Trends in Biotechnology

CellPress OPEN ACCESS

Forum

Engineering biology approaches to modulate bacterial biofilms

Clodagh M. Carr [1], Lyuboslava G. Harkova [1], and Ronan R. McCarthy [1,*,@]

Building on a productive two decades of advancements in synthetic biology, engineering biology now promises to enable the implementation and scale-up of novel biological systems tailored to tackle urgent global challenges. Here we explore the latest engineering biology approaches for the control and modification of bacterial biofilms with exciting new functionalities.

Engineering biology, which refers to the application of engineering principles (e.g., standardization, quantification, automation, simulation) to biological systems, aims to expand the potential of synthetic biology approaches for translation into real-world processes. While microorganisms have frequently been employed in biotechnological applications, this typically involves growing single strains of free-floating cells, as opposed to the dynamic, synergistic biofilm communities found in nature. Biofilms have been studied primarily from a human health perspective, where bacteria form a sticky gelatinous matrix to colonize biotic surfaces (e.g., teeth, skin, and lungs) and abiotic surfaces (e.g., water pipes and medical tubing), posing a particular threat to the health of vulnerable groups such as immunocompromised individuals. The positive attributes of bacterial biofilms have been investigated to a far lesser extent, but these biofilms have been used, for example, as part of bioremediation and wastewater treatment processes in the breakdown and removal of pollutants. By harnessing the ability of biofilms to attach to surfaces, self-repair, and withstand environmental stresses, we can develop beneficial biofilm-based solutions, and, using genetic tools, we can engineer biofilms with enhanced properties for a variety of biotechnological applications (see Figure 1).

Targets for engineering biofilms

Given that biofilm formation is mediated at a molecular level by genes, regulatory pathways, and environmental signals, we can design novel synthetic constructs that offer improved control over this powerful property. While the aim has often been to understand, disrupt, and reduce problematic biofilm formation, we can also regulate different elements to take advantage of the structural and functional complexity of biofilms. The protein and polysaccharide components of the self-secreted biofilm extracellular matrix have been investigated as engineering targets. Bacterial adhesins have been engineered for use as building blocks in the development of synthetic multicellular materials, enabling tunable cell–cell adhesion, self-assembly, and cell differentiation [1]. In another study, modified cellulose glycans were coassembled with curli fibril peptides to generate biofilm-inspired materials, where the morphological and mechanical properties of these artificial matrices could be selectively modulated, depending on the synthetic polysaccharide structure (i.e., chain length and degree or pattern of modification) [2]. In addition to extracellular matrix components, another potential engineering target is quorum sensing (QS), which facilitates cell–cell communication between microbes and the coordinated responses of microbial cells within biofilm communities. For example, the QS class of molecules acyl homoserine lactones (AHSLs) have been used to establish artificial cellular communication between synthetic liposome-based AHSL-producing cells and AHSL-responsive *Escherichia coli* cells, which produce a green fluorescent protein (GFP) reporter upon receiving the AHSL signal [3].

With any engineering biology approach, it is crucial to consider the transferability of the chosen strategy to a diverse range of microbes and the portability of newly designed synthetic constructs, particularly for the optimization of novel wild-type strains. In terms of targeting biofilm formation, many regulatory components may be specific to a given genus or may only be well studied in a model organism. A more universal approach is to manipulate a molecule that can be used to control biofilm formation across the majority of bacterial species, as exemplified by the targeting of cyclic-di-GMP to increase the biofilm-forming capacity of recombinant plastic-degrading *E. coli* [4]. Here, the *E. coli* host was engineered with native and non-native diguanylate cyclases for the synthesis of cyclic-di-GMP, increasing levels of which stimulated biofilm formation and in turn enhanced attachment to and interaction with plastic such that the efficiency of degradation was enhanced. This strategy could be extended to native plastic-associated biofilm communities that are found living in association with plastic waste to augment their natural capacity to degrade plastics [5].

Tools for engineering biofilms

Successful biofilm engineering is dependent on the availability of appropriate biofilm control and modification tools. For example, a UV-controlled QS gene expression system in which light-sensitive 2-nitrobenzyl groups were incorporated into the DNA of synthetic cells, such that transcription from the T7 promoter could be tightly controlled in an ON–OFF manner in the presence and absence of UV light, represents an inexpensive and scalable alternative to chemical induction [3]. Also, the production of cyclic-di-GMP has been brought under tight regulation by engineering a near-IR and blue light

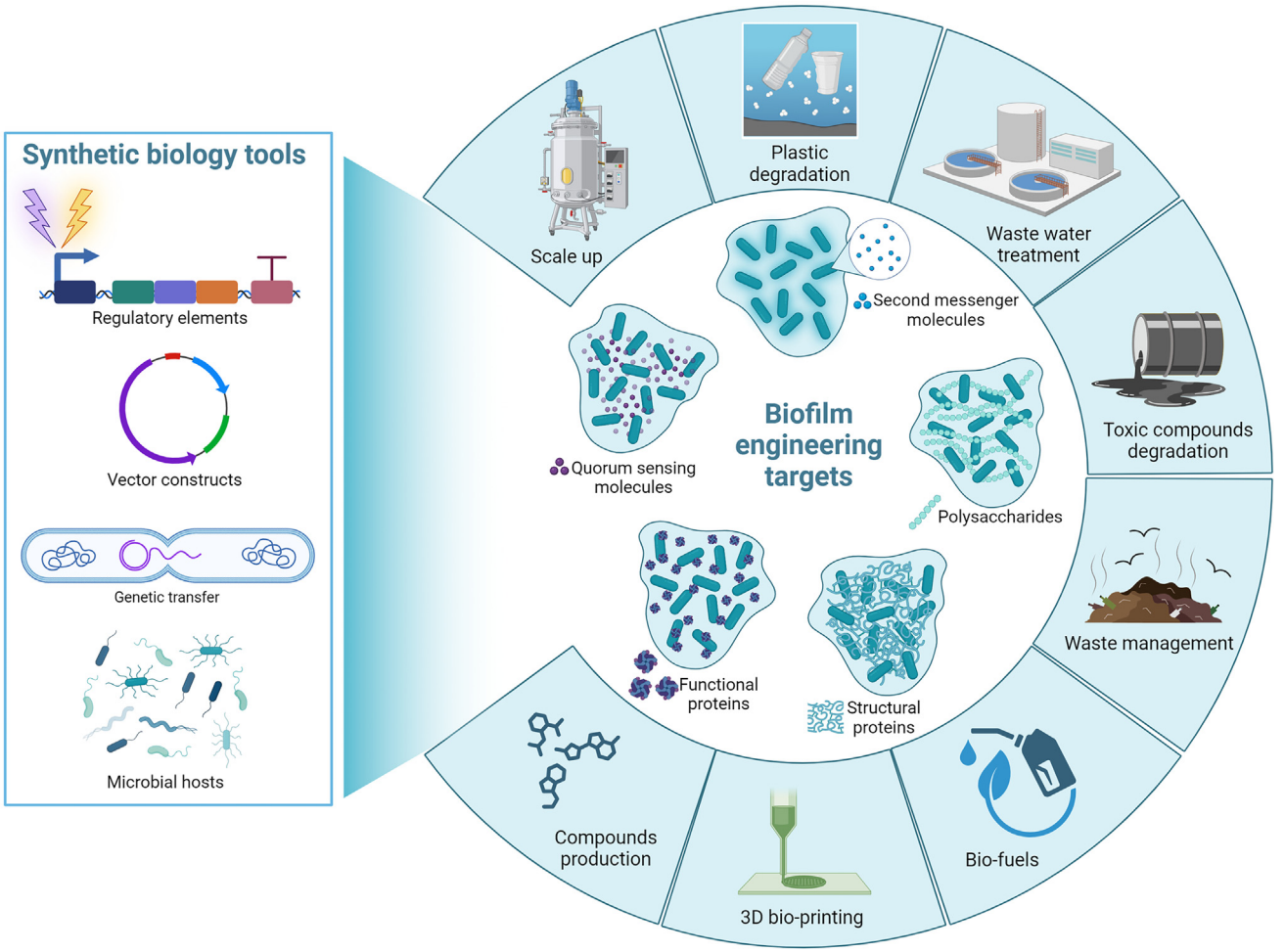

Figure 1. Toward the biological engineering of biofilms for diverse applications. Synthetic biology tools can be adapted to gain control over biofilm formation and endow biofilms with new structural and functional properties, which may subsequently be employed in a range of biotechnological applications from bioprinting to environmental bioremediation. Created in BioRender. McCarthy, R. (2024) BioRender.com/k56o466

dichromatic gene circuit for improved biofilm formation control [6] or a blue light–activated diguanylate cyclase, offering fast response time and application-specific operational range [7]. Such optogenetic approaches could be extended to the engineering of synthetic biofilms, allowing switchlike control of biofilm formation.

Examples of engineered biofilms

In an effort to move beyond current microbial engineering practices that have focused on planktonic cells, a highly controllable synthetic gene circuit was recently constructed to allow the directed assembly and decomposition of biofilms, inspired by the constant lifestyle alterations displayed by microbes in nature as they respond to changes in their environmental conditions [8]. In this system, autonomous phase transition and phase-specific gene expression enable programmable production of biomolecules across different physiological contexts. Such synthetic genetic programs help to establish biofilm-based systems as versatile and scalable platforms to address complex challenges. Biofilms have already been investigated as tunable platforms for the biomanufacturing of ordered materials, such as using self-organizing biofilms of *Cellulophaga lytica* [9]. Here, iridescent polycrystalline biofilms with controllable optical and spatial properties were characterized for the potential production of biomimetic materials at scale. Similarly, the mechanical strength and self-repairing ability of biofilm-based textile composites expressing and secreting amyloid curli fibers have been studied to assess the feasibility of delivering synthetic bio-based fabrics [10]. On the basis of these examples, the importance of developing engineered living systems that can be readily integrated into existing industrial practices is highlighted. This will require interdisciplinary cooperation and the early

identification of the challenges faced at scale and whether a synthetic biofilm engineered for a specific purpose could withstand or circumvent such obstacles.

The future of biofilm engineering will be reliant on the development of universal toolkits to readily modify microorganisms of interest, the use of simple reporter assays and characterization methods that can be widely adopted to test novel biofilm-based systems, as well as computational modeling and omics technologies to predict and understand system performance. Additionally, quantification and predictability of the microbial communities will be largely dependent on improved imaging and bioreactors coupled with biosensors. In particular, the engineering of biofilms for the biodegradation and bioremediation of environmental pollutants will be of critical importance in the sustainable management of synthetic waste. A biofilm of *Pseudomonas aeruginosa* has recently been engineered to establish a 'capture-and-release' system for the trapping and aggregation of microplastics through the exploitation of the native cyclic-di-GMP signaling system [11]. It has also been shown that biofilms can be engineered as living glue systems with the capability of autonomous repair upon damage with applications spanning from medical to industrial sectors [12]. Circuit-based control strategies that incorporate kill switches, as recently achieved in the probiotic *E. coli* Nissle 1917 strain using chemical- and/or temperature-responsive CRISPR-Cas9 kill switch cassettes, can be leveraged for the robust biocontainment of engineered biofilms in environmental applications [13]. Moreover, engineered biofilms can be used for the microbial fabrication of biodegradable bioplastic for packaging and coating, for example, by modified *E. coli* to produce protein-based hydrogels [14]. Development of multispecies microbial communities into engineered living materials provides the opportunity of customizing self-growing and self-repairing catalytic biocompatible materials and their use for various applications [15]. These living systems can be further evolved by multispecies biofilms, as exemplified by the growth of a synthetic symbiotic culture of bacteria and yeast where cellulose-rich biofilm forming *Komagataeibacter rhaeticus* bacterium, used to provide scaffolding, was cocultured with engineered *Saccharomyces cerevisiae* yeast programmed to produce specific enzymes in response to chemical or light signals [16]. Given the inherent resilience and bioactivities of natural biofilms, together with the latest advances in synthetic biology, the prospects for engineering biofilms as industrial hosts are promising. However, it is crucial to consider the long-term challenges in the scale-up and deployment of biofilm-based systems and to encourage dialogue between key stakeholders, including scientists, investors, policy makers, environmental advocates, and the general public.

Acknowledgments

All authors acknowledge support from a Biological Sciences Research Council (BBSRC), UK grant (BB/Y008332/1). R.R.M. is supported by BBSRC grant BB/V007823/1, Natural Environment Research Council grant NE/X010902/1, Medical Research Council grant MR/Y001354/1, and the Academy of Medical Sciences/the Wellcome Trust/the Government Department of Business, Energy and Industrial Strategy/the British Heart Foundation/Diabetes UK Springboard Award [SBF006\1040].

Declaration of interests

Brunel University London and R.R.M. have patent applications covering the manipulation of biofilm levels to enhance plastic degradation. The remaining authors have no interests to declare.

[1]Division of Biosciences, Department of Life Sciences, College of Health and Life Sciences, Brunel University London, Uxbridge, UB8 3PH, UK

*Correspondence:
ronan.mccarthy@brunel.ac.uk (R.R. McCarthy).
X: @McCarthy_Ronan

https://doi.org/10.1016/j.tibtech.2024.11.002

References

1. Costan, S.A. *et al.* (2024) Biophysical characterization of synthetic adhesins for predicting and tuning engineered living material properties. *Matter* 7, 2125–2143
2. Tyrikos-Ergas, T. *et al.* (2022) Synthetic phosphoethanolamine-modified oligosaccharides reveal the importance of glycan length and substitution in biofilm-inspired assemblies. *Nat. Commun.* 13, 3954
3. Smith, J.M. *et al.* (2023) Engineering cellular communication between light-activated synthetic cells and bacteria. *Nat. Chem. Biol.* 19, 1138–1146
4. Howard, S.A. and McCarthy, R.R. (2023) Modulating biofilm can potentiate activity of novel plastic-degrading enzymes. *NPJ Biofilms Microbiomes* 9, 72
5. Howard, S.A. *et al.* (2023) Enrichment of native plastic-associated biofilm communities to enhance polyester degrading activity. *Environ. Microbiol.* 25, 2698–2718
6. Mukherjee, M. *et al.* (2018) Engineering a light-responsive, quorum quenching biofilm to mitigate biofouling on water purification membranes. *Sci. Adv.* 4, eaau1459
7. Vide, U. *et al.* (2023) Illuminating the inner workings of a natural protein switch: blue-light sensing in LOV-activated diguanylate cyclases. *Sci. Adv.* 9, eadh4721
8. Kong, W. *et al.* (2023) De novo engineering of a bacterial lifestyle program. *Nat. Chem. Biol.* 19, 488–497
9. Sullivan, C.J. *et al.* (2023) Iridescent biofilms of *Cellulophaga lytica* are tunable platforms for scalable, ordered materials. *Sci. Rep.* 13, 13192
10. Cai, A. *et al.* (2023) Endowing textiles with self-repairing ability through the fabrication of composites with a bacterial biofilm. *Sci. Rep.* 13, 11389
11. Liu, S.Y. *et al.* (2021) Engineering a microbial 'trap and release' mechanism for microplastics removal. *Chem. Eng. J.* 404, 127079
12. An, B. *et al.* (2020) Programming living glue systems to perform autonomous mechanical repairs. *Matter* 3, 2080–2092
13. Rottinghaus, A.G. *et al.* (2022) Genetically stable CRISPR-based kill switches for engineered microbes. *Nat. Commun.* 13, 672
14. Duraj-Thatte, A.M. *et al.* (2021) Water-processable, biodegradable and coatable aquaplastic from engineered biofilms. *Nat. Chem. Biol.* 17, 732–738
15. Wang, Y. *et al.* (2024) Programmable bacterial biofilms as engineered living materials. *Acc. Mater. Res.* 5, 797–808
16. Gilbert, C. *et al.* (2021) Living materials with programmable functionalities grown from engineered microbial co-cultures. *Nat. Mater.* 20, 691–700