# Turning death into creative force during biofilm engineering

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Most WT bacteria thrive in multicellular communities in the form of biofilms (1). Biofilms have a highly complex 3D architecture that allows the colonization of virtually any nonshedding surface, protection against most environmental stress and a base for future surface colonization by other microorganisms. Biofilms are commonly present in water pipes, oil wells, medical implants, air conditioning, and other systems (2, 3). Much effort has been invested in combating biofilm contamination in industrial settings, agriculture, and health. They are responsible for more than 80% of all microbial infections in humans (4, 5). The remarkable mechanical and chemical properties of biofilms are attributable to a subpopulation of the cells secreting an ECM whose chemical and physical properties are now beginning to be revealed. Novel superresolution imaging approaches, such as laser scanning confocal microscopy, coupled with time-lapse video microscopy and microsensors, revealed the provoking complexity of biofilm formation and 3D architecture (6) (Fig. 1). These descriptive advances have been followed by a number of molecular genetic analyses carried out in biofilms (7, 8). The next challenge is to understand the secrets of biofilm engineering. At the cellular level, the incarceration of cells in a rigid structure requires a complex social synergy among the cells in the colony, particularly to balance and optimize cell growth and the costly production of the ECM. Because there is no individual incentive for a cell living in the biofilm to spend its resources producing ECM components, signaling pathways directing the phenotypic changes are very important. Equally important is the availability of nutrients for the matrix producers. In *Bacillus subtilis*, for example, lipopeptide surfactin not only induces a phenotypic change into matrix production, but this same subpopulation was found to also become cannibals secreting toxins (to which they are immune) that kill other cells to feed on (9). At the end of the life cycle, the cells engage in the production of resilient spores, which can be carried away and endure the trip to colonize other grounds (10).

Linked to these questions, Asally et al., in PNAS (11), focus on analyzing how the bacterial community self-organizes in space and time and determining the intricate interplay between the ECM and cell death on this elaborate process. Measuring cell displacement and cell death, together with model simulations, they discover that lateral pressures in the biofilm are relieved by areas of localized cell death (Fig. 2). Subsequent displacement of cells to these areas promotes a vertical buckling, which leads to the formation of tall wrinkly structures. These structures are crucial for the robustness of biofilms against a wide range of natural assaults and man-made treatments (as further detailed later). These tall structures were previously believed to be created by local cell growth (8), but the PNAS article by Asally et al. (11) shows that they are actually formed by cell death, a counterintuitive and provocative thought. This astonishing strategy of "turning death into creative force" during population growth in a confined space can be carried over to similar systems, including tissue development in eukaryotes.

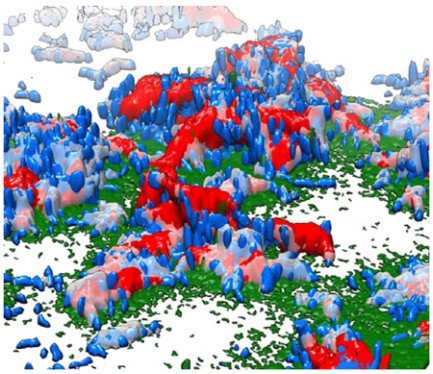

**Fig. 1.** Image of 3D reconstruction of the bacterial biofilm made by *Vibrio cholerae*. Bacterial cells (blue) attach to surfaces with a gluelike protein (green) and cement themselves together with another protein (gray). The bacterial clusters then cover themselves with a protective shell (red) made of proteins and sugar molecules (8). (Image courtesy of Veysel Berk.) Reprinted with permission from AAAS (6).

## Secrets of Biofilm Success

Biofilms are notoriously resistant to antimicrobial agents secreted by other microorganisms in the environment or applied by humans trying to eliminate their undesirable formation (2). Although single bacteria may be susceptible to antibiotics, biofilms can be 1,000 times more resistant, and most biofilm infections can only be removed surgically. Antimicrobial products are extensively used to combat biofilm contamination in health care, agriculture, and industry, and increasingly by the general public as well (12), but so far with alarmingly limited success. The same reasons that make biofilms so special also make them so hard to destroy. Secretion of the ECM forms flexible cement with remarkable structural, mechanical, and chemical properties. Composed primarily of exopolysaccharides and proteins, the ECM provides a strong and enduring yet flexible physical support structure, acting as a filter that protects and shields the cells from external chemical assaults and at the same time enables diffusion of nutrients deep into the colony. Biofilms have been reported to survive chlorine bleach for 60 min, continuous flushing with biocides in pipes for more than 7 d, and iodine solution for as long as 15 mo.

The surface of biofilms produced by *B. subtilis*, a model organism for such studies, is a strong liquid repellent against a wide variety of solvents. It is not only hydrophobic, but also resistant to liquids of lower surface tension, such as 80% ethanol. In fact, the biofilm resists even prolonged exposure to vapors. Such remarkable macroscopic properties come not only from the molecular interactions with the matrix but also from the intricate structure of the biofilm. Laser scanning confocal microscopy (13) and the "painting with light" approach (6) have recently revealed details of the biofilm architecture. A complex surface microstructure adds to the liquid repellency, and wrinkles and protuberances form tall structures that increase the surface-to-volume ratio and are thought to facilitate the dissemination of spores (10).

## Revealing Intimate Structures by Continuous Immunostaining

A new technique of continuous immunostaining allowed tracking of four separate target molecules by means of four separate fluorescent dyes (6) (Fig. 1). The use of this method together with superresolution

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light microscopy enables a closer look than ever before at the formation of the structure of biofilms. Keeping all the fluorescent probes in parallel inside the solution while imaging makes it possible to continuously monitor multiple processes, starting from a single cell all the way to a mature biofilm. This revealed that biofilms displayed three distinct levels of spatial organization: cells, clusters of cells, and collections of clusters. This complex organization begins when a single bacterium lays down glue to attach itself to a surface, then divides into daughter cells, making certain to cement each daughter to itself before splitting in two. The daughters continue to divide until they form a cluster —like a brick-and-mortar building—at which point the bacteria secretes a protein that encases the cluster like the shell of a building. The clusters are separated by microchannels that may allow transport of nutrients into the biofilm and waste products out. The process involves complementary architectural roles provided by several ECM constituents: adhesion to the surface; cell–cell adhesion; and formation of a dynamic, flexible, and ordered envelope that encases the cell clusters. Both this study (6) and the PNAS article (11) address aspects associated with the complex spatially clustered heterogeneity of the biofilms. It is reasonable to expect that applying this method to study the role of cell death during biofilm formation will provide new surprises and help expose the intimate secrets of biofilm engineering.

## Searching for Novel Strategies to Combat Biofilm Formation

The two main strategies in the war against biofilms are the development of (*i*) new antibacterial drugs (14) to eliminate existing biofilms and (*ii*) special surface coatings that use nanotechnology and smart materials (15) to prevent cell adherence to the surface. So far, bacteria seem to be able to develop ways to avoid the manmade treatments faster than we can develop new materials. This distressing situation calls for outside-the-box approaches. The recent understanding of the secrets of the special cooperative behaviors used by the bacteria during biofilm formation can provide the clues needed for the development of new strategies. For example, we can envision the development of drugs that will tamper with communication or send wrong signals that will confuse cell–cell coordination (16). Another intriguing strategy is to use bacteria as an agent to help the combat. Recently, Houry et al. (17) showed that swarming bacteria can infiltrate biofilms, and, in doing so, create transient pores that increase macromolecular transfer within the biofilm. The infiltrating bacteria can be cooperative and help the biofilm to absorb nutrients from the environment, or be competitive and cause facilitated penetration of toxic substances from the environment. Such competitor bacteria can be harnessed to combat biofilm as a Trojan horse, introducing manmade drugs. This idea can be extended to engineer "bacterial agents" that can destroy the cell–cell coordination and thus weaken the biofilm structure. Other agents, trained to degrade the ECM that the cell uses to adhere to the surface and to each other, can be used to destroy the biofilm completely.

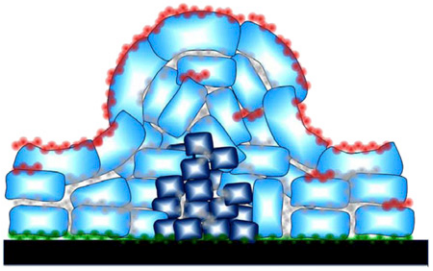

**Fig. 2.** Illustration showing how localized cell death (dark blue) can release lateral pressures and attract living cells (blue) to the vicinity of the dead cells. The resulting displacement generates the buckling and the wrinkle. As in Fig. 1, ECM is shown in green, red, and gray.

The findings in the study of Asally et al. (11) bring to mind another class of "cyberwar"-like strategy: one can envision fighting biofilms by sending signals that prevent cell death, increase fast death, or increase proliferation so that the balance between growth and cell death is destroyed.

## Looking Ahead

The new findings about the creative role of cell death during biofilm formation might provide valuable insights on why apoptosis is important for embryonic development (18). Drawing on the parallels between the two systems suggests that bacterial regulated cell death might be a rudimentary form of apoptosis. Therefore, there is the possibility that biofilms can be a valuable model to study programmed cell death in eukaryotes. By the same token, the new findings might be important for cancer research. Cancer cells are known to share similar traits with embryonic cells. Recently, it has been conjectured that they also adopt mechanisms and survival strategies similar to those exploited by bacteria (19), e.g., cell fate determination at adverse times that is stochastic on the individual cell level but controlled on the population scale (20). Put together, the new findings can help us understand the role of self-regulated cell death in tumors during tumorigenesis. How much we will be able to learn about cancer by comparing these two systems is still an open question, but provides an exciting new challenge.

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