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Controlling Geometry and Flow Through Bacterial Bridges on Patterned Lubricant-Infused Surfaces (pLIS)

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Spatial control of bacteria and biofilms on surfaces is necessary to understand the biofilm formation and the social interactions between bacterial communities, which could provide useful hints to study the biofilm-involved diseases. Here patterned lubricant-infused surfaces (pLIS) are utilized to fabricate connective structures named "bacterial bridges" between bacterial colonies of *Pseudomonas aeruginosa* by a simple dewetting method. It is demonstrated that the bacteria attached to hydrophilic areas and bacteria precipitated on lubricant infused borders both contribute to the formation of bacterial bridges. The geometry and distribution of bridges can be controlled using predesigned superhydrophobic–hydrophilic patterns. It is demonstrated that bacterial bridges connecting bacteria colonies act as bio-microfluidic channels and can transport liquids, nutrients, and antibacterial substances between neighboring bacteria clusters. Thus, bacterial bridges can be used to study formation, spreading, and development of bacterial colonies, and communication within and between isolated biofilms.

1. Introduction

Gram-negative bacteria such as *Acinetobacter spp.*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*), *Enterobacter spp.* are widely spread in natural and artificial environments.[1] The facultative pathogenic *P. aeruginosa* is a major cause of chronic infections strongly involved in cystic fibrosis patients and immunocompromised individuals.[2] Various mechanisms including active efflux of antibiotics, cell wall barrier, enzymatic inactivation of drugs, and/or antibiotic target changes/protection contribute to the antibiotic resistance of Gram-negative bacteria.[3] Except for its high level of intrinsic resistance, Gram-negative bacteria such as *P. aeruginosa* are able to achieve adaptive antibiotic resistance by living together as biofilms.[4]

Bridge or string-like structures of bacteria colonies were reported in biofilm studies previously. Thus, Jahed et al. used micropatterened poly(dimethyl siloxane) (PDMS) to form 3D nanostring of microcolonies of *Staphyloccocus aureus*.[5] Drescher et al. demonstrated that *P. aeruginosa* flowing through microfluidic channels made from PDMS formed streamer structures resembling biofilm bridges, causing clogging.[6] In our previous study, we used patterned liquid-infused surfaces (pLIS) to form arrays of homogeneous biofilm microclusters and observed string-like connections formed between such biofilm patches.[7] Since the string-like structure is observed under highly controlled conditions, it indicates that this phenomenon might be common in nature. The phenomenon of bacterial bridges could help better understand biofilms, complex 3D biofilms structure, function, or factors that can affect biofilm formation, and the removal of biofilms. It is not clear, how far micro-structures contribute to formation and adaptation of biofilms.

Bioinspired LIS have been introduced as an antifouling material.[8] The solid porous surface of LIS provides its mechanical stability and also stabilizes impregnating oil or lubricant.[9] Due to the liquid nature and smoothness of the liquid–liquid interface at the LIS' surface, bacteria cannot strongly and irreversibly attach to it.[10,11]

In previous study, detailed structure of biofilm bridges of *P. aeruginosa* was investigated and we showed a spatial distribution of bacteria and biomass in the bridges.[12] It was proposed that the biofilm bridges formed due to the migration or growth of bacteria on the hydrophobic repellent LIS regions. Nevertheless, the mechanism of the bridge formation was not known.

In this study, the pLIS are used to investigate the mechanism of biofilm bridge formation of *P. aeruginosa*. We hypothesize that with the correct understanding of the formation of biofilm bridges, we could control the geometry and distribution of bridges by using preset hydrophilic–superhydrophobic patterns. Such controlled bacterial bridge formation and structuring could be used to understand the biofilm formation and function both in vitro and in vivo. Potentially such bridges could be

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DOI: 10.1002/smll.202004575

used for bio-microfluidic applications to study the transfer phenomena through the bridges or in biofilm-involved infections.

2. Results and Discussion

2.1. Formation of Biofilm Bridges of *P. aeruginosa* and *E. coli* on pLIS

Patterned superhydrophobic–hydrophilic glass slides were used to prepare pLIS. The patterned glass slides were first immersed into water to form water droplets on hydrophilic spots, and then perfluoropolyether (Krytox GPL 103) was used to spread on the surface to form the lubricant infused borders between the water occupied hydrophilic spots. The excess of the lubricant was removed by dipping the slides into water and flushing the slides with a stream of water until that all hydrophilic spots were exposed to air. The porous structure of the surface, water contact angles and sliding angles of the pLIS have been shown in a previous study.[12] Multidrug resistant *P. aeruginosa* 49 previously isolated from wastewater and *E. coli* DSM1116 were used in this research.[13] The average height of LIS borders was 10.0 ± 1.9 µm (Table S1, Supporting Information). Previously we showed that string-like bacterial structures were observed between *P. aeruginosa* 49 attached hydrophilic spots after 3 h incubation in bacteria suspension (Basal Medium 2, BM2). Therefore, incubated surfaces for 3 h were used in this study instead of 24 h.[12] As shown in **Figure 1**a, there are three steps to achieve bridges on pLIS. First, the surfaces were incubated in bacteria suspension for 3 h at 37 °C with gentle shaking. During this 3 h, bacteria attached onto the hydrophilic spots. At the same time, bacteria precipitated onto the lubricant infused areas, but they were not able to attached to the lubricant infused borders due to their antifouling property (Figure 1a step 1). Next, the bacteria suspension was aspirated using a peristaltic pump, resulting in the dewetting of the liquid from the LIS areas exposing them to air and at the same time leading to the formation of bacterial bridges connecting bacteria clusters formed in the hydrophilic (adhesive) regions (Figure 1a step 2). Surprisingly, after the supernatant with bacterial suspension was completely gone, bridges remained on the lubricant infused borders (Figure 1a step 3).

Figure 1b and Video S1 (Supporting Information) show the bridge formation of *P. aeruginosa* 49 on the surfaces. On the surface incubated with *P. aeruginosa* 49 for 3 h, the precipitated bacteria layer was found to be heterogeneous with not only small bacterial clusters randomly growing on the surface, but also revealing 3D filamentous bacteria structures stemming from the surface into bacteria suspension (Figure 1b, white a and b; Figure S1, Supporting Information). Some of these

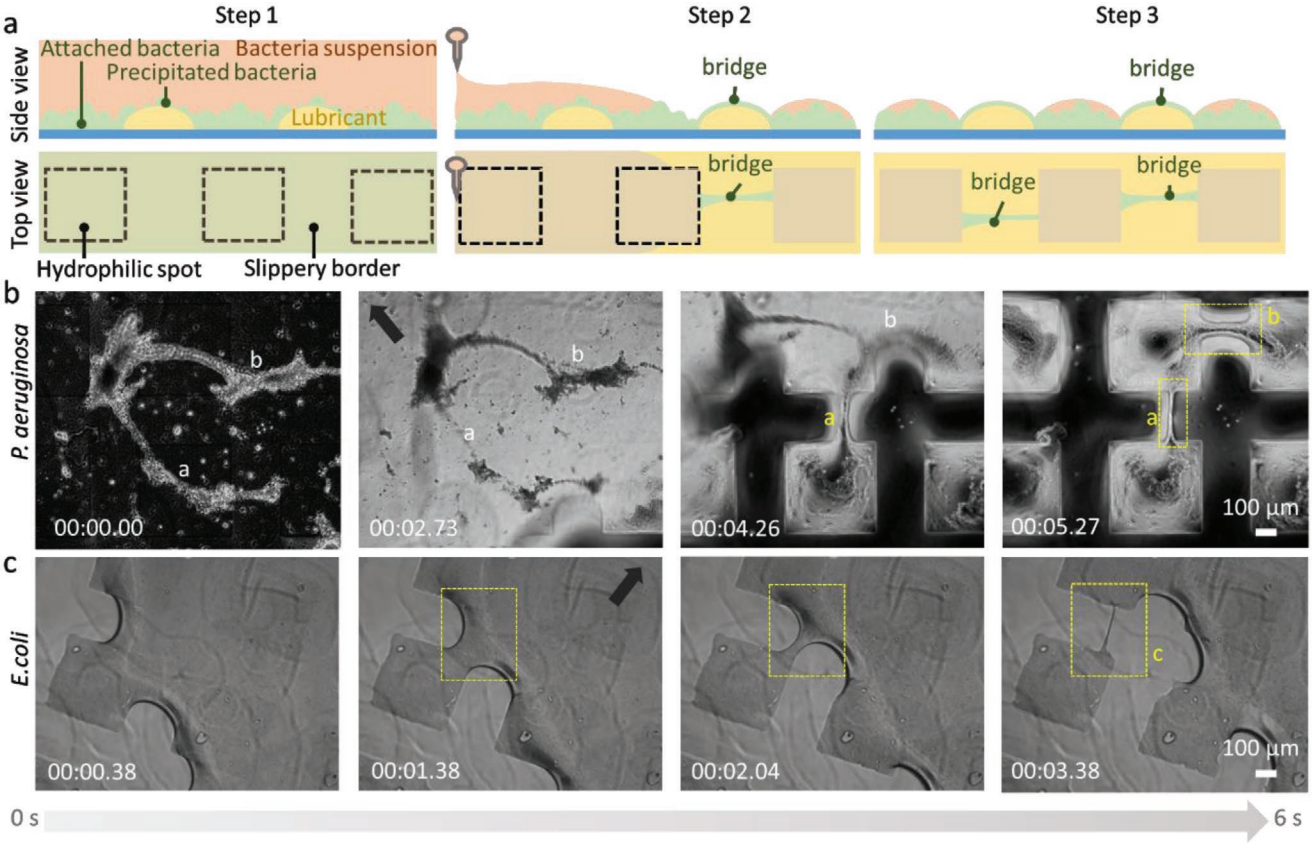

Figure 1. a) Schematic showing the bridge formation during the dewetting process on pLIS. b) Formation of bridges between *P. aeruginosa* 49 colonies attached to hydrophilic spots over lubricant infused borders. 3D filamentous structure of *P. aeruginosa* 49 in suspension is marked with "a" and "b" in white. The bridges formed are marked with "a" and "b" in yellow. c) Formation of bridges between *E. coli* colonies attached to hydrophilic spots over lubricant infused borders. Bridges formed in the area indicated by yellow dashed lines. The bridges formed are marked with "c" in yellow. Direction of liquid retraction from the surface was indicated by black arrows.

filamentous structures remained on the surface during the dewetting process, forming bridges (Figure 1b, marked with yellow color a and b). After the bridge formed, a needle was used to break the bridge. As shown in Figure S2a and Video S2 (Supporting Information), the bacteria in the bridge seemed to be not attached to the LIS border, since the two parts of a broken bridge moved towards the hydrophilic areas and did not remain fixed on the LIS border. Figure S2b and Video S3 (Supporting Information) show that bacterial bridges do not strongly attach to the LIS border even after 24 h incubation in air.

E. coli was used in order to investigate the formation of the bacterial bridges using other Gram-negative bacteria. *E. coli* formed a more homogeneous layer on the surface after incubation (Figure 1c; Video S4, Supporting Information). When the bacteria suspension was removed, bacteria attached on the hydrophilic squares remained and the bacteria precipitating on the lubricant infused borders were removed, with some of the bacteria left on the surface to form bridges. Even though the precipitated *P. aeruginosa* and *E. coli* layers showed different structure and morphology, bridges formed in both cases during the dewetting procedure.

Figure S3a (Supporting Information) shows that the bacteria number of initial *P. aeruginosa* 49 suspension was 4.5×10^7 CFU mL^{-1}. The bacteria number of the supernatant of the bacteria suspension after 3 h incubation decreased to 0.7×10^7 CFU mL^{-1}. After mixing the medium above the surface, the bacteria number of the suspension increased to 3.8×10^7 CFU mL^{-1}. This indicates that a large number of bacteria precipitated on the surface during 3 h incubation. Interestingly, as shown in Figure S3b (Supporting Information), there were no bridges formed when the surfaces were incubated vertically in bacteria suspension. It only showed attached bacteria on hydrophilic spots. This suggests that there are two requirements to form bacterial bridges. First, the surface should provide attachable region for bacteria. Only with LIS no bridge could be formed due to the antifouling property of LIS.[10,11] The second requirement is that a certain number of bacteria precipitating on the surfaces. Thus, the formation of bacterial bridges seems to be a consequence of dewetting process on pLIS covered with attached and precipitated bacteria and not due to the growth of biofilm bridges between adhesive clusters as was hypothesized in our previous study.[12]

To understand better the formation and function of bacterial bridges, it is important to investigate factors that influence its formation. Thus, we studied how nutrients presenting in the bacterial growth medium influence the bridge formation. As shown in Figure S4a (Supporting Information), no bridge was formed on the surfaces incubated in glucose-free medium, while only few bacteria colonies were observed on the hydrophilic spots. Glucose is important to form extracellular polymeric substances (EPS).[14] Absence of glucose in the medium could affect the filamentous structures of *P. aeruginosa* 49 as shown in Figure S4c (Supporting Information), leading to fewer bridges formed. There was no significant difference in the bridge number on the surfaces between samples incubated with or without DNase despite that secreted nucleic acids have been found inside bacterial bridges.[12] With lower bacteria density of the initial cell suspension (10^6 CFU mL^{-1}) no bridges were formed (Figure S4, Supporting Information). As we discussed, bacteria attached to the hydrophilic areas and also precipitated on the surface both contribute to the formation of bridges. As shown in Figure S4d (Supporting Information), fewer filamentous structures and colonies were observed on the surface incubated with bacteria suspension of lower initial cell density. Thus, we assumed that a certain initial bacterial density is required to form bridges.

2.2. Distance-Depended Formation of Bridges on pLIS

In order to investigate the distance-depended formation of *P. aeruginosa* 49 bridges, we cultured bacteria on pLIS with variable widths of the lubricant infused borders from 50 μm up to 1 mm keeping the hydrophilic adhesive spots identical (350 μm). Examples of bacterial bridges of up to 700 μm long can be seen (**Figure 2**); however, the number of bridges per square drops significantly from about one bridge per spot for 50 μm gaps down to about 1 bridge per 20 hydrophilic spots for 700 μm gaps (Figure 2f). With 350 μm hydrophilic spots, 700 μm lubricant infused borders is the limit for the formation of bridges using *P. aeruginosa* 49. In case of hydrophilic squares (length of square edge = 50, 200, 350, 500 μm) separated by lubricant infused borders of a constant width (200 μm), the bridge number increased from 0.1 per square to 0.7 per square with the increase of the size of hydrophilic squares. Therefore, bridges tend to form over short lubricant infused border with large adhesive areas. This can be useful for predicting the bridge distribution on patterned surfaces.

2.3. Control of Bridge Pattern

Understanding the mechanism of the formation of bacteria bridges allows us to create complex interconnected structures of bacterial bridges using the geometry of patterned hydrophilic-LIS structures (**Figure 3**). Since discontinuous dewetting of pLIS covered with a preincubated layer of bacteria is responsible for the formation of bacterial bridges between the adhesive regions, positioning hydrophilic spots closer to each other enables preferential bridge formation between these structures during the dewetting process (Figure 3a). In addition, since direction of liquid retraction is important for the dewetting process, it could be used to align bacterial bridges to form networks of bacterial bridges with aligned parallel lines along different directions (Figure 3a,b). Figure 3c–f demonstrates the possibility to create single bacterial bridge lines of defined geometry by positioning multiple hydrophilic spots into a certain pattern on a lubricant infused background. Such architectures might be useful to study biofilm organization and various signaling or transport phenomena within biofilms.

2.4. Bacterial Bridges as Bio-Microfluidic Channels

In order to investigate continuity of the bacterial bridges, we utilized 1 mm hydrophilic spots (square side length 1 mm, lubricant infused border between squares 500 μm) (**Figure 4**a). Bacterial bridges were formed by the incubation of pLIS with *P. aeruginosa* 49 for 3 h, followed by removing the medium to form the bridges and either leaving the structures under air

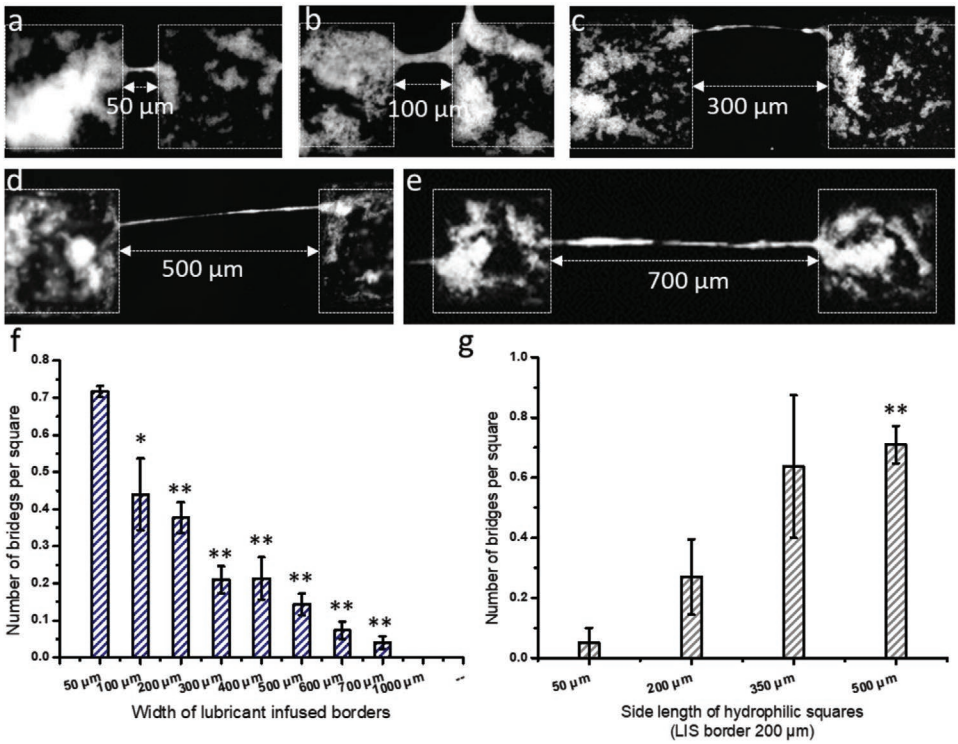

Figure 2. a–e) Fluorescence images of CTC stained bridges of *P. aeruginosa* 49 over lubricant infused borders of various widths (from 50 µm up to 1 mm). Side length of hydrophilic bacteria adhesive squares is 350 µm (indicated by white dashed lines). CTC was added into bacteria suspension from the beginning of the incubation. Images were taken after the medium was removed from the surfaces and bridges were formed due to the dewetting of lubricant infused regions. f) Number of bridges of *P. aeruginosa* 49 over lubricant infused border of different widths. Side length of hydrophilic square is 350 µm. g) Number of bridges of *P. aeruginosa* 49 on surfaces with hydrophilic squares of different sizes, while keeping the width of lubricant infused borders the same (200 µm). Data were presented as mean ± SD of three experiments with three repeats each time. The statistical significance of the experimental data was determined with a two-tailed Student *t*-test (**p*-value < 0.05, ***p*-value < 0.001).

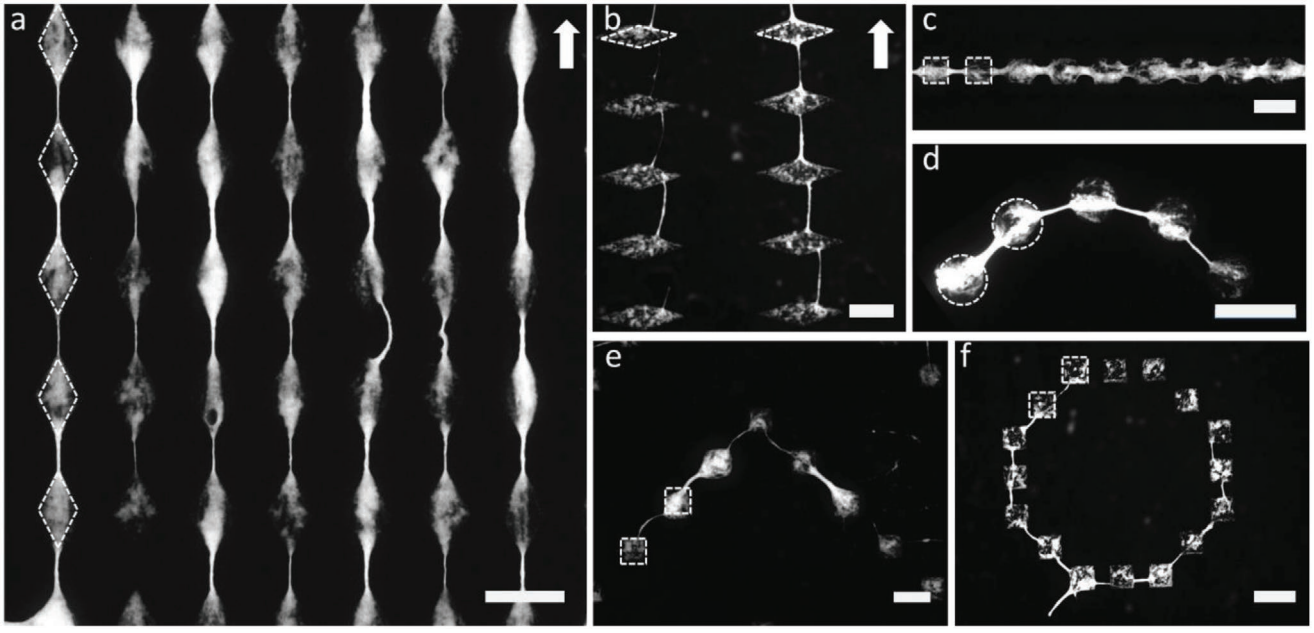

Figure 3. Creating bacterial bridge micropatterns of defined geometry. Fluorescence images of CTC stained bacterial bridges formed by *P. aeruginosa* 49 on surfaces with predesigned hydrophilic-LIS patterns. Hydrophilic spots are indicated by white dashed lines. CTC was added into bacteria suspension from the beginning of the incubation to facilitate imaging. Images were taken after the medium was removed from the surfaces. a,b) White arrows indicate the direction of liquid retraction from the surface. Scale bars: 500 µm.

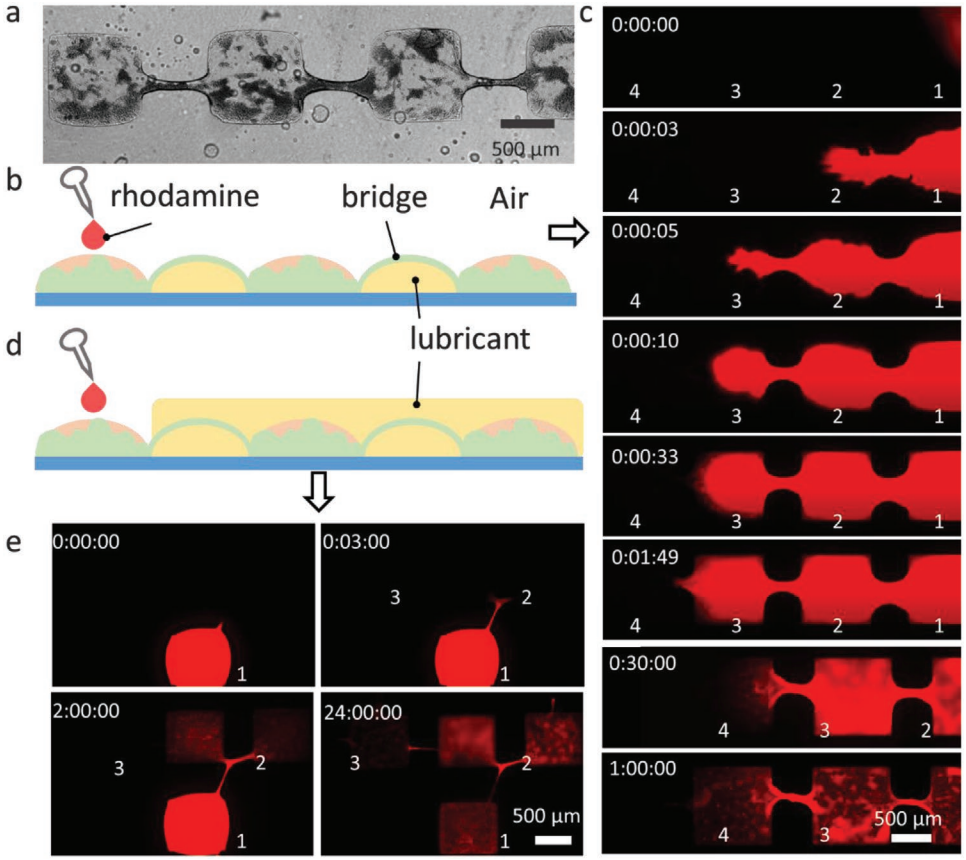

Figure 4. a) Bright field image of *P. aeruginosa* 49 on hydrophilic spots after the bacteria suspension was removed. b) Schematic representation of addition of rhodamine B solution on hydrophilic spots. The surface was exposed to air. c) Snapshot images at different time points showing the transfer of rhodamine B solution (water, 2 mg mL^{-1}) through the bridge of *P. aeruginosa* 49. d) Schematic representation of addition of rhodamine B solution on hydrophilic spots. Rhodamine solution was added on one hydrophilic spot with *P. aeruginosa* 49 which was exposed to the air. The other area around this spot was covered with lubricant. e) Diffusion of rhodamine B solution (water, 2 mg mL^{-1}) under lubricant through a biofilm bridge of *P. aeruginosa* 49. Time format shown in all images (hh:mm:ss).

(Figure 4b,c) or covering the biofilm bridges with a fluorinated lubricant (Figure 4d,e). 1 µL rhodamine B solution was added to the first hydrophilic spot (spot 1) and the spreading of the dye solution through the bridges was monitored over time (Figure 4c). Figures clearly demonstrate that the dye is spread through the bridges and does not escape from the bridges' walls. No fluorescence was observed on the lubricant infused surface outside the bridge structures. This suggests strong hydrophilicity of the bridges and their confinement. Due to the hydrophilicity of the bacterial bridges, water solution is able to wet the bridges and contributes to the transport rhodamine B to neighboring spots along bridges. The dye reached spot 2 and 3 within a few seconds and covered spot 3 within 2 min. Then the spreading of the dye slowed down and it was observed in spot 4 only after 1 h (Figure 4c). The mechanism of spreading in this case is related to wetting of the hydrophilic bridges with the aqueous dye solution. Then, we also investigated the diffusion of the dye through the bridges confined under an oil. In this case, a layer of lubricant was spread to cover the bacterial bridges as well as biofilm clusters attached to the hydrophilic squares, while keeping one hydrophilic spot exposed to air to be able to add the dye solution (Figure 4d). In this case, the spreading of the dye was significantly slower than in the open system and the dye took 2 and 24 h to reach and cover spots 2 and 3, respectively. Nevertheless, the spreading clearly demonstrated the continuity of the bacterial bridges connecting hydrophilic spots indicating its potential application to study various transfer phenomena through the bridges or bridges' functionality in vivo. The average width of the bacterial bridges was 99.4 µm while the smallest width was 18.4 µm (Figure S5, Supporting Information). These microchannels are composed of biomass and bacteria from the bacteria suspension, which makes them fully biological microfluidic channels (bio-microfluidic channels). Here we define "bio-microfluidic channels" as microstructures made of bacterial colonies, which are able to transport fluids.

To further demonstrate the connectivity of bacterial bridges as bio-microfluidic channels, we used brain heart infusion medium (BHI medium) as nutrient-rich medium and polymyxin B as effective antibiotic to *P. aeruginosa* 49 and evaluated their influence on bacteria after the transfer through bridges as shown in **Figure 5**a.[15] Water was used as control. Figure 5b shows that the number of living bacteria in spot 3 was influenced by the chemical added in the spot 1, which demonstrates that the chemical solution was successfully transferred from spot 1 to spot 3 through bridges. With BHI

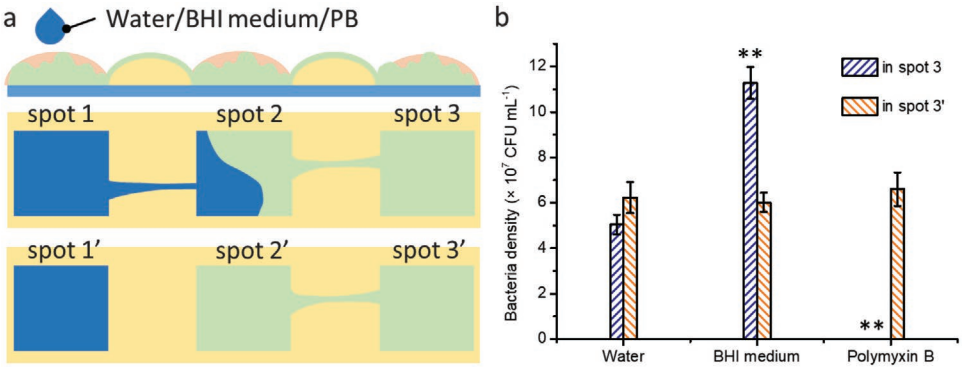

Figure 5. a) Schematic representation of experiment to study connectivity of the bridges. Side length of hydrophilic spots: 1 mm; lubricant-infused borders: 500 µm wide. Surface was kept under air after the dewetting step of the bridge formation. 1 µL water, BHI medium or polymyxin B (50 mg mL^{-1}) was added on the hydrophilic spot 1 with *P. aeruginosa* 49, respectively. b) The number of bacteria was measured either in the connected spot 3 or disconnected spot 3′ 2 h post addition of the solutions to spots 1 or 1′, respectively. Data were presented as mean ± SD of three experiments with three repeats each time.

medium added in spot 1, the number of living bacteria in spot 3 was 11.3×10^7 CFU mL^{-1}, twice as much as the number of living bacteria in spot 3 when water was added in spot 1. No living bacteria were in spot 3 with polymyxin B added in spot 1. However, there was no significant difference of the number of living bacteria in disconnected spot 3′, when different chemicals were added in spot 1′, suggesting that the solution did not spread to other spots on the surface without bridges. Therefore, the bridges have good connectivity to function as bio-microfluidic channels. Such bio-microfluidic channels could be used to study the biofilm formation, heterogeneous structure of biofilms, and the spatial variation associated cell behavior.[16] In addition, there are biofilm niches in the human body such as biofilm niches in oral cavity and in pulmonary alveoli, which are physically separated, but able to affect each other.[17] With the bridge-formed bio-microfluidic systems, it is possible to study such systems in vitro, for example, the influence of anti-biofilm compounds on heterogeneous biofilms or the transfer of signals, nutrients between biofilms communities.

3. Conclusion

Here we present a strategy to create connective bridge structures between bacterial colonies with defined geometry using pLIS in a simple dewetting process. We demonstrate the controlled spatial distribution of bridges by using specific patterns of hydrophilic spots. The ability of the bacterial bridges to transfer liquids and dissolved chemicals opens a new possibility to investigate the transfer of signals, nutrients, or small molecules in complex biofilm structures. We demonstrated that the bridges possess impressive mechanical property and were as long as 700 µm. Although the phenomenon of bacterial bridge formation has been observed by us in a synthetic artificial environment, such biofilm bridges might be common in nature. Similar wetting–dewetting conditions are present in various natural biofilm habitats. For example, lungs´ alveoli experience similar conditions and possess liquid–air interfaces where bacterial infection may lead to the formation of similar bridge structures, which might complicate proper functioning of alveoli during lung infections. Thus, investigation of this phenomenon under defined conditions and the ability to control the formation of such bridges in vitro is crucial for understanding the mechanism and function of the bridges in natural biofilm habitats.

4. Experimental Section

Materials and Instruments: Patterned superhydrophobic–hydrophilic patterned glass slides (7.5 × 2.5 cm) were obtained from Aquarray GmbH (Karlsruhe, Germany). Each slide had three compartments. Slides with hydrophilic area of different size were used. One was that each compartment had 39 × 39 square-shape hydrophilic spots. The size of each spot was 350 × 350 µm. The distance between hydrophilic spots was 200 µm. The other one was that each compartment has 14 × 14 square-shape hydrophilic spots. The size of each spot was 1 × 1 mm. The distance between hydrophilic spots was 500 µm. Slides with special designed pattern were purchased from the same company as well. Krytox GPL 103 (Dupont KrytoxR GPL 103) was purchased from H Costenoble GmbH & Co. KG (Eschborn, Germany). 5-Cyano-2,3-ditolyl tetrazolium chloride (CTC) was purchased from Polysciences Europe GmbH (Eppelheim, Germany). Brain Heart Infusion (BHI) medium was purchased from Merck (Darmstadt, Germany). DNase was purchased from Peqlab (Erlangen, Germany). Rhodamine B was purchased from Sigma-Aldrich (St. Louis, USA). Ethanol was from Merck (Darmstadt, Germany). Potassium phosphate, glucose, $MgSO_4$, $(NH_4)_2SO_4$, and $FeSO_4$ were purchased from Merck (Darmstadt, Germany). AxioImage M2 system equipped with an Apotome (Carl Zeiss, Oberkochen, Germany) was used for fluorescence microscopy.

Preparation of PLIS: PLIS were prepared as previously described.[7] Patterned superhydrophobic-hydrophilic glass slides were sterilized by dipping into 70% ethanol for 10 min. After drying in air, the slides were dipped into deionized water to form droplets in hydrophilic regions. A thin layer of Krytox GPL 103 was spread over the slides to cover the droplets of water in hydrophilic regions and infused into the hydrophobic regions. Then the extra Krytox lubricant was immediately removed by dipping the slides into water for 20 times and flushing with a stream of water for 30 s.

Formation of Bacterial Bridges on PLIS: *P. aeruginosa* strain 49 (*P. aeruginosa* 49) isolated from environmental wastewater and *E. coli* DSM 1116 (*E. coli*) were used in this study.[13] *P. aeruginosa* 49 and *E. coli* were inoculated in Basal Medium 2 (BM2; 62×10^{-3} M potassium phosphate, 7×10^{-3} M $(NH_4)_2SO_4$, 2×10^{-3} M $MgSO_4$, 10×10^{-6} M $FeSO_4$, and 0.4% glucose) separately and incubated at 37 °C with

shaking (150 rpm) overnight. The overnight culture suspensions of two bacteria were then adjust to optical density (OD) of 0.1 (≈10^7 bacteria per mL) with BM2 medium. PLIS slides were immersed into bacterial suspension and incubated at 37 °C with 50 rpm shaking for 3 h. To show the metabolic activity of bacteria, CTC was added into BM2 medium (4×10^{-3} M) from the beginning of the incubation. Slides in medium were observed with microscope after incubation. Then medium was removed with a pump set up (extraction speed: 2 mL min^{-1}) to form bridges. Samples were observed with the microscope. To investigate the influence of glucose, BM2 medium without glucose (62×10^{-3} M potassium phosphate, 7×10^{-3} M $(NH_4)_2SO_4$, 2×10^{-3} M $MgSO_4$, 10×10^{-6} M $FeSO_4$) was used for incubation. To investigate the influence of bacterial density on bridge formation, overnight culture of *P. aeruginosa* 49 was adjusted to (OD) of 0.01 (≈10^6 bacteria per mL) and used for the following incubation. To investigate the influence of DNase on bridge formation, DNase (4 U mL^{-1}) was added into the bacteria suspension from the beginning of the incubation. Bacteria suspension was then extracted after 3 h to form bridges.

Rhodamine B, and Antibacterial Chemicals Flowing through Bridges: PLIS were incubated with *P. aeruginosa* 49 suspension (≈10^7 bacteria per mL, BM2 medium) at 37 °C with 50 rpm shaking for 3 h. Then medium was removed to form bridges. 1 µL rhodamine B water solution (2 mg mL^{-1}) was placed on the hydrophilic spots with grown bacterial colonies. The flowing of rhodamine solution was recorded with the epifluorescence microscope (Axioplane 2, Carl Zeiss, Oberkochen, Germany). To investigate the flowing of rhodamine B under lubricant, the surface including the formed bridges were covered with a layer of Krytox GPL 103 again, with only one hydrophilic spot exposing to air. Then 1 µL rhodamine B water solution was placed on the hydrophilic spot with bacterial clusters exposing to air. The flowing of rhodamine B from this hydrophilic spot to the other spots through bridges was recorded with microscope.

To investigate the transfer of nutrients and antibacterial chemicals through bridges. 1 µL of BHI medium, water and polymyxin B (50 mg mL^{-1}) was added in one spot respectively. The samples were placed in box with high humidity and incubated for 2 h. The number of living bacteria in the neighboring spots was counted with plate count method, which means 1 µL of bacteria suspension was aspirated from the spot, then the bacterial suspension was diluted to proper density. Diluted bacteria suspension was spread on LB agar plates and incubated for overnight. Colony number on agar plates was counted and then the number of living bacteria in the initial spot was calculated.

Statistical Analysis: A two-sided Student's *t*-test was used for statistical data evaluation. Experiments were at least repeated three times using $n \geq 3$ samples. The statistical significance of the experimental data was determined with a two-tailed Student *t*-test (*p-value < 0.05, **p-value < 0.001).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The research was supported by the ERC Starting Grant (ID: 337077-DropCellArray). The work was further supported by the Helmholtz program BIF-TM (Helmholtz Association). W.L. is grateful to the China Scholarship Council (CSC) for the Ph.D. scholarship.

Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

antibiotic resistance, biofilms, lubricant infused surfaces, network, patterning, *Pseudomonas aeruginosa*

Received: July 28, 2020
Revised: October 11, 2020
Published online:

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