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3D printing for the fabrication of biofilm-based functional living materials

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Abstract

Bacterial biofilms are three-dimensional networks of cells entangled in a self-generated extracellular polymeric matrix composed of proteins, lipids, polysaccharides, and nucleic acids. Biofilms can establish themselves on virtually any accessible surface and lead to varying impacts ranging from infectious diseases to degradation of toxic chemicals. Biofilms exhibit high mechanical stiffness and are inherently tolerant to adverse conditions including the presence of antibiotics, pollutants, detergents, high temperature, changes in pH, etc. These features make biofilms resilient, which is beneficial for applications in dynamic environments such as bioleaching, bioremediation, materials production and wastewater purification. We have recently described an easy and cost-effective method for 3D printing of bacteria and have extended this technology for 3D printing of genetically engineered *Escherichia coli* biofilms. Our 3D printing platform exploits simple alginate chemistry for printing of a bacteria-alginate bioink mixture onto calcium-containing agar surfaces, resulting in the formation of bacteria-encapsulating hydrogels with varying geometries. Bacteria in these hydrogels remain intact, spatially patterned, and viable for several days. Printing of engineered bacteria to produce inducible biofilms leads to formation of multilayered three-dimensional structures that can tolerate harsh chemical treatments. Synthetic biology and material science approaches provide the opportunity to append a wide range of useful functionalities to these 3D-printed biofilms. In this article, we describe the wide range of future applications possible for applying functional 3D-printed biofilms to the construction of living biofilm-derived materials in a large-scale and environmentally-stable manner.

Keywords: biofilms, additive manufacturing, 3D bioprinting, synthetic biology, material sciences

Bacterial biofilms are organic platforms for sustainable nano- or biomaterials production and processing. The matrix components of naturally-occurring biofilms are resilient to extreme conditions and demonstrate self-assembly and spatial patterning[3-5]. These features explain why biofilms have recently become hotspots in emerging materials fabrication and additive manufacturing technologies. Biofilm-derived materials have been applied to a diverse range of applications from detoxification of chemicals to personalized human medicine. By using tools of synthetic biology, it is now possible to improve existing functionalities or even add new functions to biofilm-forming bacteria. Such engineered biofilms are constructed by creating genetic fusions in which desired heterologous functional peptides are appended onto biofilm matrix proteins. These chimeric proteins are then actively secreted by the engineered bacteria and self-assemble in the extracellular matrix of the biofilms[6, 7]. Synthetic biofilms can exhibit new functionalities deriving from the added peptides while simultaneously retaining their natural functionalities such as resilience, long-term viability, and self-regeneration[8]. Genetically tractable bacteria such as *Escherichia coli* and *Bacillus subtilis* have been successfully employed for the creation of synthetic biofilms and engineered materials[6, 8]. During the creation of synthetic biofilms, various factors must be evaluated, including the determination of optimal peptide fusion sites, the tolerance of the fusion protein to mutations, the toxicity of the new peptide tags to the bacterial cells, and appropriate functional assays for characterization of the novel biofilm functionalities. The resultant biofilm-derived materials can exhibit marked advantages over materials fabricated by planktonic bacteria cultures, in terms of their resistance to extreme and unexpected environments, reusability, spatial multi-scale patterning, and tunable properties.

Fabrication of biofilm-derived functional materials has been further developed with the aid of 3D printing technology. We have recently demonstrated the repurposing of commercial do-it-yourself 3D printers or construction toys to print bacteria via straight-forward alginate chemistry[1, 2]. Our simple, scalable, and inexpensive approach was used to print biofilms with sub-millimeter precision that can mimic the spatial heterogeneity of natural biofilms. The spatial resolution of the 3D-printed biofilms is determined by multiple factors including the bioink composition, the concentration of chemicals that induce expression of the modified biofilm proteins, the rheological properties of the bioink, the biocompatibility of the ink with the printed bacteria, the surface smoothness of the printing substrate, etc. 3D printing of bacteria has also been successfully achieved using bioink compositions including gelatin, agarose, hyaluronic acid, fumed silica, and κ-carrageenan[9, 10].

Previously, one major challenge of 3D bioprinting technology was the operating cost. We have addressed this problem by keeping the cost of our customized 3D bioprinters to approximately 350 US dollars[1, 2]. Additionally, some inexpensive commercially available 3D printers can perform multi-channel printing, which can mix several input components, and should in principle be able to be repurposed to print bacteria. As a first example, it has been recently shown that 3D printing of bacterial spores with good resolution can be achieved with a customized multi-channel printing system, operating at higher temperatures[10]. While this printer costs several times more than our 3D bioprinters, its multi-channel printing capability provides the option to keep the bacterial cells separated from the bioink scaffold components under different optimal conditions until printing. We expect that creation or repurposing of cost-effective 3D printers that can perform multi-channel printing without heating the samples would be ideal for 3D printing of bacteria and engineered biofilms with extended usage applications.

The combination of bacterial 3D printing technology with biofilm biology is a fascinating approach towards translation of these biofilm-derived materials into useful applications. In the following section, we describe the possible applications arising from the combination of these fields (**Figure 1**).

Materials production and processing

Given the wide repertoire of natural and artificial biopolymers, diverse synthetic biofilms could be 3D-printed for the creation of bacterially-inspired materials with tunable multi-scale patterning[7, 11, 12]. For instance, bacteria in 3D-printed synthetic biofilms could aid in the production of biopolymers such as cellulose, curdlan, and other materials with improved mechanical or electrically conductive properties with interesting biomedical and biotechnological applications[9].

3D-printed biofilms functionalized with synthetic enzymes can aid in the processing of materials even under conditions of adverse pH, temperature, or exposure to organic solvents. The desired biocatalytic transformation occurs due to the enzymes that are irreversibly immobilized in the extracellular matrix of these biofilms. The enhanced mass transfer rates and surface area in these biofilms results in increased enzymatic activities. Such biofilms could also be engineered to produce scaffolded chemical pathways, in which successive chemical reactions are catalyzed by individual stacked layers of bacteria, leading to production of a single product or a series of products via a relay of reactions. As one example, the printed bacteria could be genetically manipulated to perform complex logic gate functions[13], such that the output of one layer could serve as the input to the adjacent layer[14]. These sequential reactions would proceed more efficiently in 3D-printed biofilms due to the free diffusion of molecules between the stacked layers and their minimal separation distance, thus leading to multi-step transformations. Alternatively, templated assembly of nanoparticles on engineered biofilms could also be used to catalyze multi-step hybrid reaction systems[6, 8].

Non-engineered beneficial bacterial biofilms could be 3D-printed as an anti-fouling coating on building or marine vessel surfaces. These living functional bacteria would use up the oxygen on the surface and in turn could produce compounds that are anti-corrosive, thereby preventing corrosion and biofouling. Similarly, probiotic biofilms could be 3D-printed onto various biomedical implant surfaces to prevent device-associated infections caused by pathogenic bacteria. However, the real-time application of such approaches is far from the current realizations and demands further research.

Environmental detoxification

3D-printed engineered biofilms could be deployed for environmental detoxification purposes including bioremediation, abstraction of rare earth elements (REEs) and heavy metals, removal of assimilable organic carbon, and in wastewater treatment plants[9, 15]. Bringing together the higher metabolic potential and specific catabolic nature of active bacteria with the increased surface area and chemical resilience of the biofilm matrix would enable patterned, engineered biofilms to act as a sink capable of absorption and degradation of chemicals from processing liquid streams. Synthetic biofilms displaying selected catabolic enzymes, heavy metal binding proteins, inorganic nanoparticles, or REE-binding domains could be 3D-printed onto filters or onto pipes and reactors in treatment plants to carry out the desired degradation or abstraction activities as the contaminating streams flow past. Analytical techniques such as HPLC-MS or ICP-MS could be used to quantify the amount of chemicals absorbed onto the biofilm matrix components, and the bound residues could then be desorbed with simple acidic or alkaline washes. Metal-binding domains could be additionally added to these synthetic biofilms to facilitate their strong surface attachment such that they could resist detachment forces and withstand multiple sorption-desorption cycles. With appropriate tuning of the bioink porosity, such 3D-printed biofilms could be recyclable and reusable with minimum loss of efficiency. Incorporation of feedback-regulated genetic circuits could be used in situations involving continuous detoxification such that synthetic biofilms are produced only when the specific target chemical is sensed, thereby improving the overall absorption efficiencies.

Fundamental research

3D printing could be employed to solve fundamental research questions such as understanding the unknown interactions between bacteria species in mixed biofilms or between bacterial biofilms with their eukaryotic hosts. These experiments could be performed by (a) incorporating different bacteria in the same bioink, (b) printing different bacterial bioinks adjacent to each other with shared interfaces, and/or (c) printing layers of host cells overtop of existing mature 3D-printed biofilms or vice versa. Following appropriate exposure times, imaging techniques and -omics approaches (transcriptomics, proteomics, or metabolomics) could then be used on both the bacterial and host samples to decipher their communication and community behavior. Studying these interactions would greatly help in infectious disease management and discovery of new anti-biofilm drugs.

Development of biofilm model systems

In natural biofilms, factors like the density of the bacteria and the extracellular matrix components, the distribution of nutrients and signaling molecules, the locations of water channels, and the distribution of molecular oxygen are dynamic variables. The consequences of these variables on the emergent biological (metabolic heterogeneity and antibiotic resistance) and mechanical (cohesiveness, viscoelasticity, resistance to hydrodynamic shear and desiccation) phenotypes in biofilms are not well characterized. 3D printing could be informative in this regard to identify the design principles of biofilms by introducing individual variations in the 3D spatial distribution of biofilm constituents and studying their resultant attributes of biological and mechanical endurance. These studies could lead to development of an engineered and reproducible biofilm model system that mimics the robustness of natural biofilms whilst maintaining their structure-function relationships over time. Such model biofilms could then be used for practical applications such as testing potential anti-biofilm treatments, evaluating the adequacy of mathematical models of biofilms, etc.

Conclusions and Outlook

3D-printed biofilm-derived materials can exhibit defined spatial patterning with improved resolution and attractive functionalities. However, factors such as reusability, scalability, and potential environmental impacts must be closely investigated for individual applications. For instance, the release of genetically modified bacteria from 3D-printed devices could pose a risk to the environment or to human health, and bacterial contamination must be prevented. For societal applications such as drinking water plants, contamination risks could be eliminated by 3D printing cell-free functional extracellular matrix components that were isolated from biofilms by vacuum filtration. Such components will have longer stability and reusability compared to living bacteria and would not need constant maintenance. An interesting potential application could involve 3D printing multifunctional biofilms that can be used in dynamic settings. Such biofilms could be created by 3D printing either a bioink containing a cocktail of multiple genetically engineered bacteria possessing genetic fusions of different functional proteins and biofilm proteins, or layers of such bacteria one over the other. In either case, cross-seeding of engineered biofilm proteins could occur, leading to a combination of different functionalities in the resultant multifunctional biofilms. Another possible application of 3D-printed biofilms is the creation of responsive materials that could alter their chemical or mechanical properties based on specific environmental cues and triggers. The adaptive nature of such materials would impart them with enhanced lifetimes and continuous functionalities.

Overall, the effectiveness, stability, and versatility of 3D bioprinting approaches in combination with the distinct characteristics of bacterial biofilms offer an ideal platform for the fabrication of biofilm-derived products in materials processing and manufacturing.

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S.B. and A.S.M. developed the scope of the manuscript. S.B. conducted the literature search and prepared the first draft and the figures. A.S.M. and M.-E.A.-T. assisted in writing of the manuscript and critically reviewed the manuscript. All authors subsequently modified the manuscript jointly. The final manuscript was approved by all the authors.

Notes

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References

1. Lehner, B. A. E., Schmieden, D. T., and Meyer, A. S. (2017) A straightforward approach for 3D bacterial printing, *ACS Synth Biol 6*, 1124-1130.
2. Schmieden, D. T., Basalo Vazquez, S. J., Sanguesa, H., van der Does, M., Idema, T., and Meyer, A. S. (2018) Printing of patterned, engineered *E. coli* biofilms with a low-cost 3D printer, *ACS Synth Biol 7*, 1328-1337.
3. Bjarnsholt, T., Buhlin, K., Dufrene, Y. F., Gomelsky, M., Moroni, A., Ramstedt, M., Rumbaugh, K. P., Schulte, T., Sun, L., Akerlund, B., and Romling, U. (2018) Biofilm formation - what we can learn from recent developments, *J Intern Med 284*, 332-345.
4. Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., and Kjelleberg, S. (2016) Biofilms: an emergent form of bacterial life, *Nat Rev Microbiol 14*, 563-575.
5. Felz, S., Vermeulen, P., van Loosdrecht, M. C. M., and Lin, Y. M. (2019) Chemical characterization methods for the analysis of structural extracellular polymeric substances (EPS), *Water Res 157*, 201-208.
6. Nguyen, P. Q., Botyanszki, Z., Tay, P. K., and Joshi, N. S. (2014) Programmable biofilm-based materials from engineered curli nanofibres, *Nat Commun 5*, 4945.
7. Chen, A. Y., Deng, Z., Billings, A. N., Seker, U. O., Lu, M. Y., Citorik, R. J., Zakeri, B., and Lu, T. K. (2014) Synthesis and patterning of tunable multiscale materials with engineered cells, *Nat Mater 13*, 515-523.
8. 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, T. K., and Zhong, C. (2019) Programmable and printable *B. subtilis* biofilms as engineered living materials, *Nat Chem Biol 15*, 34-41.
9. Schaffner, M., Ruhs, P. A., Coulter, F., Kilcher, S., and Studart, A. R. (2017) 3D printing of bacteria into functional complex materials, *Sci Adv 3*, eaao6804.
10. Gonzalez, L. M., and Voigt, C. A. (2019) Resilient living materials built by printing bacterial spores, *bioRxiv*, 537571.
11. Nguyen, P. Q. (2017) Synthetic biology engineering of biofilms as nanomaterials factories, *Biochem Soc Trans 45*, 585-597.
12. Chen, A. Y., Zhong, C., and Lu, T. K. (2015) Engineering living functional materials, *ACS Synth Biol 4*, 8-11.
13. Tamsir, A., Tabor, J. J., and Voigt, C. A. (2011) Robust multicellular computing using genetically encoded NOR gates and chemical 'wires', *Nature 469*, 212-215.
14. Osmekhina, E., Jonkergouw, C., Schmidt, G., Jahangiri, F., Jokinen, V., Franssila, S., and Linder, M. B. (2018) Controlled communication between physically separated bacterial populations in a microfluidic device, *Commun Biol 1*, 97.
15. Tay, P. K. R., Basavanna, A.M., and Joshi, N.S. (2018) Repurposing bacterial extracellular matrix for selective and differential abstraction of rare earth elements *Green Chemistry 20*, 3512.

Figures:

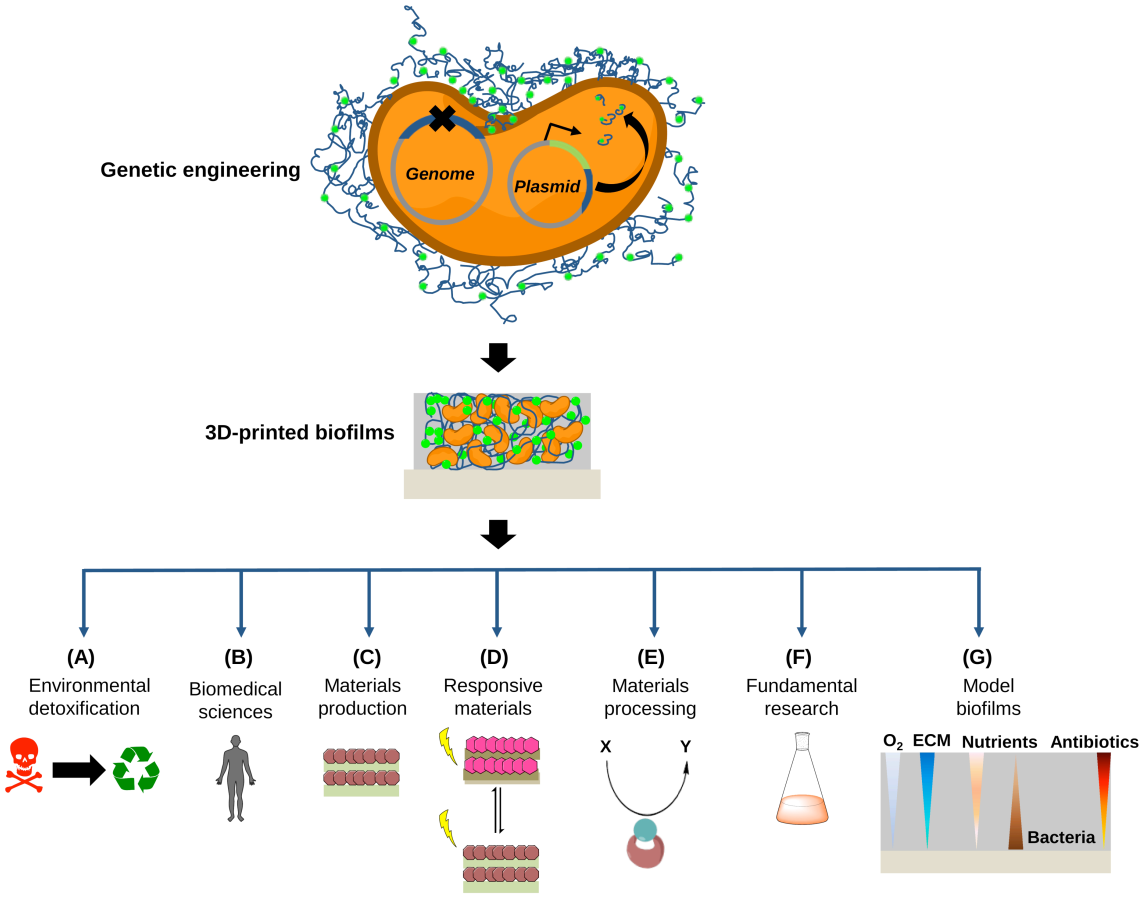

Figure 1: Possible applications of 3D-printed synthetic biofilms. Bacteria can be genetically engineered to produce structural biofilm proteins (in blue) decorated with specific functional peptides (in green) via heterologous expression in a bacterial strain that has a genetic deletion for structural biofilm proteins. By combining these engineered bacteria with 3D bioprinting, 3D-printed engineered biofilms can be created with multiple potential applications, including **(A)** Environmental detoxification and bioremediation, **(B)** Biomedical applications, **(C)** Tunable materials production with improved mechanical and/or conductive properties, **(D)** Fabrication of responsive materials, **(E)** Biocatalysis-driven materials processing, **(F)** Addressing fundamental research questions, **(G)** Creation of reproducible model biofilm systems for studying the structure-function relationships of bacterial biofilms.

Abstract graphic