Trends in Biotechnology

Review

Engineered Living Materials: Taxonomies and Emerging Trends

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At the intersection of synthetic biology and materials science, the field of engineered living materials (ELMs) has evolved into a new, standalone discipline. The fusion of bioengineering's design–build–test–learn approaches with classical materials science has yielded breakthrough innovations in the synthesis of complex, biologically active materials for functional applications in therapeutics, electronics, construction, and beyond. However, the transdisciplinary nature of the ELM field – and its rapid growth – has made holistic comprehension of achievements related to the tools, techniques, and applications of ELMs difficult across disciplines. To this end, this review proposes an emergent taxonomy of ELM research and uses the categorization to discuss current trends and state-of-the-art advancements, significant opportunities, and imminent challenges for scientists and engineers in the field.

Highlights

The fusion of synthetic biology with classical materials science has yielded breakthrough materials innovations and spawned a new biotechnology field: engineered living materials (ELMs).

Taxonomic classifications of ELM research highlight states of the art, opportunities, challenges, and emerging trends.

Opportunities abound to engineer bacteria, fungi, plants, mammalian cells, and diverse consortia of those organisms to produce – and persist within – complex, biologically active materials for applications in therapeutics, electronics, construction, and beyond.

Principal challenges for the maturing ELM field concern scaling beyond the biofilm, increasing production rates and volumes, engineering consortia of microorganisms, and developing policies related to biocontainment, standardized metrology, and workforce development to address global scientific and engineering challenges.

Engineered Living Materials: Where Synthetic Biology Meets Materials Science

Nature manufactures a multitude of complex, hierarchical, multifunctional 'living' materials – including bone, wood, tissue, and organs. Advances in modern biotechnology over the past few decades have enabled scalable manufacturing of multiple 'non-living' materials (e.g., chemicals, fuels, pharmaceuticals) produced by a variety of **wild-type** (see Glossary) and engineered microorganisms. However, synthetic replication of hierarchical living materials largely eluded scientists and engineers until the advent of a new field – **engineered living materials (ELMs)**.

To engineer living materials, ELM researchers integrate the disciplines of **synthetic biology** and **materials science**. Synthetic biology concerns the application of design–build–test–learn approaches to enable the rational design and re-design of living organisms by genetically engineering them to have new – or enhanced – abilities or useful purposes (e.g., material production, sense-and-respond functionality). By contrast, classical materials science concerns the fundamental study of process–structure–property relationships of natural and engineered materials. Contemporary research has now proven feasible the genetic programmability of organisms to produce – and to persist within – materials with tailorable properties [1–3]. Inclusion of a living component genetically tailored to participate in material manufacturing and to provide continued biological functions distinguishes ELMs from other science and engineering fields (Figure 1).

ELMs are more formally defined as engineered materials composed of living cells that form or assemble the material itself and modulate the functional performance of the material post-production. The ELM field has been rapidly growing and evolving over the past several years. ELM researchers continue to boost natural **biosynthesis** pathways (or integrate artificial ones) in organisms for the express purpose of producing biologically active materials for applications in a number of fields beyond biomedical and tissue engineering.

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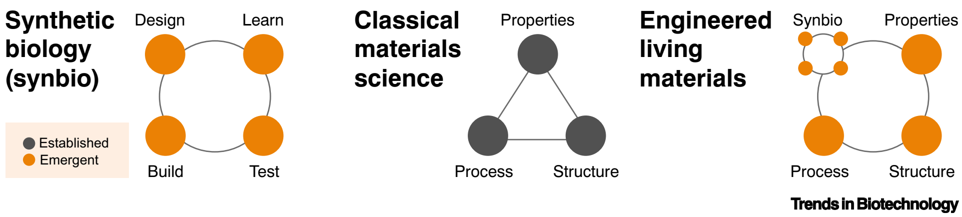

Figure 1. Integration of Synthetic Biology, Namely a Design–Build–Test–Learn Methodology to Design and Re-design Organisms for the Express Purpose of Material Production, with Classical Materials Science Defines the New Field of Engineered Living Materials.

While a number of comprehensive reviews of ELM literature currently exist [1–3], the purpose of this work – and what distinguishes it from other reviews – is to address the critical need for taxonomic classifications of ELM research – and products thereof – for scientists and engineers to best understand ELM states of the art, opportunities, challenges, and emerging trends that currently exist in the field. A structured, taxonomic classification of ELM research would help current and future researchers define and describe their own identities and perspectives within the discipline. Furthermore, a **taxonomy** would aid in articulating successes and identifying persistent challenges in ELM research, all the while promoting the development of a common vocabulary – a vital necessity to advance such a multidisciplinary field.

This review is organized as follows. First, a taxonomical categorization of ELM research is presented. Second, the taxonomy is used to highlight recent advancements and trends in the ELM field within each taxon. Finally, challenges that scientists and engineers must overcome to progress the nascent ELM field to maturity – and opportunities for the ELM field to address global grand challenges of the 21st century – are discussed in the concluding remarks.

Taxonomy and Trends of ELM Research

Figure 2 presents a taxonomic classification of ELM research. These classifications, which include taxons such as scale, organism, and application, are further discussed in light of recent achievements and existing obstacles that must be addressed to advance states of the art.

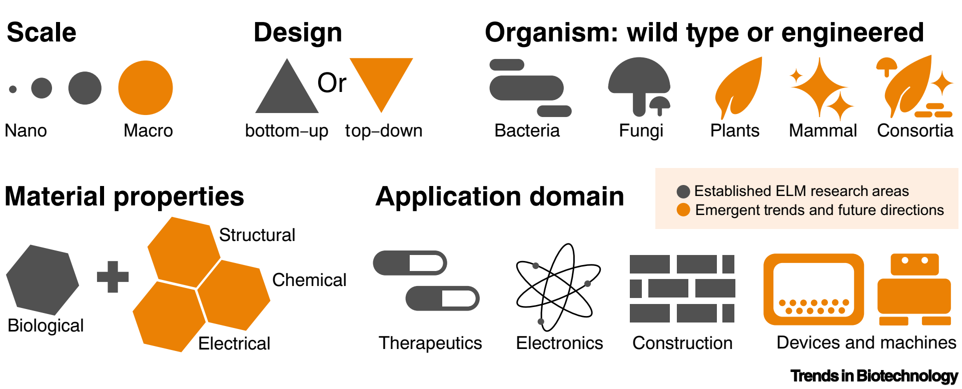

Figure 2. A Taxonomy of Engineered Living Material (ELM) Research Organized by Maturity from Left to Right within Each Taxon. With historical roots in synthetic biology and microbiology, the vast majority of established ELM research areas (denoted in dark gray) concern small-scale, engineered control of bacteria, fungi, and plant growth into living material architectures with applications in medicine, electronics, and the built environment. Emergent, alternative approaches (denoted in orange), including macroscopic, top–down materials design or working with plants, mammalian cells, or consortia of cells, have been pursued by researchers in recent years. Very few researchers are designing complex, multifunctional (e.g., biologically active structural, chemical, and electrical) devices and machines – an emergent ELM application space.

Glossary

Amyloid: aggregates of fiber-like proteins.
Biomineralization: the formation or accumulation of minerals by organisms especially into biological tissues or structures (e.g., bones, teeth, and shells).
Biosynthesis: the multistep, enzyme-catalyzed process where substrates are converted into more complex products in living organisms.
Consortia: the concurrent cultivation or growth of multiple organisms. A microbial consortium is a group of two or more different species that work together and function at a higher level than they could alone.
Engineered living materials (ELMs): as artifacts, engineered materials are composed of living cells that form or assemble the material itself or modulate the functional performance of the material in some manner. As a field, the integration of synthetic biology, namely, the rational design–build–test–learn methodology to design and re-design organisms for the express purpose of material production, with classical materials science.
Genetic tractability: a qualitative measure of how straightforward it is to create a mutant organism that either completely lacks a gene or to create a mutant organism in which a gene product is expressed for the first time and/or in excess.
Materials science: the field concerning the discovery and design of new materials. Materials scientists study how processing a material influences its structure and how its structure affects its properties and performance.
Monoculture: the cultivation or growth of a single organism.
Scaffold: a supporting material framework (e.g., hydrogels, hydrogel–sand composites) in or on which cells can grow or proteins, polymers, or minerals can be affixed.
Synthetic biology: a field of science and bioengineering that involves application of engineering principles (i.e., design–build–test–learn) to design (and re-design) organisms for useful purposes or to engineer them to have new abilities.
Synthetic morphogenesis: a field of synthetic biology that aims to engineer and program a single cell to differentiate and produce complex tissues, structures, and biological systems.

Scale and Design: Nanoscale and Microscale Bottom–Up Approaches

Principal challenges in ELM research concern scale, the first taxon, and design approach, the second. The holy grail of ELM research is to produce complex, hierarchically ordered, living materials *de novo* from a single cell (in the case of bacteria or fungi) or seed (in the case of plants) within a short timeframe. To address this challenge, synthetic biologists have embraced bottom–up approaches, which target the nanoscale and microscales, to induce higher-order structure beyond the cell wall.

The most common bottom–up tactics for growing functional living materials include (i) biofilm formation via secretion of designer extracellular proteins, polysaccharides, enzymes, and other molecules [1]; (ii) surface display and modification of patterned structural proteins [4] or enzymes [5] which can serve as 2D **scaffolds** for ordering at higher length scales; and (iii) **biomineralization** of silica or carbonate minerals that can occur on, near, or beyond the cell surface [6] (Figure 3). 2D and 3D living biofilms have been produced by secretion of modified extracellular polymers [1,7], primarily by integrating and manipulating expression of the functional **amyloid** CsgA (to produce Curli fibers and modifications thereof) and TasA [8,9]. Other approaches have leveraged the SpyTag-SpyCatcher [10] system and adhesin-mediated trapping [11], as well as chitin [12], curdlan [13], and bacterial cellulose secretion. Recently, Caro-Astorga and coworkers [14] further advanced this approach by using *Acetobacteraceae* to produce extracellular bacterial cellulose spheroids and demonstrated their potential use as building blocks to grow 2D and 3D ELM architectures.

While biofilm formation essentially results in the entrapment of living cells, an alternative bottom–up approach enables the cell surface to serve as a nanoscale scaffold for autonomous induction of higher-order structure. Charrier and colleagues [4] biologically engineered the paracrystalline surface-layer (S-layer) protein of *Caulobacter crescentus* to display SpyTag peptides that could form irreversible isopeptide bonds to SpyCatcher-modified proteins, nanocrystals, and

Taxonomy: an orderly classification. In the context of this review, ELM taxons include scale, approach, organism, function, and end-use application.
Topology optimization: a mathematical method that optimizes material layout within a given design space for a given set of forces, boundary conditions, and constraints with the goal of maximizing the performance or structural efficiency of the entire system.
Wild type: a strain, gene, or other organism characteristic that prevails in natural conditions, as distinct from an atypical mutant (or engineered) type.

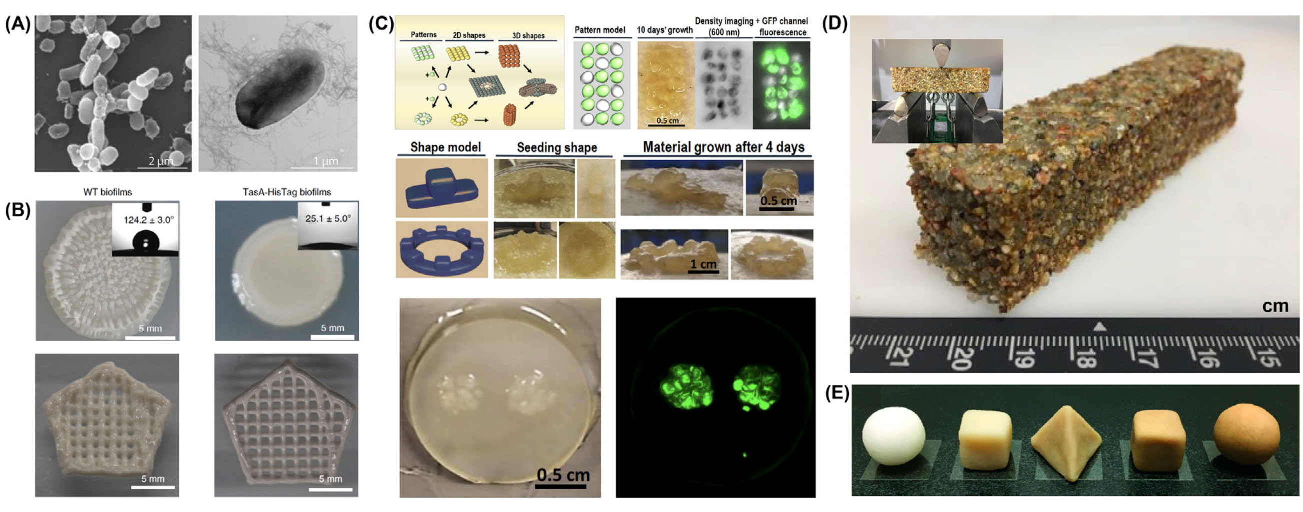

Figure 3. Examples of Engineered Living Materials (ELMs) Produced at Multiple Scales. (A) Scanning electron microscopy image of *Escherichia coli* in curli biofilm with a transmission electron microscopy image of a single cell with curli fibers. Reproduced, with permission, from [28]. (B) Optical image showing the differences in morphology of wild-type and engineered *Bacillus subtilis* biofilms and 3D-printed biofilm structures. Reproduced, with permission, from [8]. (C) Schematic and growth of simple and complex structures that can be created from cellulose spheroid building blocks from *Komagataeibacter rhaeticus*. Reproduced, with permission, from [14]. (D) Gelatin-sand scaffold biomineralized by *Synechococcus* sp. PCC 7002 to create a living, photosynthetic structural material. (E) Representative yeast-embedded cellulose bioinks with unique appearance and various hand-molded geometries. Reproduced, with permission, from [22]. Abbreviation: WT, wild type.

biopolymers on the cell surface. Autonomous precision assembly of hierarchical structures using the cell itself as a scaffold is a distinct advantage of this method.

Other researchers have achieved high-order structure in soft and hard materials beyond the single cell through other biochemical, bioelectrical, and biomineralization approaches. In terms of soft materials, crosslinkers have been exogenously added to biogenic styrene produced by genetically engineered *Escherichia coli*, for example, to make polystyrene [15]. Fan and colleagues [16] approached styrene production from another direction, exogenously adding molecular precursors but utilizing *Shewanella oneidensis* as a living electrode to control radical polymerization in lieu of crosslinkers. These results, which interestingly show biological persistence in the presence of toxic molecules (e.g., styrene), illustrate a clear potential for living cells to simultaneously aid in the processing and refinement of material matrices and persist in them to overcome the challenges of scale from a bottom–up perspective.

For hard materials, microbially induced carbonate and silica precipitation have been used as a microscale strategy to produce tough, hard hierarchical mineral architectures. Heveran and coworkers [6,17] recently proved for the first time that the morphology and nanomechanical properties of microbial-precipitated calcium carbonate could be tailored by modulating the precipitation kinetics of *E. coli* through genetic engineering. Other researchers have applied genetic engineering approaches to study the morphology and structure of siliceous diatoms [18,19]. Understanding and, ultimately, directing mineral growth by mimicking and manipulating the biosynthesis pathways and templating mechanisms in these organisms would enable significant ELM achievements in scale using a bottom–up approach.

Scale and Design: Macroscale Top–Down Approaches

Materials scientists have embraced 'top–down' approaches to address issues with scale by embedding living cells in artificial scaffolds and substrates in similar methodologies to tissue engineering. Utilization of artificial scaffolds and embedment of living organisms in non-living substrates enable bulk ELM production at the centimeter and meter scales via mixing, casting, molding, or other advanced approaches, like additive manufacturing (i.e., 3D printing; Figure 3).

In contrast to autonomous biofilm formation, which *de facto* provides adequate environmental conditions for living cells to thrive, the primary challenge using an embedment approach concerns compatibility and long-term viability of living cells, which ultimately depends on the conditions that can be maintained within the artificial scaffold. While natural biopolymer scaffolds have an ability to be autonomously produced, synthetic biopolymers that are used as scaffolds for living materials offer distinct advantages. For example, synthetic biopolymers exhibit more consistent and predictable properties and can also be used in higher concentrations than are normally producible *in situ* by microorganisms. Most ELM researchers use only hydrogels as artificial scaffolds (e.g., dextrans [11], gelatins [20], alginates [21], celluloses [22]), agaroses [23]), which retain sufficient moisture and nutrients under the proper conditions. Some researchers have embedded *E. coli* in naturally produced bacterial cellulose to create low-strength artificial living membranes [24].

Civil engineers have explored the feasibility of embedding [25] and encapsulating [26] bacteria in structural concrete – a ceramic – for the express purpose of self-sealing upon crack formation via microbial biomineralization. The high ionic strength and pH (>12.5) of concrete pore solution, however, have rendered preservation of microbial viability of bacteria difficult. In most studies, less than 1% of the initial bacteria inoculum survives longer than a few weeks [20].

Heveran and coworkers [20] overcame the viability issue in structural concrete by creating macroscale structural ELMs by inoculating a sand-hydrogel scaffold with a biomineralizing photosynthetic cyanobacterium. The researchers achieved cell viabilities two to three times higher compared with bacteria embedded in concrete and compressive strengths on par with cementitious mortars. Similarly, bioMASON and other researchers [27] have used a *Bacillus* strain to biomineralize an inorganic substrate to produce a concrete-like material. Companies like MycoWorks and Ecovative use a similar approach to create bulk-scale ELMs using fungal mycelium as the binding agent, although both companies produce products that are ultimately rendered inert postproduction. Taken together, these examples illustrate either (i) the importance of judiciously choosing an appropriate matrix in which to embed cells or (ii) the potential to enable the organism (or organisms) to serve as the primary binding agent for other particles to create macroscale ELMs.

In addition to directly embedding bacteria in artificial matrices, advances in additive manufacturing (i.e., 3D printing) and regeneration/regrowth approaches have further enabled materials scientists to overcome the ELM challenge of scale. Researchers have created 3D-printable living fibers, filaments, and bioinks by embedding bacteria in cross-linkable hydrogels (e.g., alginate). Bulk and layered composites produced via 3D printing have been demonstrated to remain biologically functional [21,22]. González and colleagues [23] created resilient ELMs via 3D printing of *Bacillus subtilis* spores and agarose and demonstrated their resilience to environmental stressors, including desiccation, solvents, osmolarity, pH, ultraviolet light, and γ-radiation [23]. Genetic engineering further enabled the bacteria to respond to external stimuli or produce chemicals on demand. Researchers have also created bulk ELMs with spatially distributed properties through light-mediated patterning of living bacteria on a variety of substrates (e.g., fabrics, ceramics, plastics) [28]. Despite these advances, a major gap in the ELM field related to scale concerns investigating other natural and synthetic substrates and probing the bond strength at biotic–abiotic interfaces with scanning electron microscopy, atomic force microscopy, nanoindentation, or other material characterization technique.

One exciting potential of ELM research is the potentiality for on-demand growth and regeneration. In a seminal study concerning bulk-scale regeneration of ELMs, Heveran and coworkers [20] proved that microorganisms encapsulated within a structural ELM (Figure 3) were capable of on-demand exponential regeneration in response to temperature and humidity switches. Three child generations of ELMs were grown from one parent generation, demonstrating the feasibility of exponentially 'growing' ELMs at-scale. Related challenges to this manufacturing approach, however, concern genetic drift and cross-contamination – challenges that are also relevant to varying degrees in all ELM applications.

From Single Organisms to Consortia

Organism type is the third taxon. While the number and type of living organisms that can be used to fabricate ELMs are as diverse as nature itself, the most common microorganisms leveraged in ELM production include genetically tractable *E. coli*, as well as *Bacillus* (e.g., *B. megaterium, B. subtilis), Shewanella, Pseudomonas, Geobacter, Geobacillus, Gluconacetobacter, Caulobacter, Sporosarcina, Saccharomyces* (and other cellulose-producing yeasts), and *Synechococcus*, among others. Specific trains are chosen for various advantages, including **genetic tractability**, S-layer features, photosynthesis, extremophile characteristics, desiccation tolerance, and mineralization abilities. Other researchers have used engineered (i.e., genetically modified) and wild-type yeast and fungal mycelia [29,30] to grow living materials with enhanced protein expression and secretion [31] and new biologically integrated circuit capabilities [32].

Plant cells, mammalian cells, and **consortia** of organisms, by contrast, have been less explored in the ELM field. Although the germination time scale of plants has largely favored the use of bacteria or fungi in ELM research, some examples of living tree-based structures exist in the literature [33], as well as plants genetically engineered to serve as chemical sensors [34].

Enhancing or augmenting mammalian cells could result in new classes of smart responsive living materials. Mammalian cells serve complex functions, like chemical and electrical sensing and protein post-translational modification [35–37]. Seminal ELM research has resulted in breakthrough innovations in mammalian cell-derived therapeutics [35,36] and soft robotics [37]. Cachat and coworkers [38] recently reported a proof-of-concept study concerning **synthetic morphogenesis**, which would enable genetic programmability of a single cell to differentiate into sophisticated tissues and structures. Mammalian cell-derived ELMs, favored for their innate ability to differentiate and create complex, hierarchical patterns, are uniquely positioned to impart structure, actuation, sensing, and control in advanced robotics applications. Combining optical stimulation with mechanical contraction, for example, could yield mobile, living 'swimmer'-type soft biohybrid robotics at scales beyond a single cell [37]. However, mammalian cells do have notable disadvantages. Like many living cells, however, they are susceptible to intracellular and extracellular stressors, especially in non-native environments. They require more delicate (i.e., physiological) conditions and necessitate higher quantities of nutrients, on average. Furthermore, more stringent regulation barriers exist with mammalian cells versus other microorganisms. However, opportunities abound for mammalian cell-derived ELMs if these technological and regulatory barriers could be overcome.

Very little ELM research exists in terms of engineered consortia. Consortia offer the potential advantages of resource sharing (i.e., producing a necessary chemical or molecule that is needed by other organisms, thereby reducing the cost and efficiency burdens associated with exogenous addition) and enhanced environmental stability [39]. In one study, biofilm formation was manipulated using two engineered strains of *E. coli* [40]. In another study, cellulose-producing *Acetobacter* was used to encapsulate photosynthetic microalgae to produce soft, living matter [41]. Researchers at the University of Colorado Boulder measured the engineering properties of living lichen, a naturally occurring consortium of fungi and bacteria, and found superior moisture buffering performance (and potential building energy savings) compared with traditional building materials (B.K. Kreiger, MS dissertation, University of Colorado at Boulder, 2020). While the results are promising for real-world applications of innocuous, lichen-based ELMs, the slow growth of lichen is a challenge for commercial applications. Furthermore, successful examples of scalable lichen synthetics do not yet exist, given the inherent difficulties in engineering stable consortia – especially across genera or other biological classifications [39].

Beyond Biological Production and Biomedicine

Material properties and application represent the fourth and fifth taxons, respectively. While modern materials applications of biotechnology have largely concerned using microorganisms to 'produce' useful chemicals, fuels, and pharmaceuticals, the field of ELMs strives to enable organisms to 'persist within' materials, thereby combining biological functionality with other material properties (e.g., structural, chemical, electrical) for applications beyond biomedicine. Indeed, breakthrough innovations in biomedical engineering have enabled high-fidelity engineering of living cells and tissues [42]. For example, synthetic organelles with higher-order structure have been engineered [43]. The design and application of SynNotch [44] approaches have enabled cell–cell communication approaches that underscore the myriad of approaches to developing chemical biosensors. These achievements will certainly advance therapeutics and the field of precision biomaterials.

In recent years, primary advancements in applied ELM technologies have centered on non-medical applications for design problems in other industries (e.g., electronics, construction, devices, and computing). Engineers have produced electrically conductive biofilms and advanced electrical biosensors using Curli fibers [45–48] and bacterial cellulose pellicles [49]. Examples of enabling technologies and proofs of concept also exist in the literature related to engineering living photovoltaics [50], electronics [51], and photonic materials [19].

The predominant application of living materials to construction concerns self-healing [25,26] and biologically cemented concrete [27], mycelium foams [52], and, most recently, living building materials [20]. Further development of organisms as electrochemical biosensors [53] could lead to advanced applications as living wallpaper (e.g., bacterial pigments, electrically conductive bacterial displays), sensors and indicators of indoor environmental quality (e.g., humidity, temperature, CO_2, CO, and harmful volatile organic compounds), and active bioelectronics for structural health monitoring. While recent advances in synthetic biology have enabled the production of useful construction minerals [6,17] and polymers (e.g., polystyrene) [15], further demonstrations and feasibility studies of construction-relevant biomaterials production are needed in addition to ELM research, given the challenges of scale and associated economics that accompany such a commoditized industry.

Another emergent application space for ELMs concerns robotics and computing devices [37] capable of providing structure, actuation, sensing, and control. Efficient mechanical energy conversion from chemical energy produced by living cells would enable marked advances in the robotics field. In computing, living bits [54] have been created and related nucleic acid-based storage (in which information is stored in DNA) [55] is being progressively investigated. Living materials are also being explored for enhanced human–computer interaction [56], but these applications lack maturity compared with other application spaces.

Concluding Remarks

Nature architects complex, hierarchical biomaterials that sense, adapt, respond, and heal on multiple length scales. Over the past 10 years, the fusion of synthetic biology and materials science toolkits has yielded breakthrough innovations across the ELM field that together illuminate the possibilities – and the obstacles – that must be overcome for scientists and engineers to progress the nascent ELM field to maturity (see Outstanding Questions). The most significant scientific and engineering challenges for the field include the following.

Scale

Hierarchical growth challenges beyond biofilms exist for researchers pursuing bottom–up approaches. Continued fundamental study of natural templating mechanisms, such as those found in diatoms, coccolithophores, shells, bone, and teeth, would help elucidate the molecular basis for hierarchical structuring that could be leveraged in overcoming issues with scale. Investigating the feasibility of using microorganisms to template macroscale structures from microscale units in controlled bacterial ‘swarms’ – by using biomineralizing magnetotactic bacteria controlled by external magnetic or electrical fields, for example – could enable quantum leaps in scale.

Given the material efficiency benefits that come with **topology optimization** and structural form finding, researchers using top–down approaches should further embrace additive manufacturing techniques to advance ELMs from the materials to the structural scale. All should consider cost and simplicity in developing advanced manufacturing approaches to address issues with scale.

Outstanding Questions

Can the grand challenge of scale be overcome using bottom–up approaches to ‘grow’ functional, living materials *de novo* from a single cell? Could top–down approaches enable more rapid deployment of scalable ELM technologies in the area of hybrid living materials?

How will additive manufacturing (i.e., 3D printing), patterning, directed growth, and form-finding approaches advance ELM fabrication beyond the biofilm?

How can long-term robustness and organism viability be best preserved? Could enzyme-based stabilization, synthetic coatings, microfluidic techniques, or boosting natural protective mechanisms of organisms be effective sustainment strategies?

Which organisms are useful for which applications? What benefits will engineered consortia bring to the field? Could engineering higher-order organisms, like insects and other invertebrates, enable research advances in electrochemical sensing, mechanical actuation, and protection?

What standardized metrologies must be developed for consistent, repeatable manufacture, measurement, and reporting? Who will lead such an initiative?

What biocontainment strategies and safety protocols are required to enable advancements and applications beyond the benchtop?

How do we best train the next generation of ELM researchers with equal strength in synthetic biology and materials science to address the challenges of the 21st century?

Production Rates and Volumes

Regardless of approach, production rates and volumes are currently a limiting factor for ELMs. Increasing production from the milligram/liter to kilogram/liter scale is no easy feat, especially considering that organisms produce materials at a variety of time scales, from hours (in the case of bacterial cellulose) to decades (in the case of trees). Thus, realistic time scales, rates, and volumes must be calculated in aligning choice of organism and material with the target application. ELM biosensors, for example, would require significantly less volume than ELMs designed for construction.

In general, it is a good strategy to boost the natural abilities of wild-type organisms that make target materials to produce larger quantities. Metabolisms of non-native organisms genetically engineered to produce materials, like *E. coli*, can often be compromised, slowing rates of production. Similarly, while it might seem an efficient approach to engineer organisms to produce multiple materials or serve multiple functions (e.g., secretion, cell-cell communication, mineralization), metabolisms can be significantly burdened if organisms are over-engineered.

Engineered Consortia

To address rates and volumes, the strategy to engineer consortia of organisms to maximize efficiency without compromising metabolic activity is assuredly likely to become a more common trend. Consortia of microorganisms can exhibit distinct advantages over **monocultures**, such as improved environmental resilience, resource production, and resource utilization. Engineered higher-order organisms, too, including insects and other invertebrates would enable research advances in electrochemical sensing and mechanical actuation. However, few examples of consortia approaches exist in the ELM literature, indicating it will be a significant – yet worthwhile – challenge for researchers in the field.

Viability and Robustness

Long-term viability of living organisms has been a challenge for ELM researchers. ELM applications in therapeutics, tissue engineering, and living biofilms have been most successful, since *in vivo* (and biofilm) moisture contents, temperatures, and pHs are highly conducive to sustained viability. Researchers creating ELMs for other applications, however, are facing significant challenges with ensuring not only long-term viability, but also long-term robustness in physically and chemically challenging environments. Some approaches and evolving techniques to ensuring long-term viability and durability include enzyme-based stabilization, synthetic coatings, and microfluidic techniques to deliver precise quantities of specific nutrients to sustain the ELM. Harnessing natural protective mechanisms of organisms and imparting that capability to ELMs would be another meaningful strategy.

Biocontainment

Fundamental science and engineering advances are just the starting point. Issues concerning biocontainment strategies, regulatory frameworks that will enable applications, and material safety protocol need be developed for advancement of the ELM field [57]. Currently, almost all ELM research exists in a laboratory setting. The few commercial products available, such as Ecovative's commercial mycelium products, for instance, are rendered inert via heat treatment postproduction. Some researchers are instead considering biological routes to make living materials innocuous [30]. To keep ELMs biologically functional, however, a significant effort regarding regulation and safety must be put forth to overcome the so-called valley of death for academic research.

Standards, Design Rules, and Workforce Development

Major challenges for the ELM field include development of a standardizing metrology, definition of design rules for different ELM categories, and education and training of a new generation of scientists and engineers that will continue to define and shape the discipline and address the science and engineering grand challenges of our time. Inconsistencies in experimental methods and reporting standards will stifle advancement. Thus, the ELM community should establish canonical measurement and reporting methods that are used consistently across disciplines, for example, pairing canonical biochemistry analytical techniques (e.g., microscopy, spectroscopy, colony forming unit analysis) with material characterization (e.g., American Society of Testing and Materials) standards. Furthermore, the inherent variability imparted by both nature (i.e., genetics) and nurture (i.e., environmental conditions) of organisms will ultimately dictate the structure and performance of living materials, thus rendering holistic understanding of the entire design space for designer ELMs difficult if not impossible and rendering consistent and reliable manufacturing difficult but not impossible to guarantee.

To this end, development of select ELM design rules will necessarily involve solving specific 'engineering' design challenges. Addressing these challenges will require a new ELM workforce well versed in the tools, techniques, and fundamentals of synthetic biology and materials science, given that both are needed to achieve marked advancements in the field. While it is, indeed, worthwhile for the new ELM generation to produce ELMs that display very interesting and novel functionalities, concerted efforts to design and produce ELMs that help solve the global grand challenges of our time should be of paramount importance. Global decarbonization, water quality and sanitation, autonomous manufacturing, and circular economy efforts, for example, can all be supported by biological production of – and biological persistence within – new, functional material architectures if the field so boldly takes on the challenges that humanity will face in the 21st century and beyond.

Acknowledgments

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