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Engineered Living Materials I

Foundations, Classifications and Future Potentials

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Summary

Engineered Living Materials (ELMs) incorporate living organisms in the synthesis process or seamlessly integrate them into conventional technological substrates, giving rise to novel functional materials. The transformative impact of ELMs spans various length scales and domains, such as construction, biomedicine and wearable technology. This impact arises from their exceptional attributes, including self-repair mechanisms, environmental responsiveness and inherent biocompatibility. This NanoTrust Dossier thoroughly examines the current state of ELMs production, offering a comprehensive systematic classification based on various parameters and elucidates recent strides in this dynamic field. Exploring ELMs unveils their scientific intricacies and underscores their potential to revolutionize and redefine material technologies in various applications.

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Introduction

Engineered Living Materials (ELMs) stand at the forefront of biomaterial innovation, representing a distinctive category that leverages living organisms – ranging from bacteria, fungi, algae and plants to (in-)vertebrate cells – coupled with a spectrum of traditional construction materials spanning synthetic polymers to ceramics and metals. This incorporation of biological entities with non-living constituents marks the inception of a paradigm shift, aptly termed “Biology meets Engineering” transcending scales from the nano- to the macroscale. ELMs exhibit specific properties and functionalities that surpass the capabilities of conventional non-biogenic counterparts, although they may not achieve the characteristics of specifically engineered high-performance materials[1].

As bioengineered composites emerge, living organisms actively participate in material formation, modification and response to environmental stimuli. The inherent biological processes intrinsic to living components bestow ELMs with the potential for adaptability, self-regeneration and self-healing – a paradigm shift in material science.

Living organisms sustain themselves through locally available resources and undergo natural recycling processes at the end of their life cycle. The synergy between the living components of ELMs and the required biocompatibility of the non-biogenic elements holds tremendous promise in addressing sustainability challenges.

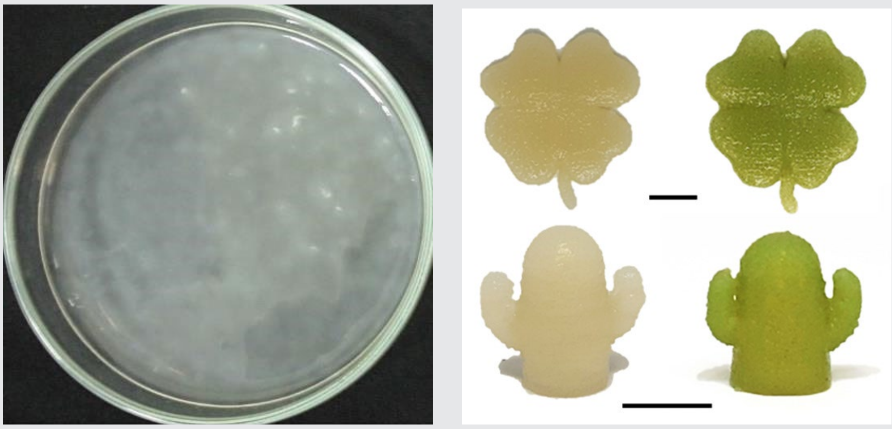
The interplay between living and non-living constituents, particularly at the nano- and microscales, imparts ELMs with unique adaptability, biocompatibility and functional versatility. This NanoTrust Dossier sets the stage for a comprehensive exploration of the emerging field of ELMs, elucidating their scientific intricacies and vast potential across multifaceted applications.

Evolution of ELMs: Historical and Contemporary Applications

Ancient civilisations across the globe have long recognized the robustness, adaptability and versatility of ELMs. For instance, using mud bricks, also known as unfired bricks, in construction by the Ancient Egyptians exemplifies an early understanding of sustainable building materials. These bricks often contained organic components, which enhanced their durability and thermal properties, illustrating an early form of ELMs[2]. In the wet and forested landscapes of Meghalaya, India, indigenous communities have ingeniously cultivated living bridges utilising the aerial roots of the Ficus elastica tree for centuries, showcasing the historical incorporation of ELMs in structural applications[3]. These ingenious techniques persist in contemporary practices, as evidenced by the avant-garde approach of furniture growing[4]. A wood-staining mould (Aureobasidium genus) is utilised as an ELM finish for oil-treated woods, serving as a protective, self-healing and decorative treatment for wood in modern construction[5]. Another contemporary example is the emergence of kombucha leather. This leather comprises cellulose nanofibrils produced by bacteria and yeast, which emerged as part of the sustainable fashion movements in the 2000s at the Davis University of California[6], highlighting the progressive intersection of biotechnology and fashion. These diverse instances underscore the enduring symbiosis between human ingenuity and Nature’s resilience, illustrating the historical and contemporary utilization of ELMs across various cultures and applications.

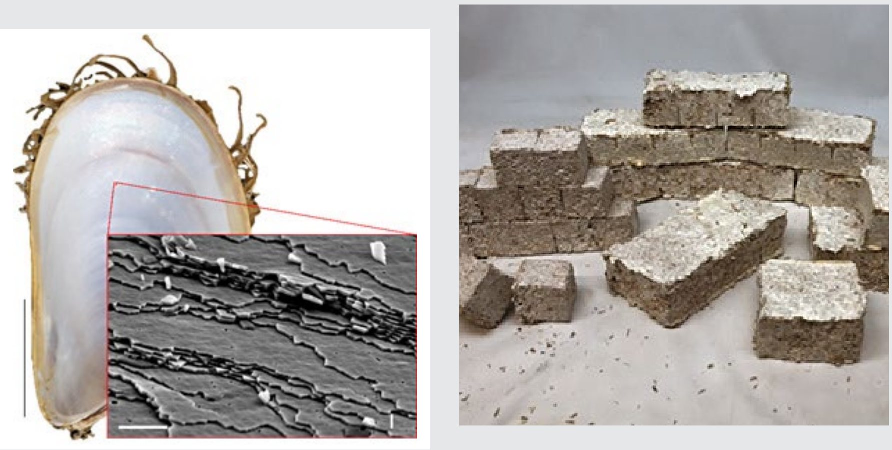
Emerging ELMs in Contemporary Research

Scientific exploration of ELMs has been spanning only a few years. Despite this brief duration, ELM research has manifested a remarkable diversity of trajectories. Based on this diversity, we want to comprehensively overview the evolving landscape and its discrete subdomains in this NanoTrust Dossier by employing a multidimensional classification of ELMs. Initially, we focus extensively on the categorization based on various organism types while providing succinct



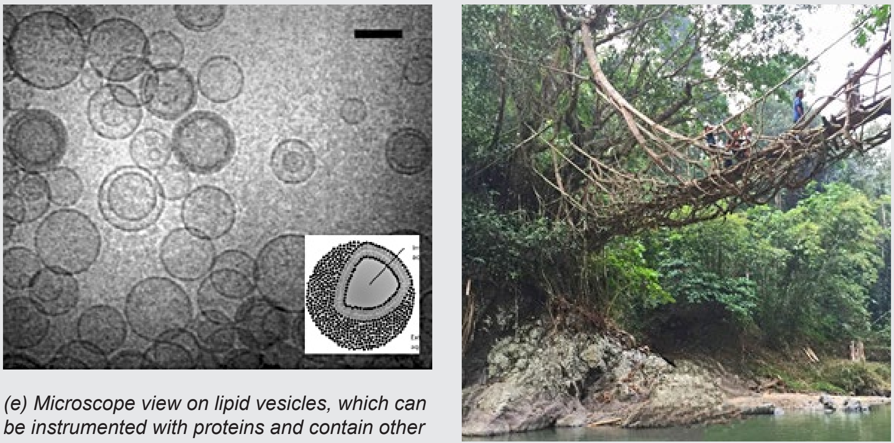
(a) Bacterial cellulose is produced by fermentation utilizing Acetobacter xylinum in coconut water. A. xylinum has been known to produce pure cellulose for more than 100 years[7].

(b) Microalgal in a hydrogel scaffold, ELMs in the shape of cacti and clovers 14 days after incubation under autotrophic conditions.



(c) Nacre (insert right with a scale bar of 10µm)[8] of a Modiolus barbatus[9] with a scale bar of 10mm.

(d) Sterile Ganoderma sp. is combined with raw non-sterile feedstock and grown in a mould before the form-stable moulded biocomposite is post-processed[10].



(e) Microscope view on lipid vesicles, which can be instrumented with proteins and contain other organisms such as bacteria as organelles. Such vesicles are essential components of bottom-up synthetic biology.

(f) Living root bridge (July 2015, Kanekes, Java, Indonesia)[1].

Figure 1: *Cells and tissues from various organisms are utilised as the living component for ELMs. Examples of the different main categories using bacteria (a), algae (b), the principles of nacre (c), synthetic biology (e), mycelium of fungi (d) and plants (f) showcase the broad spectrum of living entities serving as foundational elements in developing innovative ELM technologies*

glimpses into additional classifications, which will be explored in the subsequent NanoTrust Dossiers on ELMs. Moreover, we elucidate some formidable challenges intrinsic to ELM research, addressing pertinent aspects such as safety and regulatory concerns. This systematic exploration aims to equip the reader with a nuanced understanding of the current state of ELM research, laying the foundation for an in-depth exploration of its intricacies and future directions.

Taxonomy Categorizing ELMs Based on Biological Constituents

Cells from diverse organisms serve as foundational elements or integral components within ELMs. The distinctive properties exhibited by these ELMs are contingent upon the specific cell types utilised and the various advancements along the different research trajectories. Therefore, the taxonomic classification of ELMs in this dossier is structured and founded on the classification of organism types.

Bacterial-based materials

Bacterial cellulose (BC) has been selected as a representative member of this category. Also called nano-cellulose, BC stands out as a cost-effective and versatile biomaterial synthesized by various bacteria species such as Gluconacetobacter xylinus and Acetobacter xylinum[11]. BC is an archetypal example of bacterial-based ELMs and possesses unique properties such as versatility, moldability in situ and biocompatibility. Its nano-fibrillar structure retains a significant amount of liquid[12] and offers high mechanical strength in wet states due to its hydrophilic nature and high surface area-to-mass ratio. In contrast to plant-based cellulose, BC is not integrated with other components such as lignin, pectin and hemicellulose[13].

BC shows a subdued inflammatory response, good cellular adhesion, proliferation and differentiation, no genotoxic effects and low cytotoxicity in vivo. These properties position BC as a compelling candidate for exploration in reconstructive surgery and tissue engineering applications. The biocompatibility further makes it a valuable material for medical applications such as wound dressing and skin regeneration[14], where BC absorbs exudates from wounds and conforms to the body shape over extended periods. The characteristics of BC can be modified to support forming material similar to cartilage, increasing porosity, or reducing the coagulation effect on polyester vascular grafts and stents[15]. Due to its bio-based nature and biodegradability BC provides an environmentally sustainable alternative for textiles[16] and leather-like materials.

While various applications of BC focus on its use as a bulk material to be further refined, researchers were able to create various free-standing 3D shapes by utilizing the bacterium Komagataeibacter xylinus to produce BC in superhydrophobic moulds, such as hollow BC spheres that were used to support the growth of mouse embryonic stem cells[17,18].

Certain BC-producing bacteria, such as Komagataeibacter rhaeticus, generate substantial pellicles and millimetresized spheroids at the air-water interface under specific environmental conditions. In 2021, researchers established a reproducible methodology for producing such BC spheroids. This approach allows BC spheroids to be utilised as constructional elements in functional 2D and 3D ELMs. To underscore this capability, researchers successfully employed BC spheroids to assemble and mend disjunct pieces of BC materials, as documented in the work by Caro-Astorga et al.[19], thus paving the way for an entirely novel research trajectory in the realm of BC-based ELMs.

Mycelium-based materials

Mycelium constitutes a macroscopic network composed of interconnected tubular cells extending down to the nanoscale, formed through the inherent growth pattern of filamentous fungi. These fungi play a pivotal role in the ecological recycling of biomass, contributing to the breakdown and recycling of diverse biomass sources within terrestrial and marine ecosystems. Leveraging the unique properties of mycelium-forming fungi, various biotechnological applications have emerged, harnessing their capabilities to accomplish various functions in different contexts[20]. In situ, manufactured mycelium-based bio-composites offer a cost-effective alternative to local construction materials for space travel to the moon and Mars[21].

ELMs that utilize mycelium are often composite materials that incorporate mycelium as a binding element into their structure – for example, inoculating lignocellulosic waste that acts as a substrate for fungi growth with saprotrophic fungi species. The substrate particles are bound together by the growing fungal filaments that fill out the space between them. The growing phase can be suspended by dehydrating the fungi; notably, this does not implicate the death of the fungi[22,23]. Reactivating the growing phase of the fungi via rehydration will induce a self-healing development[24].

Pure mycelium biomass is derived either by isolating the growing organism from its substrate or through the complete absorption of the substrate by the fungi. The material properties of pure mycelium are intricately influenced by the biological characteristics of the organism, the specific growth conditions employed and the subsequent post-processing steps. A diverse collection of consumer products, ranging from meat alternatives (e.g., Atlast™) to leather substitutes (such as Mylo™, Reishi™ [25], Mylea™, Forager™), have already been developed, accentuating the versatility and potential applications of pure mycelium-based materials. Beyond its fundamental applications as primary materials, mycelium demonstrates potential in coatings, contributing to the fabrication of protective, self-healing and aesthetically enhanced biotreatments for wood substrates.

Plant-based materials

Plant-based materials have been used by humanity since humans learned to use tools. Bamboo, for instance, has been used in Asia for millennia, not only for its strength but also its flexibility[26]. Straw usage in adobe bricks or thatch roofing similarly dates back thousands of years in various cultures[27].

Ancient Egyptian medicine incorporated plant-based poultices that included living materials[28]. Similary old are the Indian living bridges constructed from aerial roots. These examples illustrate early human functional manipulation of living plant material.

Recently, a surge of interest evolved in harnessing plants' regenerative and adaptive properties[29,30]. Plant ELM research combines these properties to engineer novel, eco-friendly materials. These materials can be constructed using various techniques, from utilising living plants (such as vertical greenery systems[31] and as flax fibres based pavilion[32]) and controlling the growth of plants (by understanding growth factors in plants[33,34]) to futuristic plant-based living materials that are a dynamic fusion of living plant cells and traditional structures. These materials integrate biology and engineering, where the metabolically active plant cells lend their inherent and unique properties to static materials, promising biobased, biodegradable and self-repairing capabilities. Due to the longevity of certain plant-based organisms and their natural ability to incorporate dead and non-living components in their matrix, such composite materials offer enormous potential for novel materials and applications for various industries.

Algae-based materials

Algae, encompassing a polyphyletic assortment of organisms with disparate genetic lineages, share standard features[35,36]. Certain green algae represent early divergent lineages within the broader spectrum of green plants[37]. Microalgae species prove particularly well-suited for ELMs due to their singular nature as single-cell photosynthetic organisms. These microalgae can seamlessly integrate into various matrices, provided they can access water, light and essential nutrients. Certain algae exhibit remarkable resilience, enduring compression within narrow tubing of extruderbased 3D printers to craft diverse structures, including CO_2-absorbing hydrogel configurations[38]. Additionally, specific algae called diatoms can polymerise nanostructured biogenic glasses at room temperature[39]. These biogenic glasses, serving as versatile materials, find application as mechanical or optical components within ELMs[40], thereby contributing to sustainable fabrication processes with minimum energy input.

Animal tissue-based materials

Animals exhibit distinctive features that distinguish them from other organisms. Unlike plants and algae, which synthesise nutrients through photosynthesis, animals are heterotrophic, relying on the chemical energy stored in external organisms for nutrient acquisition. Characterised by a unique extracellular matrix (ECM) composed of diverse components, including collagen, animal tissue cells operate within a distinctive structural framework. Unlike multicellular organisms with cell walls that anchor cells in place, animals possess a flexible framework that facilitates progressive tissue development. Remarkably, animal tissue can undergo biomineralisation of the ECM, forming robust scaffolds that give rise to structures such as bone and spicules[41,42]. This capacity underscores animal tissues' dynamic and adaptable nature in their developmental processes.

Using living animal tissue in ELMs poses the enormous challenge of the more complex interconnected metabolism of the organism compared to the simpler ones mentioned before. While individual parts of plants and algae can mostly live autonomously, animal tissue depends on other functional elements of the organism to survive. Theoretically, this dependence could be utilised in medical applications of animal cell-based ELMs by connecting them to human organism functions. Recent progress in bio-hybrid robots exemplifies the utilizes of muscle tissues as actuators[43]. Some materials produced by animals that can be harvested are akin to other ELMs. One of them is nacre, commonly known as mother-of-pearl. Mollusc species produce layered nacre as an inner shell or when an irritating microscopic object becomes trapped within its mantle folds, resulting in a pearl[44]. Nacre has a unique combination of toughness, strength and beauty, making it an intriguing research subject. Nacre consists primarily of aragonite but is about 3,000 times tougher. This strength stems from the hard aragonite platelets and the soft organic matrix, which distributes stress and prevents cracks from propagating[45,46]. Natural nacre growth involves a complex interplay of mineralisation and organic matrix secretion. Understanding and integrating nature's self-assembly technique of nacre into ELMs could open pathways for biocompatible high-performance materials[47].

Synthetic biology-based materials

Synthetic biology[48] employs microbiologic tools to engineer living cells and organisms, likening the process to programming machines. The ELMs discipline seamlessly merges engineering principles from synthetic biology and materials science within materials synthetic biology. This integration facilitates the bottom-up or re-design of living systems, transforming them into dynamic and responsive materials endowed with emerging and programmable functionalities[49,50,51]. The hazards of synthetic biology are open for discussion and will be discussed in Section *"Biosafety and Biosecurity considerations*".

The basic principles of biology, chemistry, and engineering are utilised for the (bottom-up) assembly of biological cells. Lipid or polymer vesicles are widely used as model membrane systems to investigate the translocation of solutes and the transduction of signals by membrane proteins[52]. The bottom-up assembly and functionalisation of such artificial compartments enable complete control over their composition. Artificial cells can thus provide specifically optimised environments for synthetic biological processes.

In the context of this study, genetically modified organisms remain in their host organism categories, i.e. that of the genetically unmodified organism. This dossier understands bottom-up synthetic biology as BUSB to differentiate it from genetically altered organisms. The bottom-up approach allows the isolation of functionality and structures thereby expanding the fundamental understanding of biology. As a significant step in that direction, synthetic biology is underway to synthesise a minimal cell with artificial metabolism[53].

The synthesis of tissues (fabrication of complex cellular constructs) consisting of synthetic cells is still a future perspective[54]. However, the toolbox of BUSB enables advances in other material sciences, such as the work towards engineering and producing synthetic spider silk based on research on spider silk proteins[55]. Another prospect of BUSB is the transfer of the BC synthesis from bacteria to synthetic cells, resulting in bacteria-free BC. Bacterially synthesised BC is typically contaminated with lifeless bacteria. Bacteria have more metabolic needs than synthetic cells. Hence, the cellulose-producing synthetic-cells-based ELMs reduce the complexity in production drastically. However, simultaneously, the complexity at the synthetic cellular level (synthetic pathways) increases[56].

Synthetic biology also offers the prospect of creating ELMs in approaches that can neither be classified as belonging entirely to the field of BUSB nor that of genetic engineering. By capturing and processing spatially segregated bacterial colonies within individual microdroplets, ELMs can be assembled into membrane-bound structurally and morphologically complex synthetic cells[57].

Synthetic biology is still a grey area in the current biosafety regulations. However, as soon as biogenic DNA is incorporated into the system, the strict regulations and guidelines of genetic engineering must be fulfilled.

Composite materials

Unlike the previously mentioned ELMs that combine a single living organism into an ECM, also multiple combined organisms in a single composite ELM are possible. The combination enables the exploitation of the synergetic interactions between different living entities to manifest improved or entirely new properties. By including multiple organisms, a broader range of functionalities are opening up as adaptive responses within the material and optimisation of material behaviour for specific applications. For instance, while one organism might provide structural integrity, another might confer self-healing or responsive effects such as moisture dependent stiffness.

As an example, fungal-bacterial biocomposites grown on lignocellulosic in a mold, which are regenerative structures where the bacteria can be enhanced for biosynthetic and sensing capabilities[10]. Such regulations limit the utilisation of specific enhanced organisms when exposed to the environment to prohibit unwanted interactions with various ecosystems.

Further Categorisations

The categorisation of the diverse ELMs landscape based on organism type represents a multifaceted approach; however, additional categorisations become imperative to encompass the entire ELMs field comprehensively. Our subsequent NanoTrust Dossiers on ELMs expands upon ELMs for material types, production methodologies, distinctive characteristics and the implicated length scales. Simultaneously, a thorough examination of the challenges within these domains is discussed. These elaborations include cultivated ELMs wherein no living organisms remain in the final material, ELMs simulating living behaviour to achieve specific functionalities and ELMs interfacing living tissue with technical materials, exemplified by soft robots leveraging muscle cells.

Current Challenges in ELMs

The widespread adoption and production of ELMs still faces various challenges. We spotlight some of these in the context of the categorisation presented in this dossier.

Understanding growth

Controlling ELMs growth speed is a fundamental challenge. Growth that is too fast might impair the material's structure or become unmanageable. The living component might not support the material's viability if growth is too slow. A consistent growing rate throughout an ELM structure ensures homogeneity, which is vital for many applications, especially when mechanical and other physical properties are essential. Cells in ELMs require specific nutrients to grow. Nutrients accessibility, composition, quantity and allocation will influence growth patterns. However, ensuring a consistent nutrient supply, especially in large structures, remains a challenge[58]. As cells grow, they also produce waste products and if they are not designed as an integral part of the material, accumulating these waste products can inhibit further growth. Efficient waste management systems are another essential part of ensuring sustained growth. Other external factors, such as temperature, humidity and pH value, also influence growth. Enabling consistent growth in various environmental conditions is crucial for the widespread applicability of ELMs.

For dynamic material properties and structure control, the growth process must be handled by self-assembly and external control (through light, chemicals, or other signals). Managing growth at the interface with traditional materials is challenging. All living materials have the potential for mutation, and evolutionary pressures will alter the growth characteristics of ELMs. The genetic stability over generations of organisms depends on various factors, such as the fact that every species has specific intrinsic genome stability. Maintaining genetic stability over long periods is a concern, especially when researchers often select organisms with easy genetic tractability[59]. Over time, aging cells can enter a state called senescence, where they no longer divide. Understanding and governing this process would be instrumental in creating long-lived materials or structures. Especially for ELMs, controlled homeostasis would facilitate form-stability while preserving other vital living capabilities. It is further known that cells communicate with their neighbours to coordinate growth patterns, especially in complex structures. Further research is necessary, as the mechanisms and reliability of this communication under various conditions still need to be fully understood[60].

All these factors illustrate that understanding growth in ELMs is not solely about the growing process itself; it is about controlling and directing growth predictably. This complex task at the intersection of biology, materials science and engineering will take a lot of effort and time to understand.

Acceptance of (Epi)Genetic engineering and Biomanipulation

Manipulating organisms to fulfil specialised functions in an ELM is challenging and usually done by (epi)genetic manipulations. However, the selective breeding of microorganisms for ELMs also requires extensive research and biocontainment until environmental safety can be guaranteed.

Public perception of (epi)genetic engineering and other biomanipulation often intertwines with ethical, safety and environmental concerns, especially given the long-term implications of releasing genetically modified organisms into the environment. Public views of (epi)genetic engineering are polarised: Some view it as a revolutionary scientific breakthrough, while others express strong reservations about potential unforeseen ecological and health impacts. Building trust with the public will take time, but it is essential for successfully integrating ELMs into society.

Conclusion

This dossier serves as an introductory exploration into the intricate realm of ELMs, endeavouring to provide a comprehensive overview of its multifaceted aspects. The focal point of this compilation is the characterisation of the field along a unifying dimension – the foundational organism within the ELM. By adopting this approach, we engage in a nuanced examination of both the advantages and obstacles associated with diverse living organisms.

The encountered challenges span a spectrum, ranging from the intricacies of managing the growth of living organisms to sustaining life within a given material matrix. Moreover, the imperative to confine viability outside the material is paramount to mitigate potential environmental risks. These challenges underscore the necessity for further research across multiple disciplines and the significance of establishing a robust regulatory framework. Such a framework is crucial for addressing ethical considerations and ensuring comprehensive biosafety within the realm of ELMs.

Further challenges

Our subsequent NanoTrust Dossiers on ELMs examines additional challenges regarding multiscale complexity, vascularization, composite structures and interfacing.

Regulatory Considerations for ELMs

As with any new technology, regulations must keep pace with scientific development to ensure safe and ethical use of the technology and minimize the risk of adverse environmental side effects. By their very definition, ELMs offer great opportunities for novel technological materials. ELMs may also pose specific threats to the ecosphere if not adequately controlled from an environmental, ethical, and general acceptance (media) perspective.

Biosafety and Biosecurity considerations

The field of ELMs is intricately linked with those of genetic engineering and synthetic biology in many regards, especially because genetic modifications may often be the key to unlocking features of organisms not possible in conventional ELMs (based on organisms without genetic changes) design. Incorporating genetically engineered organisms into consumer products or devices means overcoming several challenges, as they have to remain alive and functional during the lifetime of the material. The risk of releasing the modified cells into the environment must be controlled, as they pose a potential bio-hazard and could trigger unforeseen changes in an ecosystem. Such considerations are critical in biodegradable ELMs. Questions about cell survival and activity after biodegradation of the scaffolding material are of high importance, as the circumstances during the degradation of the scaffolding material may still provide an environment where the cells can survive.

Depending on the applied method, detecting whether a cell has been genetically altered is impossible, hindering the traceability of such cells in the environment once released. To address this challenge, the Intelligence Advanced Research Projects Activity (IARPA) of the U.S. Intelligence community together with Gingko Bioworks™ and Draper™ conducted the FELIX (Finding Engineering-Linked Indicators) program, which aimed to develop new ways of detecting genetically engineered biological systems[61,62].

Several legislation frameworks already deal with the ramifications of genetic engineering, the minimisation of associated risks and biological containment strategies to hinder modified cell survival in the absence of human control. Researchers need to be familiar with the legal frameworks they operate in and stay informed about their evolution.

Ethical considerations

ELMs reside on the interface between biology and material science. Living organisms produce or they feature living components that facilitate their unique properties. As such, the methods employed in production and their uses as finished products are subject to ethical considerations[63]. Researchers should, of course, adhere to ethical guidelines set by their institutions and governments. However, as this field is still emerging, appropriate and comprehensive policies and legislation for ELMs may still need to be created. Therefore, it is essential to be active in developing such regulations to ensure ethical and safe conduct.

Glossary

biocompatibility: Biocompatibility specifies the ability of a material to interact harmoniously with living organisms without causing harm or any other adverse reactions.

lignocellulosic: Lignocellulose (biomass) is a biomass composed of plant dry matter. It is the most abundantly available raw biomaterial on Earth. It comprises mainly two carbohydrate polymers, cellulose and hemicellulose and an aromatic-rich polymer called lignin.

mycelium: Mycelium is a network of fungal threads or hyphae.

polyphyletic: Polyphyletic groups are organisms with homologous characteristics that evolved independently and have no common ancestor.

saprotrophic fungi: Fungi that obtain their carbon by the decomposition of dead biogenic material.

Acronyms

2D	Two dimensional
3D	Three dimensional
BC	Bacterial cellulose
BUSB	Bottom-up Synthetic Biology
DNA	Deoxyribonucleic acid is the molecule that carries genetic information for the development and functioning of all living organisms
ECM	extracellular matrix
ELM	Engineered Living Material

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Declarations

All authors declare that they have no conflicts of interest.

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