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Perspective

Title: Make Engineered Living Materials Carry Their Weight

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ABSTRACT

Engineered living materials (ELMs) are a new class of materials designed to be synthesized and/or populated by living organisms. ELMs have the potential to reduce energy costs in material manufacturing and provide novel material functionalities including self-healing and sensing. However, energy costs from material manufacturing are primarily due to the production of rigid materials used for construction and machines. To substantially reduce carbon emissions, ELMs must be able to replace some of these rigid materials. However, naturally occurring materials synthesized by living cells are not sufficiently stiff to replace most rigid engineering materials. Furthermore, the cellular viability in the current stiffest ELMs is not yet adequate for achieving the potential sustainability benefits of these materials. The need for rigid ELMs will require new approaches to enhance resident cell viability and/or combine living cells with rigid scaffolds. Among the naturally occurring materials, bone is a rare example of a rigid material that is synthesized and functionalized by cells that maintain viability for years. Bone is expected to provide valuable lessons for surmounting challenges in achieving the requisite viability and mechanical properties of ELMs intended for load-bearing purposes.

1.0 Introduction

Materials that *do more, using less* are key to fulfilling and anticipating societal needs in a resource-limited future. Population growth and climate change will both increasingly strain the availability of raw material and fuel resources for traditional material manufacturing. Engineered living materials (ELMs) are an emerging class of materials that have the potential to address these sustainability challenges[1]. ELMs contain a living component that confers unique functionalities to the structure, such as material synthesis, self-repair, or environmental sensing[2–4]. The living component of an ELM can include engineered or naturally occurring microorganisms, such as bacteria, algae, fungi, or even microbial consortia[2,4,5]. ELMs are already enabling disruptive new technologies in tissue engineering, wound care, flexible biosensors, and energy generation and storage[6–9]. In many ELMs, cells synthesize part or all of the material, thereby reducing energy and environmental costs during manufacturing. While prior reviews have focused on molecular synthesis approaches related to ELMs and their resulting functions[3,5,10,11], here we instead focus on a key aspect of application of ELMs: mechanical performance. We argue that rigid engineered living materials are required for ELMs to meet their sustainability potential.

A major motivation for engineered living materials is their potential for drastically lower energy and environmental costs during manufacturing. As much as 25% of annual global carbon emissions are attributed to materials manufacturing[34,35]. Many material manufacturing processes (e.g., cement kilning, metals melting and heat processing) require high temperatures and, correspondingly, large energy expenditures generating a high carbon footprint. Manufacturing materials using much lower temperature, microbial-driven processes have the potential to substantially reduce greenhouse gas emissions. Further, ELMs have the potential to be produced at the point of application, thereby reducing energy costs associated with materials' transportation to the location of application. ELMs also have the potential for additional functionality not possible with traditional engineering materials, such as self-assembly, self-repair, environmental sensing, or remediation, and constitute an improved use of limited material, time, and energy resources. ELMs could also enable new approaches for engineering resilient systems and infrastructure. For instance, these materials could facilitate building in, or responding to, inhospitable situations such as climate disasters, marine environments, or conflict zones.

While ELMs can be produced with substantially lower energy and environmental costs, if they do not have sufficient mechanical properties the sustainability impact will be small; 80% of the carbon footprint generated by material manufacturing is attributable to materials used for construction, machines, vehicles and other durable goods[35]– i.e., applications that require rigid, load-carrying materials. To date, engineered living materials that are sufficiently rigid (Young's modulus > 1 GPa) to replace some common materials in load-carrying applications have not been demonstrated. Here we argue that for ELMs to have a transformative effect on sustainability, they must be sufficiently rigid and durable to be capable of replacing load carrying materials in engineering devices. As an example of what is possible in this space, we describe the interactions between living cells and extracellular matrix in the only existing rigid, living material: bone.

2.0 The importance of improving the mechanical properties of ELMs

Conceptual representations of engineered living materials frequently involve the replacement and/or re-envisioning of materials used in buildings. Research efforts to date, however, have focused on biological synthesis of materials and/or novel sensing mechanisms and not the mechanical performance of the materials. The vast majority of engineered living materials demonstrated to date have been in soft hydrogels. A few, more rigid ELMs have been developed, including biomineralized aggregate or hydrogel scaffolds or even dehydrated bacterial pellets[12–14]. However, even these implementations have achieved only modest stiffness and strength (Young's modulus as high as ~300 MPa, often less than 10MPa; compressive strength ~1-5 MPa)[12,13,15]. The strengths of these ELMs are on par with light duty cement-based mortar materials and thus their usefulness in the built environment is limited to low-load bearing applications (e.g., paving tiles)[16]. As strength improves towards that of concrete (e.g., >20 MPa), more applications will become available for ELMs for building and infrastructure applications.

The mechanical properties of naturally occurring biological materials provide some guidance in terms of what mechanical properties might be possible for ELMs that are manufactured by living cells. Bone, wood and coral are among the most rigid naturally occurring biological materials. These three biological materials have mechanical properties per unit density that are comparable to some commonly used engineering materials (Table 1).

Given that natural biomineralized materials can meet or exceed the strength characteristics of concrete, the prospect of replacing a portion of concrete materials with living functional materials is readily imagined. However, even if ELM strength increases towards the properties of bone or wood, replacing very stiff materials, such as aluminum or steel, may be much more challenging and require substantial modification of structural design. For example, the Young's modulus describes the stiffness of a material and is a key material property for load-carrying applications by enabling a structure or device to carry loads without undergoing excessive deformation. Wood (pine) is a commonly used natural material in building and has a Young's modulus of 7 GPa[17]. If wood (or a wood-like ELM) were to replace structural steel, the structure would still need to be ~30 times larger in area (a column) or flexural rigidity (a beam) to carry the same load with similar amounts of deflection. This simple comparison illustrates the fact that even the most rigid naturally synthesized living materials cannot easily replace the mechanical function of some of the most frequently used engineering materials. Hence, ELMs that are intended for load carrying applications must either surpass the mechanical performance of naturally occurring living materials and/or require completely new structural design strategies. To be sure, there are many applications in which the full mechanical function of the engineering material is not needed and an ELM with less robust mechanical properties could still replace the traditional material; for example, sidewalks, pavement, and some framing applications. As discussed later, hybrid materials that integrate a living component with a very stiff engineering material substrate may offer new ways to add living functions to stiff structures.

Table 1. The mechanical properties of naturally occurring living materials are low compared to engineering materials used load-carrying applications[17].

Material	Young's Modulus (GPa)	Yield Strength in Tension (MPa)	Compressive strength (MPa)	Specific Stiffness (E/ρ, GPa/(g/cc))	Specific Strength (σ_y/ρ, MPa/(g/cc))
Bone (cortical)	17	133	193	5.66	44.33
Wood (pine)	7.38	23.4	3.5	12.79	40.55
Coral	76	6	20	25.8	2.04
Concrete	30	5	20-30	0.013	0.002
Structural Steel (ASTM-A36)	200	290		25.64	37.18
Aircraft Aluminum (7075-T6)	72	503		25.6	179.00

3.0 Challenges in the design of rigid ELMs

There remain multiple technical challenges that must be overcome to create ELMs that are sufficiently strong to replace engineering materials used in load-carrying applications. Here, we review the current progress and persistent challenges in synthesizing rigid ELMs: (1) increasing strength, (2) maintaining prolonged cellular viability, and (3) using the living component to extend the service life of these materials.

(1) Increasing the strength of ELMs

The most rigid of ELMs reported so far employ microbial biomineralization. Microorganisms can be utilized to directly or indirectly produce biominerals. Microbial biomineral production can be intracellular, including membrane-bound iron oxide or iron sulfide nanocrystals produced by magnetotactic bacteria species[18,19] or silica polymerized intracellularly and then incorporated into the cell wall as performed by diatoms[20,21]. However, most biomineral production for the purpose of stiffening ELMs has involved microorganisms associated with extracellular biomineralization[22]. Often, the biomineral formed is calcium carbonate (i.e., microbial-induced calcium carbonate precipitation, MICP) [12,22–28]. Numerous wild-type bacteria alter their surrounding saturation state to induce local calcium carbonate precipitation as a byproduct of metabolism (e.g., urea hydrolysis and other mechanisms[12,23–28]). Calcium carbonate polymorphs produced by microbes, such as calcite and vaterite, can achieve microscale Young's moduli between 30-70 GPa[29–31], and are therefore able to stiffen the structure when deposited in sufficient quantity. MICP has been abundantly used to directly bridge sand or soil particles[22,23]. Alternatively, hydrogel or bacterial cellulose scaffolds can be stiffened by colonizing with MICP-performing bacteria[12,13]. These materials are reported to achieve strength between ~1-5 MPa[12,13]. It is possible for these types of ELMs to develop much higher strength, as demonstrated in mineralized gelatin-sand materials that do not include cells. In these materials, extensive calcium carbonate mineralization was achieved via carbonic anhydrase together with abundant CO_2 and calcium[32]. The compressive strength of these materials exceeded 9 MPa and was further increased to nearly 12 MPa by glutaraldehyde crosslinking. These results suggest that increasing the quantity of biomineral is a reasonable path to improving the strength of ELMs. However, increasing biomineral volume may have important impacts on the sustainability and health impacts of these biological processes. Increased inputs of microbes and/or biomineralization media generates considerable waste [23,33]. Further, in the case of urea hydrolysis, ammonia is produced. Therefore, improving the strength of ELMs through more extensive biomineralization can impact their sustainability and introduce waste products that require management.

Some ELMs are stiffened by encouraging biomineralization through altering microbe protein expression. *Bacillus subtilis* was engineered to display proteins that encourage silica nucleation, which was utilized to stiffen a proteinaceous scaffold also produced by the microbes[33]. In another strategy, biofilm formation was induced by light and served to encourage hydroxyapatite nucleation [34]. While microbes can generate small nodules mineral with high stiffness, translation beyond microscale - milliscale specimens to macroscale ELMs remains challenging. Some of the principal challenges involve inducing biomineralization where it is most needed (and perhaps inhibiting it where not desired) and achieving sufficient mineralization of the structure for high strength development. [23,35]

Other strategies for stiffening an ELM, usually to a much lesser degree than biomineralization, can include bacterial production of cellulose or curli fibers[36–40]. Crosslinking a hydrogel can also stiffen the ELM, although some crosslinking agents can decrease microorganism viability through toxicity or interference with cellular functional groups[41,42]. Inclusion of another, load-bearing nonliving element (i.e., sand) to ELMs can effectively increase strength. ELMs containing sand have achieved stiffness and strength on par with light-duty cementitious mortar[12,13].

(2) Maintaining long-term cellular viability in ELMs

Maintaining the long-term viability of cells living within ELMs is the next major challenge. Microbial viability requires delivery of nutrition, removal of waste, and protection from environmental stressors, including dehydration, harsh environments, and toxins. These criteria have been accomplished by some groups by incorporating microorganisms into a soft hydrogel[11–13,43–46] or within a capsule of bacterial cellulose[9,47]. Other approaches that have been useful for prolonging viability within soft ELMs have included using synthetic biology to improve the stress resistance of microorganisms[48–50], utilizing photosynthetic microbes on or near the surfaces of materials[38,51], or creating consortia of multiple microorganism species that together extend the viability and environmental tolerance of the microbial community[52–54].

Currently, strengthening ELMs comes with a tradeoff for viability. For instance, stiffening hydrogels through dehydration decreases or eliminates microbial viability[12,14]. Some strategies can partially decouple this tradeoff. Including the desiccation protectant trehalose in hydrogel-sand ELMs increased the viability of *Synechococcus* sp. 7002 as well as an engineered ureolytic *E. coli* strain to the point where 18-40% of initial inoculum remained viable twelve days after the initiation of drying methods[13]. While these methods are promising, currently there are no rigid ELMs with substantial microbial viability longer than ~4 weeks[55]. It is necessary for cells within a rigid ELM to survive for years or longer for the material to function as a living structure in more than an ephemeral sense.

Where do microbial cells source the precursors for making materials without constant and costly human intervention? Is it practical to expect bacterial cells to remain metabolically active for years, at least enough for basal sensing and remodeling capacities?

(3) Extending the useful life of engineered living materials

An effective means of reducing environmental and energy costs of a material is to increase its useful life. One strategy to do so is to have the living component of the ELM provide

for self-repair or adaptation of the material. Self-repair requires that 1) the living component maintains viability for an extended time and/or can be re-introduced if lost; and 2) that the living cells can sense damage and then reinforce these damaged locations. Some efforts have attempted to add living cells to cement and concrete to achieve self-healing of cracks through microbial biomineralization[16,56–60]. While this approach is possible in concrete, the microbial viability of bacteria within the concrete is poor due to stress from the initial mixing, aggressive pH and ionic strength of the cement pore solution, and mechanical stresses when the cells are constrained within small pores in the cement matrix[61]. As a result, only a very small component of the inoculum (<1%) survive beyond 1-2 weeks in these structures[62,63]. Using bacterial spores instead of vegetative bacteria can improve their long-term viability. Bacteria spores with a protective coating, such as expanded clay, diatomaceous earth, or a hydrogel encapsulant, can survive several months in concrete in a non-metabolically active state[59,64]. These spores respond to damage when the cracks withing the material introduce the bacteria to nutrients[59,64–66]. These spore-based mechanisms are single use – after responding to a crack the cells do not sporulate and are therefore not present if a subsequent crack appears[65,66]. Spores have also been utilized in non-concrete systems, such as endospores produced by recombinant *Bacillus subtilis* retained within silica biomineralized protein scaffolds[33]. New strategies are needed for engineering systems in which microbes sense and respond to damage to extend the useful life of the structure, or potentially even adapt the structure so that it can respond to changing demands or requirements. As we will argue in the next section, rigid ELM design should look towards load-bearing living materials (i.e., bone) for lessons in overcoming this and other key challenges.

4.0 Surmounting Barriers to Designing Rigid ELMs: Lessons from Bone

Although the idea that living organisms could grow and/or populate a material is one key trait of an engineered living material, there are few examples of naturally occurring durable, rigid, living materials. Wood, coral, and seashells are all examples of rigid materials created by living cells; however, the load-carrying components of these materials do not contain living cells. For instance, in trees, the living cells are present in a thin layer (the cambium), not the load-carrying wood. Similarly, the living cells in coral and seashells reside on the surfaces of the rigid mineralized shells, not embedded within the rigid material. We are aware of only one living material that is both rigid and populated and maintained by living cells throughout its volume: bone.

Bone is a material synthesized and populated by living cells. Living cells synthesize bone extracellular matrix[67–69]. After matrix formation, living cells continue to reside within the bone, sensing damage and other stimuli and directing remodeling and repair processes[70–72]. Cells embedded within bone matrix live for decades and maintain the material such that mechanical failure (fracture) is not observed in most individuals even after decades of life[70–72]. Bone also displays a relatively high stiffness and strength per unit density (Table 1)[73]. The material properties of bone have been of interest for hundreds of years. Indeed, Galileo Galilei used examples of bone structure when inventing the concept of mechanical stress[74]. Importantly, we are not suggesting the design of living structures that are strictly biomimetic of bone structure or physiology. In addition to its exquisite structure and multicellular coordination, the skeleton is supported by, and itself supports, many other physiological systems. Instead, we distill three key traits of bone that may be useful, alone or together, for inspiring new strategies to surmount persistent challenges in the design of rigid ELMs.

(1) *Bone is efficiently constructed into a strong material*

Bone is a hierarchical composite material comprised of carbonated hydroxyapatite mineral (~65 wt%), organic matrix (~25%), and water (~10%) (Figure 1) [69,75–77]. The formation of bone follows a complex biomineralization process. Bone cells called osteoblasts secrete the organic component of bone which consists primarily of collagen with small amounts of non-collagenous proteins[69,75]. Mineral nucleates within and between organic collagen fibrils as controlled by non-collagenous proteins[68,77]. Bone cells do not make mineral but do produce matrix vesicles that can participate in transporting hydroxyapatite precursors[78]. Bone rapidly mineralizes after synthesis of the organic component, accruing ~70% of its mineral content within the first few days after formation, which then gradually grows and matures[68,79,80].

Bone mineralization of an organic scaffold under the control of specific proteins provides a potential design template for the efficient assembly of biomineralized ELMs with desirable properties. Bacterial cellulose scaffolds can be biomineralized to stiffen the structure[81]. Scaffolds are also produced through engineering microbes to produce a scaffold, such as by the Spycatcher-Spytag system, which promotes the covalent attachment of cells to surfaces and to each other[33,82]. Some of these systems also include proteins that encourage mineral nucleation[33]. However, appreciating these lessons from bone does not strictly require that cells make the supporting scaffold. Encapsulating cells within a polymer matrix is already a widely used method for manufacturing soft ELMs[3] and is sometimes employed in stiffer ELMs to bridge aggregate particles before mineralization[12,13]. Electrospinning produces scaffolds that can be populated by cells and stiffened by biomineralization, which is used with success in applications including bone tissue engineering[83]. Improving spatiotemporal control of biomineralization in ELMs may also benefit from 3D printing technologies, which have been used to pattern locations of cellular growth in soft ELMs[84–87]. While these technologies demonstrate progress towards the efficient manufacturing of stiffer ELMs, considerably higher mineral volume within scaffolds needs to be achieved to generate sufficient structural strength.

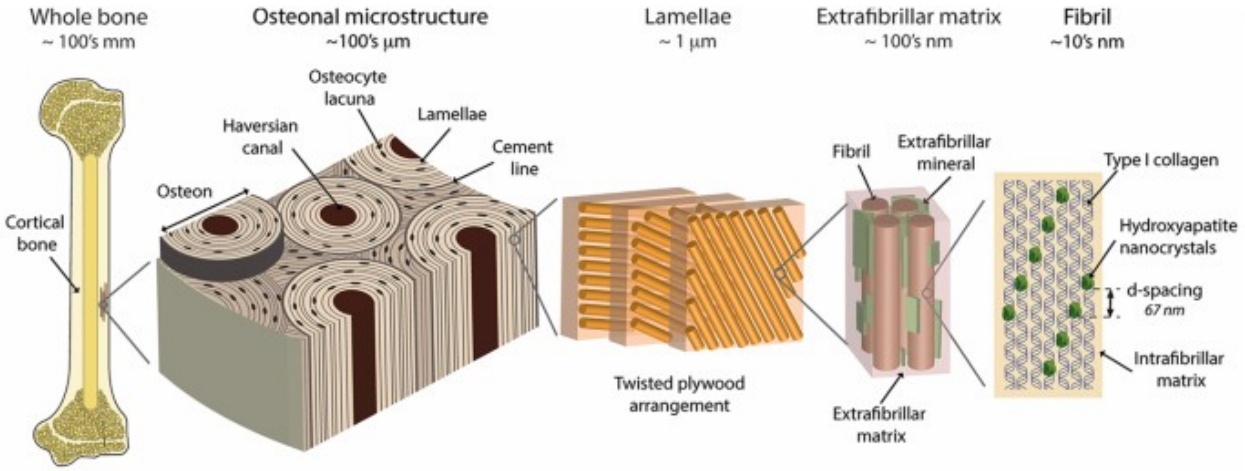

Figure 1: The hierarchical structure of cortical bone contributes to high strength and toughness. At the nanometer-micrometer scales, a collagen scaffold stabilized by crosslinks is partially mineralized by hydroxyapatite. Arrays of collagen fibers, assembled from mineralized collagen fibrils, assemble into fiber arrays called lamellae. These lamellae assemble into cylindrical structures called osteons that host blood vessels and nerves in their central Haversian canal. Reprinted with permission from reference [88].

(2) Bone maintains the viability of resident cells through fluid delivery systems

Many of the cells that synthesize bone matrix become encapsulated within the rigid matrix and differentiate into a cell population called osteocytes. Osteocytes serve a critical role in coordinating self-repair (discussed in next section) and sensing and directing changes in bone density and geometry in response to mechanical stimuli. Osteocytes live for decades trapped within stiff bony matrix and therefore provide an example of how to maintain cell viability in ELMs.

For osteocytes to maintain their prolonged viability, they must have access to nutrition and removal of waste products. These functions are possible because these cells are embedded within a vast porous network within bone. Osteocytes reside within microscale pores ('lacunae', ~10-20 μm length) that are connected to one another and to the external surfaces by nanoscale channels ('canaliculi', 0.3-0.5 um width)[89–91]. Dense cortical bone has very high nanoscale porosity. In a 1 mm^3 volume of bone matrix there are 20,000-30,000 osteocyte lacunae, each with 50-100 nanoscale canaliculi connected to other lacunae[89] (Figure 2A). In dense cortical bone, these porous networks are arranged within concentric rings of bone (osteons, Figure 1, Figure 2B) that each contain a Haversian canal through which blood vessels and nerves can travel (Figures 1, Figure 2C). Fluid transport within the network of nanoscale channels is complex, yet sufficient to maintain the viability of the embedded osteocytes [92–94].

A key lesson from bone in surmounting viability issues in rigid ELMs is to design an analogue of the osteocyte lacunar-canalicular system that enables fluid transport to living cells. This goal could be realized through many potential strategies, such as utilizing porous scaffolds, selectively inhibiting mineralization, or utilizing microorganisms that can dissolve or tunnel through their surroundings.

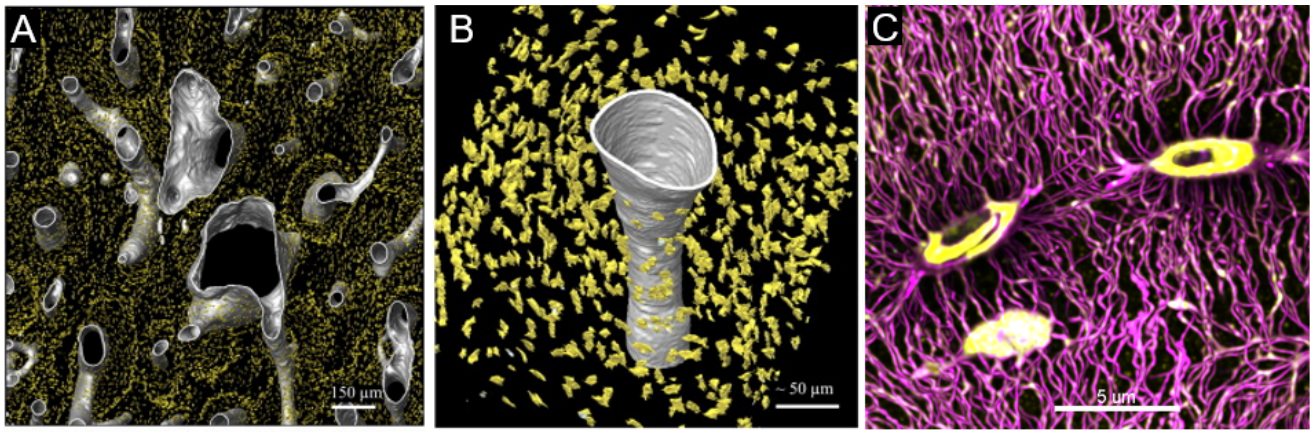

Figure 2: A) 3D rendering of Haversian canals and surrounding osteocyte lacunae from human femur cortical bone, imaged using synchrotron radiation microtomography. Reprinted with permission from reference [95]. B) The Haversian canal (grey) is surrounded by osteocyte lacunae (yellow)[95]. C) Osteocyte lacunae (yellow) are densely connected by canaliculi (magenta), visualized from confocal laser scanning microscopy of basic fuchsin stained cortical mouse bone. Image credit Ghazal Vahidi, Montana State University.

(3) *Bone repairs and replaces itself and can adapt to use*

Most bones in the body provide mechanical function for an entire lifetime without undergoing mechanical failure. Such a long service life is almost unheard of in engineered

devices such as vehicles and machines. Bone is able to maintain excellent material properties for much longer than most engineering materials because of the coordinated repair and replacement activities performed by resident bone cells. Complex teams of bone cells are continuously turning over bone extracellular matrix through a process called bone remodeling[67,96–98]. During bone remodeling one type of bone cell (the osteoclast) removes a small portion of the matrix and soon after another type of bone cell (the osteoblast) deposits new extracellular matrix in the newly made pore [67,96–98]. The remodeling process is accelerated in the presence of microscopic and submicroscopic cracks that are sensed by local osteocyte populations[99–101]. The result is that bone remodeling is a self-repair process that is efficiently targeted to regions of damage[70]. This daily maintenance is often sufficient to delay the formation of much larger cracks that can compromise the structure.

In addition to turnover of damaged bone matrix, the entire bone structure can be modified in response to changes in habitual mechanical demand. Osteocytes within the bone matrix sense habitual mechanical stress and strain and release chemical cues to surrounding bone cells. Surrounding bone cells may then add or subtract matrix from external surfaces – adding bone matrix if habitual mechanical stresses are too large and removing bone matrix if mechanical stresses are too small. The changes in geometry of the bone, by altering the mechanical stresses within the bone matrix, drive the mechanical stresses experienced by embedded cells toward a preferred physiological range. As a result of this process, the geometry of bone matches mechanical demands: individuals who have large mechanical demands develop larger and/or denser whole bones[102]. Notably, this adaptation process is similar to topological optimization approaches used in the design of structures, except that the living tissue continues to adapt throughout life[103].

For an ELM, self-repair and/or adaption similar to bone could be achieved through the presence of a living component that is sensitive to changes in mechanical stress associated with damage and/or increased mechanical demands. Some mechanosensitive systems in bacteria may be a good fit for this strategy. Bacteria have mechanosensitive channels and regulate osmotic force to improve survival in changing osmolarity[104,105] . Other mechanosensitive systems have also been identified, such as the two-component VxrAB[106,107]. Controlling this mechanosensation may offer novel methods to monitor the internal stresses of a load-bearing material and repair or adapt the material through activating bacterial metabolism or matrix production. Several notable challenges must be overcome to exploit bacterial mechanosensation. Most importantly, the continued viability of microbes within a material, or delivery of new microbes to the target locations, must be achieved.

4.0 Discussion

Engineered living materials sufficiently rigid to replace traditional engineering materials have the potential for transformative impacts on sustainable manufacturing, reducing energy, material, and labor costs. Further, the novel functionalities possible with ELMs, such as self-repair or environmental sensing, would be expected to facilitate new strategies for extending the service lives of these structures or to serve more than one purpose. The challenge of creating ELMs with sufficient mechanical performance to replace traditional engineering materials, however, is substantial. In considering these challenges, we see three major priorities for research.

A first priority must be increasing the viability of cells within rigid materials. The benefits of ELMs in terms of self-healing and sensing require living and metabolically active

resident cell populations. Recent demonstrations of stiff ELMs have achieved only short lifespans of resident cells and/or relied on metabolically inactive spores. Increasing ELM viability to the point where stiff materials can have useful service lives of years to decades will almost certainly benefit from engineering "vasculature"[108]. Although vascular systems have been proposed as a means of passively distributing healing agents inside materials, here such a system would instead be used to deliver nutrients, remove waste, facilitate intercellular communication, or even re-inoculate the structure with living microorganisms. Such vasculature would also greatly facilitate transport of biomineralization media to useful locations to participate in strengthening or repairing the structure. Bone offers a design template for the organization and function of this vasculature that could be leveraged by ELM researchers (**Figure 2**). An important consideration for engineering vasculature systems in ELMs is how to minimize costly human intervention, such as in monitoring microbial viability and introducing either new microorganisms and/or supportive media. Another consideration is how long microorganisms can maintain sufficient metabolic activity to confer useful functions to the material and when their re-inoculation is required. Besides vasculature, increasing the viability of cells within stiff ELMs may also benefit from approaches useful in softer living materials, such as using microbes engineered for greater stress resistance or multiple microbial species capable of symbiotic functions to increase survival of the community[1].

A second priority is advancing the state of "hybrid" ELMs which consist of living cells residing on a scaffold made of non-living engineering material with more desirable mechanical properties.[109,110] While stiff materials are a relatively recent goal for ELM design, the success of biofilms in establishing for years on stiffer, non-living surfaces has been known for considerable time. Biofilms are found on natural rock and on historic stone monuments, where their impacts can range from positive (e.g., crack healing through biomineralization) to negative (e.g., discoloration, crack formation)[111]. Oftentimes, such as in biomedical materials, biofilms found on ceramic and metal surfaces are unwelcome[112]. By contrast, biofilms established on stiff media or surfaces have been used for beneficial applications such as removing heavy metals from wastewater[113]. Microbial fuel cells can employ stiff anodes, such as porous ceramics or glass[114]. These hybrid designs can help surmount the current tradeoff between viability and dehydration in hydrogel-based ELMs where the matrix has dual roles of supporting cellular viability and contributing to structural strength. Thus, exploiting the ability of biofilms to grow, and persist, on stiff surfaces is likely to improve the success of generating truly living functional materials. Such an ELM would not be as effective at reducing energy costs in manufacturing as a non-hybrid ELM because the engineering material scaffold would be manufactured using traditional techniques and then transported to the application site. However, these limitations could be offset if the living component could extend the service life by helping to repair and/or maintain the load-carrying parts of the structure. Small increases in service life can have large effects on energy costs in material manufacturing: studies in the construction industry have indicated that a 20% increase in service life of structures, in some situations, can reduce carbon costs associated with manufacturing new construction materials by as much as 30% per year[109,110].

A third priority is redesigning load-bearing materials and devices with ELMs in mind. Materials synthesized by living cells will likely have substantially lower stiffness, strength or fracture toughness as compared to traditional engineering materials. Even ELMs that mimic naturally occurring living materials like wood and bone would not have sufficient mechanical properties to replace the most commonly used (and energy-intensive) engineering materials, like

steel and aluminum. As a result, the application of ELMs to load carrying applications will require substantial redesign of structures that account for the reduced stiffness of ELMs.

As progress is made towards resolving the primary challenges of generating strong, living, functional structures, other pressing considerations will come into focus. Life cycle assessments that consider the energy and resources needed to grow and sustain living materials will be needed to inform where and at what scale we should employ ELMs to achieve sustainability targets. A positive public perception of living materials is also an essential criterion for widespread usage. To make the needed progress for ELMs to fulfill the strength and viability requirements to become candidate materials for widespread applications, and to prepare for how these materials are to be used and interacted with in society, it is time to bring together teams of researchers across many disciplines. Advancing this field requires not only synthetic biologists who design the living components, but also experts in biomechanics who have long studied the mechanical performance and maintenance of biological materials (bone or otherwise), civil and mechanical engineers who have a keen understanding of the potential application space for newly designed ELMs, and experts in the social, economic, and legals aspects of integrating living materials into society. As of the time of writing this manuscript, there is not a common space (e.g., journal or dedicated conference) where ELM researchers across each of these communities share ideas. Initiatives that bring researchers across communities together to collaboratively work towards bringing rigid materials to life are much needed for realizing a sustainability revolution in materials manufacturing and usage.

CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest.

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