 (**a**) The experimental evidence of *P. aeruginosa* swarming colonies. It demonstrates branching patterns and how it is disrupted by hyperswarmers. (**b**) *P. aeruginosa* (wild-type) have a single flagellum (left panel). Lab experiment explores a highly dispersive hyperswarmer mutants (with multiple flagella) and the bacterial colonies without branching patterns (right panel) (Adapted from Deng et al. 2014)

$$L_{+}(d) \propto 2^{-\left(\frac{d}{d_1}\right)^{h_1}} \tag{3.9}$$

where h_1 sets the shape. The d_1 is the distance where the colonization rate $L_+(d)$ is half of its maximal. Let us consider the second process (counteracts dispersal) as follows:

$$L_{-}(d) \propto 2^{-\left(\frac{d}{d_2}\right)^{h_2}} \tag{3.10}$$

This process implies a negative effect (i.e., crowding, repulsion). The resulting spatial kernel can be represented by

$$f(d) = b \times 2^{-\left(\frac{d}{d_1}\right)^{h_1}} - 2^{-\left(\frac{d}{d_2}\right)^{h_2}} \tag{3.11}$$

where b scales the strength of the positive process relative to the negative. One can apply cellular automata model with this spatial kernel (SIMSWARM). Then the simulation generates a range of colony shapes as shown in Fig. 3.3a. If the shape factor is high, the colony becomes round. On the other hand, branched colony appears at low shape factor. In particular, the colony branching is possible (with

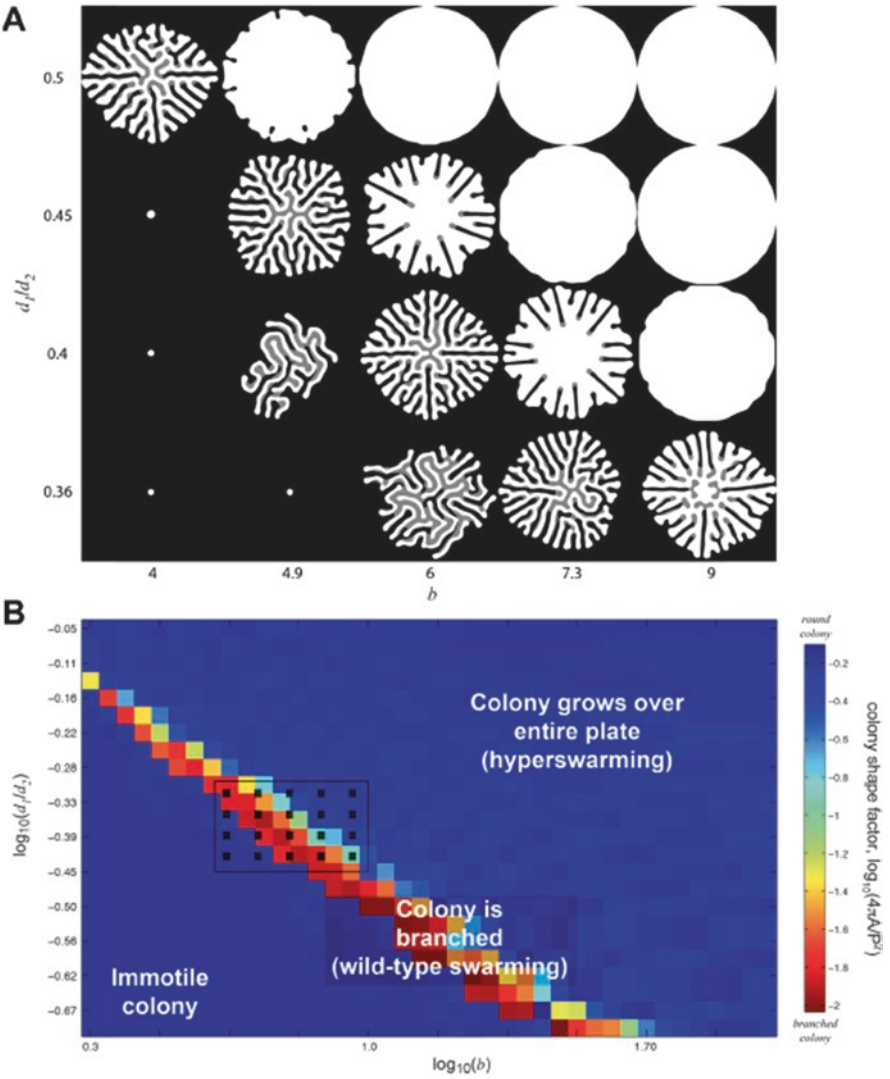

Fig. 3.3 Bacterial colony morphology induces by spatial kernel. (**A**) The colony shapes aregenerated by SIMSWARM. Parameter b (strength of the positive process) and $\frac{d_1}{d_2}$ (scale ofpositive interaction) varies across row and column respectively. The white color shows the bacteria occupied niches (currently) and grey color shows niches that were at some point occupied by bacteria. (**B**) All stimulation is conducted with $h_1 = h_2 = \infty$. Simulations shows that for each value of $\frac{d_1}{d_2}$ branching occurs in narrow windows of b (Adapted from Deng et al. 2014)

$h_1 = h_2 = \infty$) in a very narrow region of parameter space. One can find this parameter region by plotting circularity of colony shapes (see Fig. 3.2b) (Deng et al. 2014).

3.5 Recent Observations on Biofilms

- The combination of theoretical and experimental results describes that the effective cell–cell interaction potential captures the emergent architecture and growth dynamics of biofilm (Hartmann et al. 2019).
- The printable and programmable *B. subtilis* biofilms are made, which represents a completely advanced kind of living functional material. They are self-regenerating, tunable, and multifunctional. This living functional material has application in medicine and biotechnology (Huang et al. 2019).
- *P. aeruginosa* biofilm formation on the cornea is responsible for keratitis. *P. aeruginosa* biofilm infection (corneal) is limited by the neutrophilic recruitment (Thanabalasuriar et al. 2019).
- In vitro model can be useful for fundamental studies on electrochemical treatment of biofilm infections (electroceutical dressings) (Dusane et al. 2019).
- Polymer sensor array can identify bacterial species rapidly (interaction between biofilm matrix and polymer sensor elements) (Ngernpimai et al. 2019).
- Bacterial evolution and ecology are shaping by the bacteriophages. Phages induce biofilms. Biofilm formation is considered as the bacterial defense strategy (Hansen et al. 2019).
- A high intensity focused ultrasound can be used to distort the matrix of the biofilms (Bharatula et al. 2019).
- A new technological advancement (lattice light sheet microscopy) is used to find out details of biofilms development and gives crucial information on it (Zhang et al. 2019).
- Bacterial biofilms are active matter systems, which have large-scale ordered structures. Biofilms have mechanical instabilities, intrinsic length scales, and topological defects (Yaman et al. 2019).
- Exotic mechanical state is observed by the interplay between cell verticalization and cell growth. Agent-based model also reproduces the dynamical feature of biofilms (Beroz et al. 2018).
- Distant biofilm communities couple through ion-channel mediated electrical signaling mechanism. A synchronization is observed in the growth dynamics of the couple biofilms. Moreover, nutrient competition between biofilms is resolved by time-sharing behavior (Liu et al. 2017).

References

Aggarwal S, Hozalski RM (2010) Determination of biofilm mechanical properties from tensile tests performed using a micro-cantilever method. Biofouling 26(4):479–486

Beroz F, Yan J, Meir Y, Sabass B, Stone HA, Bassler BL, Wingreen NS (2018) Verticalization of bacterial biofilms. Nat Phys 14(9):954
Bharatula LD, Rice S, Marsili E, Kwan JJ (2019) Sounding out bacteria: microstructural effects of therapeutic ultrasound on bacterial biofilms. J Acoust Soc Am 145(3):1894
Birjiniuk A, Billings N, Nance E, Hanes J, Ribbeck K, Doyle PS (2014) Single particle tracking reveals spatial and dynamic organization of the Escherichia coli biofilm matrix. New J Phys 16(8):085014
Branda SS, Vik Å, Friedman L, Kolter R (2005) Biofilms: the matrix revisited. Trends Microbiol 13(1):20–26
Cheong FC, Duarte S, Lee SH, Grier DG (2009) Holographic microrheology of polysaccharides from *Streptococcus mutans* biofilms. Rheol Acta 48(1):109–115
de Smet I, Jürgens G (2007) Patterning the axis in plants–auxin in control. Curr Opin Genet Dev 17(4):337–343
Deng P, de Vargas Roditi L, van Ditmarsch D, Xavier JB (2014) The ecological basis of morphogenesis: branching patterns in swarming colonies of bacteria. New J Phys 16(1):015006
Dusane DH, Lochab V, Jones T, Peters CW, Sindeldecker D, Das A, Roy S, Sen CK, Subramaniam VV, Wozniak DJ, Prakash S, Stoodly P (2019) Electroceutical treatment of *Pseudomonas aeruginosa* biofilms. Sci Rep 9(1):2008
Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S (2016) Biofilms: an emergent form of bacterial life. Nat Rev Microbiol 14(9):563
Fuqua C, Winans SC, Greenberg EP (1996) Census and consensus in bacterial ecosystems: the LuxRLuxI family of quorum-sensing transcriptional regulators. Ann Rev Microbiol 50:727–751
Galy O, Latour-Lambert P, Zrelli K, Ghigo JM, Beloin C, Henry N (2012) Mapping of bacterial biofilm local mechanics by magnetic microparticle actuation. Biophys J 103(6):1400–1408
Gray KM, Passador L, Iglewski BH, Greenberg EP (1994) Interchangeability and specificity of components from the quorum-sensing regulatory systems of *Vibrio fischeri* and *Pseudomonas aeruginosa*. J Bacteriol 176:3076–3080
Hansen MF, Svenningsen SL, Røder HL, Middelboe M, Burmølle M (2019) Big impact of the tiny: bacteriophage–Bacteria interactions in biofilms. Trends Microbiol. https://doi.org/10.1016/j.tim.2019.04.006
Hartmann R, Singh PK, Pearce P, Mok R, Song B, Díaz-Pascual F, Dunken J, Drescher K (2019) Emergence of three-dimensional order and structure in growing biofilms. Nat Phys 15(3):251
Helmuth BS, Sebens KP, Daniel TL (1997) Morphological variation in coral aggregations: branch spacing and mass flux to coral tissues. J Exp Mar Biol Ecol 209(1–2):233–259
Hohne DN, Younger JG, Solomon MJ (2009) Flexible microfluidic device for mechanical property characterization of soft viscoelastic solids such as bacterial biofilms. Langmuir 25(13):7743–7751
Huang J, Liu S, Zhang C, Wang X, Pu J, Ba F, Xue S, Ye H, Zhao T, Li K, Wang Y, Zhang J, Wang L, Fan C, Lu TK, Zhong C (2019) Programmable and printable Bacillus subtilis biofilms as engineered living materials. Nat Chem Biol 15(1):34
Lambert G, Bergman A, Zhang Q, Bortz D, Austin R (2014) Physics of biofilms: the initial stages of biofilm formation and dynamics. New J Phys 16(4):045005
Liu J, Martinez-Corral R, Prindle A, Dong-yeon DL, Larkin J, Gabalda-Sagarra M, Garcia-Ojalvo J, Suel GM (2017) Coupling between distant biofilms and emergence of nutrient time-sharing. Science 356(6338):638–642
Majumdar S, Mondal S (2016) Conversation game: talking bacteria. J Cell Commun Signal 10(4):331–335
Majumdar S, Pal S (2016) Quorum sensing: a quantum perspective. J Cell Commun Signal 10(3):173–175
Majumdar S, Pal S (2017a) Cross-species communication in bacterial world. J Cell Commun Signal 11(2):187–190
Majumdar S, Pal S (2017b) Bacterial intelligence: imitation games, time-sharing and long - range quantum coherence. J Cell Commun Signal 11(3):281–284

Majumdar S, Pal S (2018) Information transmission in microbial and fungal communication: from classical to quantum. J Cell Commun Signal 12(2):491–502
Majumdar S, Roy S (2018a) Relevance of quantum mechanics in bacterial communication. NeuroQuantol 16(3):1–6
Majumdar S, Roy S (2018b) Mathematical model of quorum sensing and biofilm. In: Implication of quorum sensing system in biofilm formation and virulence. Springer, Singapore, pp 351–368
Majumdar S, Roy S (2019) Bacterial intelligence. Acta Sci Neurol 2(4):7–9
Majumdar S, Datta S, Roy S (2012) Mathematical modelling of quorum sensing and bioluminescence in bacteria. Int J Adv Appl Sci 1(3):139–146
Majumdar S, Roy S Llinas R (2017) Bacterial conversations and pattern formation. bioRxiv 098053. https://doi.org/10.1101/098053
Metzger RJ, Klein OD, Martin GR, Krasnow MA (2008) The branching programme of mouse lung development. Nature 453(7196):745
Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Ann Rev Microbiol 55(1):165–199
Monds RD, O'Toole GA (2009) The developmental model of microbial biofilms: ten years of a paradigm up for review. Trends Microbiol 17(2):73–87
Ngernpimai S, Geng Y, Makabenta JM, Landis RF, Keshri P, Gupta A, Li CH, Chompoosor A, Rotello VM (2019) Rapid identification of biofilms using a robust multichannel polymer sensor array. ACS Appl Mater Interface 11(12):11202–11208
Pavlovsky L, Younger JG, Solomon MJ (2013) In situ rheology of *Staphylococcus epidermidis* bacterial biofilms. Soft Matter 9(1):122–131
Rogers SS, van der Walle C, Waigh TA (2008) Microrheology of bacterial biofilms in vitro: *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Langmuir 24(23):13549–13555
Shapiro JA (1998) Thinking about bacterial populations as multicellular organisms. Ann Rev Microbiol 52:81–104
Tero A, Takagi S, Saigusa T, Ito K, Bebber DP, Fricker MD, Yumiki K, Kobayashi R, Nakagaki T (2010) Rules for biologically inspired adaptive network design. Science 327(5964):439–442
Thanabalasuriar A, Scott BNV, Peiseler M, Willson ME, Zeng Z, Warrener P, Keller AE, Surewaard BGJ, Dozier EA, Korhonen JT, Cheng L, Gadjeva M, Stover CK, DiGiandomenico A, Kubes P (2019) Neutrophil extracellular traps confine *Pseudomonas aeruginosa* ocular biofilms and restrict brain invasion. Cell Host Microbe 25(4):526–536
Vert M, Doi Y, Hellwich KH, Hess M, Hodge P, Kubisa P, Rinaudo M, Schue F (2012) Terminology for biorelated polymers and applications (IUPAC recommendations 2012). Pure Appl Chem 84(2):377–410
Williams P, Winzer K, Chan WC, Camara M (2007) Look who's talking communication and quorum sensing in the bacterial world. Philos Trans R Soc Lond Ser B Biol Sci 362(1483):1119–1134
Yaman YI, Demir E, Vetter R, Kocabas A (2019) Emergence of active nematics in chaining bacterial biofilms. Nat Commun 10(1):2285
Zhang M, Zhang J, Wang J, Achimovich AM, Aziz AA, Corbitt J, Acton ST, Gahlmann A (2019) 3D imaging of single cells in bacterial biofilms using lattice light-sheet microscopy. Biophys J 116(3):25a