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Viewpoint

Toward Programming Bacterial Behavior via Synthetic Interfaces: Physicochemical Nanopatterning, Decoupling Surface Properties, and Integrating Material and Biological Insights

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A. INTRODUCTION: FROM ANTIFOULING MATERIALS TO GREATER CONTROL OVER BACTERIAL BEHAVIOR

Controlling the behavior of living organisms is a central topic in biomaterials design. Synthetic materials, empowered by rapid advances in surface engineering capabilities, present a highly programmable medium that may interface with living organisms in close proximity and gain broader control of the population via communal signaling. Interface-based control strategies are distinct from drugs and signals delivered via liquid phase and bring some unique advantages. For example, they help to reduce systemic side effects, enable targeted (and possibly more pronounced) effectuation, and even permit spatiotemporal programming of the properties of material surface, allowing multiplexed control over cellular and communal behaviors.[1] It shall be noted that here the concept of "surface properties" encompasses a holistic collection of structures and properties, including topography (e.g., static or dynamic), native and derived chemistry (e.g., surface energy, surface charge, surface-immobilized ligands and proteins), and mechanical properties (e.g., stiffness).

A plethora of research on biomaterials and biointerfaces has focused on modulating the behaviors of *mammalian* cells, such as attachment, growth, orientation, mobility, differentiation, and functions, using various surface properties.[2,3] In comparison, material-centric control of *bacterial* behaviors is underexplored, despite the ubiquity and importance of bacteria in essentially every environment in nature.[4] For example, bacterial and mammalian cells often compete in occupying the surface of biomaterials (such as orthopedic implants),[5] a process termed "race for the surface".[6,7] While the biological activities involved in the adhesion and surface growth of mammalian cells are well understood, reports on bacteria–material interactions often treat bacteria as passive colloids and resort to simplified physicochemical explanation for the bacteria–material interactions.[8] Here, "physicochemical" refers to any or all of the following interactions, e.g., hydrophilic/hydrophobic, electrostatic, van der Waal's, steric interactions, etc., that do not depend on bacterial sensing, biochemical signal transduction, and responses to effectuate. As a first step toward controlling bacterial behaviors via the synthetic interface, it is imperative to recognize bacteria to be complex and dynamic living organisms and to understand bacteria–surface interactions to predict potential biological effects elicited by synthetic materials.

Driven by the need to reduce biofilm formation, considerable research efforts have been focused on understanding initial bacterial attachment through combined microbiological and biophysical approaches. For example, on an individual cell level, obstructed retraction of surface-bound Type IV pili serves as a mechanical cue for *Caulobacter crescentus*, triggering holdfast synthesis;[9] on a communal level, *Pseudomonas aeruginosa* cells visiting material surfaces at different point in time can coordinate their surface exploration by leaving behind exopolysaccharide (e.g., Psl) trails, leading to the emergence of microcolonies.[10] Such fundamental bacterium-centric discoveries may have important implications for developing novel material-centric intervention strategies beyond what purely physicochemical perspectives have offered. The resultant progress will benefit a wide variety of human activities ranging from healthcare, food safety, and water treatment to marine antifouling.

To achieve the above-mentioned material-centric programming of microbial functions, we must go beyond the material functions of antifouling and antimicrobial and seek answers to two fundamental questions: (i) How do bacteria adapt their metabolism and signaling in response to surface properties? (ii) What are the key design parameters (e.g., material properties) that can *reliably* predict the response of attached bacteria? Answers to these two key questions are inextricably linked. Fundamental understanding of the effect of surface properties on microbial behaviors is an essential step to achieving a comprehensive predictive model, and the predictive model will guide the rapid exploration of a broader scope of surface properties for targeted microbial behaviors. The complex and intricate interplay among the three primary components of this system–material surface, bacteria, and the aqueous environment may preclude a singular theoretical framework. Nonetheless, as Curtis and Wilkinson pointed out two decades ago, the initial

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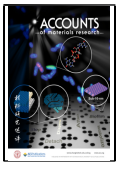

exploration must involve *systematic* examination of the reactions (of bacterial cells in this case) to "simple well-defined" surfaces.[11] Engineering of such surfaces, however, is anything but simple, especially given the potentially vital role played by sublithographic nanostructures (as described under "Need I" below).

As summarized in Figure 1, in this Viewpoint, we aim to share our insight on three prominent needs in the pursuit of understanding bacterial response to synthetic interfaces via "simple well-defined" material design. As evidenced by the nature of these needs detailed in the following section, multidisciplinary collaboration involving expertise in surface science, nanoengineering, data science, and microbiology will prove essential to meeting such needs.

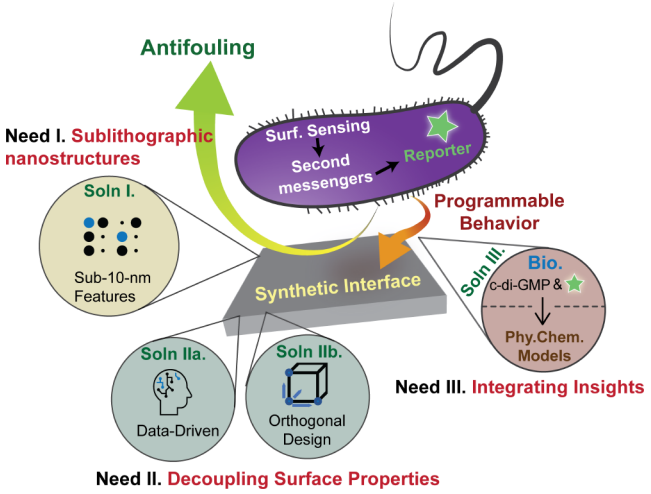

Figure 1. Three prominent needs to be addressed in order to elucidate fundamental bacteria−surface interactions and some promising strategies (in circles) covered in this viewpoint to address them.

B. RESEARCH NEEDS IN UNDERSTANDING FUNDAMENTAL BACTERIA−SURFACE INTERACTIONS

Need I: Nanopatterning beyond Conventional Lithography Limit

Compared to the extensive research devoted to understanding how mammalian cells interact with nanoengineered surfaces, we just started to scratch the surface of how bacterial cells interact with nanoengineered surfaces. This is in part because bacterial cells (of size ∼1 μm) are generally about 1 order of magnitude smaller than mammalian cells. Their surface appendages (e.g., fimbriae and flagella) and envelope proteins (e.g., OprF[12] and PilY1[13]), which are often responsible for environmental sensing, are smaller still, with typical fingerprint widths of 1−10 nm on surfaces.[14−17] Correspondingly, in order to create surface features (such as topographical and chemical patterns) that can interface with the sensory apparatus (e.g., to elicit differential interactive strengths), an important prerequisite is to manipulate surface properties on a length scale comparable to or even smaller than those bacterial appendages and membrane proteins, i.e., sub-10 nm nanoengineering (or sublithographic engineering).

The evidence from multiple studies suggests that surface nanoscale features with characteristic length less than hundreds of nanometer (i.e., the lithographic length scale) could affect three aspects: (i) *Multilevel bacterial response to surfaces*: e.g., surface arithmetic roughness (R_a) on the order of 10^2 nm, denoted "$O(10^2)$" nm, altered morphological, proteomic, and transcriptional responses of surface-bound *Escherichia coli* and *P. aeruginosa*, typically featuring down-regulation of fimbriae (e.g., Types I & IV) and transport proteins (e.g., porins) while up-regulating proteins involved in stress response (e.g., osmotic pressure-inducible membrane protein, OsmC).[18,19] (ii) *Conditioning layer characteristics*: e.g., it is evident that morphology of nanofeatures dictates the amount, speciation, orientation, and conformation of surface-adsorbed proteins,[20] thus influencing the properties of protein conditioning layers that bacteria may sense and respond to. (iii) *Local interfacial free energy landscape*: e.g., compared to microfeatures, nanofeatures could exert greater influence on interfacial forces, thanks to their substantially larger surface-to-volume ratio and more frequent presence of sharp projections (e.g., pore walls, pillars, cones).[11,21,22] That imbalanced local distribution of interfacial free energy may influence not only the nonspecific physicochemical interactions between bacteria and the nanostructured surfaces[23] but also bacterial mechanosensing of such surfaces. Taken together, improved understanding of how bacteria respond to topographical patterns could be achieved with advances in nanoengineering capability, which calls for well-defined nanotopographical patterns with tunable shapes and sizes.[23]

However, lithographic and sublithographic (i.e., sub-10 nm) engineering face different challenges. For engineering with lithographic length scale [$\sim O(10^1)-O(10^2)$ nm], mature nanofabrication technologies such as photolithography and nanoimprinting lithography offer excellent reproducibility, precision, and design flexibility; nevertheless, patterning on curved surfaces remains a challenge. This challenge is especially evident during the translation of benchtop discoveries to real-world applications such as patterning on the curved surfaces of orthopedic implants. As for sublithographic engineering, technological feasibility has been a long-standing bottleneck.

Studies in the past decade have demonstrated early promises for sublithographic patterning by electron beam lithography[24] and directed self-assembly techniques (e.g., block copolymers,[25] nanoparticles,[26] and anodization[27]). In addition to the nanoscale surface patterns, nanostructures fabricated using bulk materials, such as the nanopores in anodic aluminum oxide (AAO), offer a useful platform for studying bacteria−surface interactions.[21,28,29] Existing anodic etching protocols have produced hexagonally arranged nanopores (circular, triangular, or squarely shaped[30]) with tunable aspect ratios (e.g., diameter, 6−500 nm; pore length, tens of nm to hundreds of μm)[27,31,32] distributed over macroscopic areas (on the order of cm^2 to m^2), without requiring a clean room. The chemical stability of aluminum oxide also provides a resilient foundation for subsequent engineering of surface chemistry and biofunctionality, for understanding decoupled effect of surface properties on bacterial behaviors, as discussed below.

Need II: Decoupling Surface Topographical and Chemical Properties

Since all surfaces exhibit topographical and chemical (and mechanical in some cases) properties simultaneously to the cells they interface with, decoupling of these properties thus becomes pivotal to understanding the relative contribution of each property to bacterial behaviors.[33] As discussed in the Introduction, systematically varied and precisely characterized

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Table 1. Comparison between Two Decoupling Strategies for Understanding Fundamental Bacteria−Surface Interactions[a]

	data-driven decoupling	orthogonal-design decoupling
	Data	
nature of decoupling	mathematical decoupling	physical decoupling
data set requirement	large data sets; typically, > $O(10^2)$ training data are required	compatible with small data sets
orthogonal engineering capability	not essential, but helps to reduce the minimum data set size needed	essential
precise characterization of surface properties	Essential	essential
currently achievable feature spatial resolution	$\sim O(10^2)$ nm	$\sim O(10^1)$ nm
emerging examples and solutions	high-throughput topography fabrication and ML;[35] chemistry-topography dual screening and ML[44]	sequential orthogonal engineering (e.g., AAO + iCVD)
	Modeling for Data Interpretation	
models appropriate for data	inductive; regression-based	deductive; mechanistic
interpretability	trade-off between predictive power and interpretability[39]	more interpretable; terms usually have physical and/or biological meaning
generalizability	generally, predictions only valid within the scope of the data supplied	once validated, model can be applied to various systems as long as the key assumptions are satisfied
	Outcome	
nature of conclusions (e.g., on surface properties-bacterial behaviors relationship)	primarily statistical/correlational	more suited for deriving mechanistic/causal relationship
suitable/preferred questions	"what work?" "how?" (may require human insights)	"how?" & "why?"

[a]This table is inspired by the work reported in ref 41.

surface properties are crucial to progress in this regard.[11] Nevertheless, there have been few studies reporting successful systematic variation, i.e., the capability to vary one property at a time, or precise characterization of these surface properties (e.g., the intrinsic surface energy of curved surfaces) in the context of bacteria−material interactions. For example, surface chemistry could be inevitably altered during topographical modification by chemical etching, due to introduction of chemical impurities or affinity of crystallographic facets to specific ligands.[33] Conversely, unintended surface topographical changes could be introduced during surface chemistry modification, due to side reactions (e.g., self-polymerization of silane) or nonconformal coating on nanostructures.[34] Confounding surface properties can lead to inconsistent trends regarding the surface topography−attachment or surface chemistry−attachment relationships, thus impeding the progress on understanding bacteria−surface interactions.

Two strategies have shown promise in decoupling the effects of surface topography and chemistry (and other surface properties) (Table 1): (i) data-driven decoupling, which relies on generation of big data via high-throughput material screening platforms followed by pattern recognition via machine learning (ML)/artificial intelligence (AI) to statistically isolate the effects of each surface property on bacterial behaviors,[35,36] and (ii) orthogonal experimental design and surface fabrication (i.e., "orthogonal-design decoupling"), which leverage ultraconformal coating techniques (such as chemical vapor deposition) in combination with the aforementioned nanofabrication techniques (see Need I) to enable independent control over surface topography and surface chemistry in two separate steps.[28,37]

The data-driven decoupling of the biological effects of surface properties takes full advantage of the rapid-advancing ML/AI capabilities, which has become increasingly good at answering the "what"-type questions: e.g., identifying exemplary materials in an existing material library for a given performance target, or even informing the design of new molecules or materials with optimal performances from an abstract latent space.[36,38] Efforts have also been made to increase the interpretability of ML models (often at the expense of model predictive power[39]) so that we can learn "how" the ML models learn the underlying patterns and make predictions: e.g., isolating influential surface features or descriptors (e.g., molecular fingerprints) from a high-dimensional feature space based on their weights.[39,40] Nevertheless, ML cannot yet uncover the physicochemical or biological mechanisms underpinning the performance of each surface property (i.e., the "why"-type questions)—for example, is the bacterium avoiding the surface due to the repulsive physicochemical forces or biochemical signaling? (See Need III for more discussion.) The correlations predicted by ML may reveal new patterns, which can serve as evidence for novel hypothesis. But the formulation of mechanistic hypotheses often hinges on human input.[41] Furthermore, application of the data-driven approach requires high-throughput experimental techniques to generate sufficiently large training set [typically $\sim O(10^2)$ or more for predicting bacteria−surface interactions[36,40,42]], many of which are still under development. For example, the smallest lateral feature dimensions achieved thus far by the high-throughput fabrication (via photo- and nanoimprinting-lithography) are ∼100−200 nm,[35,43] which are more than 2-fold greater than the target topographical length scale for exploring the new frontier of bacteria−surface interactions (as discussed in Need I). Notwithstanding this size limitation, recent promise unveiled by chemistry-topography dual screening platforms[44] has provided an tantalizing glimpse of ways to devise combinations of surface properties (i.e., complex cues) that surpass the bacteria-instructive performance achievable by any property alone.

In contrast, the experimental decoupling of topographical and chemical features is well suited for generating key insights from a much smaller yet thoughtfully constructed data set, which in turn serves as a basis for generating and testing novel hypothesis and mechanistic models.[41] A potentially effective way of implementing this approach is through orthogonal engineering: for example, well-defined surface nanotopography can first be generated through the above-mentioned anodization technique, followed by modification of surface chemistry/mechanical properties via conformal coating technologies.[45] Conformal coating technologies are key to enabling the orthogonal

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engineering because it allows for chemical modification of a surface without changing its nanotopography. Unlike most solution-phase coating techniques with limited conformality due to undesirable surface tension effects, vapor-based treatments, such as CVD, are ideal candidate technologies due to the Knudsen diffusion enabled by the vacuum condition. Furthermore, the coating technique should be sufficiently benign to avoid damage to the underlying nanostructures. The room-temperature initiated CVD (iCVD) thus represents a suitable option for orthogonal engineering, given its compatibility with over 70 functional monomers,[46] outstanding conformality on ultrahigh aspect ratio nanostructures,[47] and ability to deposit ultrathin (<10 nm) polymer films.[48] Other characteristics of iCVD-synthesized ultrathin films, such as high retention of functional groups, adjustable degree of cross-linking, being solvent free, etc., further enables precision synthesis of the surface chemistry, controllable mechanical properties, and compatibility with solvent-sensitive substrates (such as paper-based devices).[1,49]

Need III: Integrating Materials Insights and Microbiology

Bacteria−surface interactions are ultimately determined by the interplay between physicochemical interactions (e.g., electrostatic, van der Waal's, hydrophobic, hydrodynamic, steric interactions)[8] and the biological effects (e.g., environmental sensing and subsequent adaptation, metabolism, group behaviors).[50] For example, interactions between bacterial sensing molecules/appendages and material surface moieties (e.g., FimH-mannose binding) can be tuned by engineering the surface physicochemical properties, which subsequently triggers signal transduction within a bacterial cell.

Given this interplay between physicochemical and biological effects, it follows that a comprehensive model to describe the effects of microbe−material interactions require input from both fields.[51] A potential path forward is for physicochemical models to provide the backbone of a predictive framework in which detailed biological effects can be added.[52] For example, many prior studies employed the (extended) Derjaguin−Landau−Verwey−Overbeek (DLVO) theory to capture bacterial attachment and biofilm formation on various surfaces (e.g., glass and metal-oxide, and AAO with varied nanotopography) with some success.[21,53,54] While the (extended) DLVO theory captures the thermodynamic driving forces during bacterial adhesion, it has two major limitations: (i) its predications are often made based on properties (e.g., surface zeta potential and contact angles) measured and averaged over macroscopic length scales (e.g., millimeter or centimeter), orders of magnitude greater than the typical length scale [i.e., $O(10^{-1})$−$O(10^{1})$ nm] on which "specific" interactions such as molecular recognition operate;[55] (ii) it treats bacteria as passive, simple-shape colloidal particles, which obscures the sophisticated environmental adaptability that is characteristic of living organisms; this simplification also overlooks the dynamic nature of the cellular body (hence the dynamic interacting geometry with material surfaces) and the role of its surrounding appendages during bacteria−surface interactions.

Similar to decoupling surface properties (Need II), in order to further advance our understanding of how physicochemical effects and biological factors interplay, it is also important to decouple the contribution of "passive" physicochemical driving forces (e.g., electrostatic interactions between bacteria and a surface) and "active" bacterial sensing and adaptation (e.g., driven by enhanced nutrient intake or escape from predators) to the observed bacteria−surface interactions (e.g., bacterial adhesion to synthetic surfaces). To achieve this, one approach is by comparing the adhesion of fixed bacterial cells (ideally with fixation performed without affecting their surface physicochemical properties) and that of live cells as representations of "passive-only" and "passive-plus-active" scenarios. Along this line, *E. coli* and *P. aeruginosa* were discovered to adhere to polydimethylsiloxane (PDMS) with varying stiffness in a similar trend as carboxylate-terminated polystyrene beads (showing more adhesion on softer PDMS surfaces), which seemingly suggested that passive driving forces dominated the adhesion.[56] Nevertheless, the number of adhered bacteria was at least an order of magnitude greater than the number of adhered polystyrene beads, hinting at the non-negligible effects of "active" settlement.[12,57]

Alternatively, one could assess the importance of different mechanisms of attachment by tracking the bacterial signal transduction processes using reporter molecules—akin to reading the bacterial "mind". For example, cyclic-3′5′-diguanylate (c-di-GMP), a universal second messenger conserved across bacterial genera for regulating the transition between planktonic and sessile states, has garnered increasing interest as a proxy for such bacterial decision. Recently, real-time *in situ* monitoring of c-di-GMP levels has been made possible by using an engineered plasmid that enables production of intracellular GFP reporters, thereby green fluorescence, in proportion to the c-di-GMP level.[58] By tracking c-di-GMP expression in various isogenic mutants, envelope protein PilY and Type IV pili were identified as mechanosensors deployed by surface-bound *P. aeruginosa*, for sensing fluid flow at the interface and adapting to this dynamic environment.[59] Compared to transcriptomics and proteomics, which provide more comprehensive views of the biological activity landscape (but often demand at least ~10^9 cells to perform the assays[19]), the use of c-di-GMP reporter approach stands out in its real-time monitoring, single-cell resolution (and thus enabling the investigation of cellular variation), and relatively low cost. Simultaneous labeling of multiple second messengers involved in different bacterial sensing pathways may further expedite the progress toward decoding the bacterial sensing and decision-making processes key to interfacing with synthetic materials. Ultimately, future research effort should be guided toward integrating the vast biological information with the physicochemical models to arrive at more comprehensive models with greater predictive power.

C. CONCLUDING REMARKS AND OUTLOOK

With rapid development of surface nanoengineering techniques, application of ML in materials and microbiology, and bioengineering and biological characterization tools, we are more equipped than ever to achieve the goal of decoding fundamental bacteria−surface interactions, which in turn paves the way for encoding cues via synthetic interfaces to control bacterial behaviors. To fully realize the potential of material-centric programming of biological functions, fundamentals of bacteria−surface interactions must first be scrutinized and understood in a rigorous and quantitative manner. Bacteria-instructive interfaces can learn from and expand on similar research done on mammalian cells. Nevertheless, more emphasis needs to be put on precise sub-10 nm patterning of various surface topography, which can be further extended to chemical and mechanical patterns on that length scale, to uncover surface-associated cues recognizable by bacteria (Need

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I). The ability to decouple the surface properties, via application of data-driven statistical decoupling and/or experimental decoupling such as orthogonal nanoengineering, will prove pivotal in clarifying the individual and synergistic effect of surface properties on bacteria−surface interactions (Need II). Finally, quantitative evaluation of the relative contribution of the "passive" physicochemical interactions and the "active" bacterial sensing and adaptation activities will also serve an important step toward fully capturing the complexity of bacteria−surface interactions (Need III).

Surface features on the molecular level, their spatiotemporal distribution and evolution on the nanoscale, and microscopic properties may be analogous to the "alphabets", "words", and "sentences" of a language for communicating with bacteria. We envisage that as we decode this language, surface cues may be engineered to incentivize desired bacterial behaviors, such as biosynthesis, self-repair, and electron transfer.

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Notes

The authors declare no competing financial interest.

Biographies

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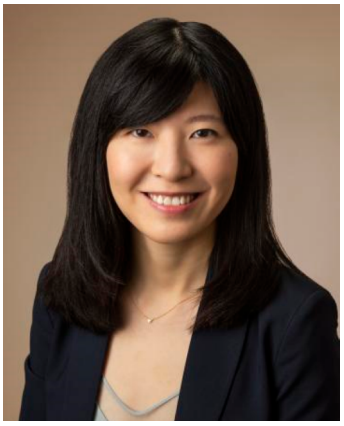

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