Perspective
Taxonomy for engineered living materials
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SUMMARY
Engineered living materials (ELMs) are the most relevant contemporary revolution in materials science and engineering and aim to outperform current examples of “smart,” active, or multifunctional materials, enabling countless industrial and societal applications. The “living” materials facilitate unique properties, including autonomy, intelligent responses, self-repair, and even self-replication. Within this dawning field, current literature has classified ELMs mainly into biological ELMs (bio-ELMs), which are solely made of cells, and hybrid living materials (HLMs), consisting of abiotic scaffold and living cells. Considering that the most relevant feature of ELMs is the living cell colonies or micro-organisms, we consider that ELMs should be classified and presented differently, more related to life taxonomies than to materials science disciplines. Toward solving the current need for the classification of ELMs, this study presents the first complete proposal of taxonomy for these ELMs. Here, life taxonomies and materials classifications are hybridized hierarchically. Once the proposed taxonomy is explained, its applicability is illustrated by classifying several examples of bio-ELMs and HLMs, and its utility for guiding research in this field is analyzed. Finally, possible modifications and improvements are discussed, and a call for collaboration is launched for progressing in this complex and multidisciplinary field.

INTRODUCTION
Taxonomies are schemes for classification, typically hierarchical, which help organize and index knowledge and research fields. Apart from classifying what already exists, taxonomies can be used to foresee and organize what may still be developed or created, especially in the cases of taxonomies dealing with novel research fields. Life sciences researchers have been trying to organize life since antiquity, although modern taxonomy starts with the works of Linnaeus1 and with subsequent improvements aimed at incorporating evolution into it. 2,3 Even today, the task of organizing life is overwhelming and a source of intense and fruitful debate, 4 also illustrated by the recent development of the PhyloCode 5 as an alternative to more traditional taxonomies.

In any case, taxonomies for life will surely need to be reformulated to adequately account for creations from the realm of synthetic biology. For more than a century (the term “synthetic biology” dates back to around 1910), 6 life scientists and physical scientists have joined efforts to regenerate or “engineer” damaged tissues, to edit pieces of genetic material, to artificially promote immune responses, and to create synthetic cells from basic constituents, to cite some examples, pursuing advanced therapies and a better understanding of the fundamental mechanisms of life. 7,8 These research directions have set the foundations for the synthetic biology field.
Engineered living materials (ELMs) are considered the most relevant contemporary revolution in materials science and engineering. These ELMs aim to outperform current examples of “smart,” active, or multifunctional materials and, hence, enable countless industrial and societal applications, in which the “alive” materials improve autonomy, intelligent responses, self-repair, and even self-replication. In the last 2 decades, the first examples of true living materials have been achieved, normally incorporating living cells to scaffolding structures or entirely by self-assembly of living cells. Respectively, these two approaches are commonly referred to as bottom-up synthesis of “biological engineered living materials” (bio-ELMs) and top-down creation of “hybrid living materials” (HLMs) (Figure 1). The more relevant pioneering endeavors and results of ELMs, both HLMs and bio-ELMs, have been which also deals with the design and materialization of living machines and living materials, the central topic of this study.

Figure 1. Scheme of engineered living materials
(A and B) Engineering living materials and its categorization into (A) biological engineered living materials (bio-ELMs) and (B) hybrid living materials (HLMs). Few examples of the applications of bio-ELMs and HLMs are also depicted in (A) and (B), respectively. It should be noted that the applications of ELMs are not limited to the ones represented here.
(C) An example of bio-ELM, which shows growth and patterning into 3D geometries of cellulose spheroids from Komagataeibacter rhaeticus bacteria. Reprinted with permission from Caro-Astorga et al. Copyright 2021 Springer Nature.
(D) An example of HLM, where a soft robot was fabricated using the synergic activities of cardiomyocyte cells and carbon nanotubes, mimicking the crawling movement of caterpillars. Reprinted with permission from Sun et al. Copyright 2019 Wiley.
The emergent ELMs field is already populated with hundreds of examples. Figure 2 presents the annual number of publications while using “engineered living materials” as the keywords in Web of Science and Google Scholar. It should be noted here that the term engineered living materials was first used in a publication in *Biochemical Society Transactions* in 2017 by P.Q. Nguyen. However, this publication is not indexed in Web of Science; it projects another publication by Nguyen in the journal *Advanced Materials* in 2018 detailing the concept and future prospects of ELMs as the first publication. Therefore, no publication was found in Web of Science before 2018 using the keyword. As the Google Scholar search engine is not as restricted as Web of Science, a few related publications appeared before 2018. However, there are also several publications before 2018 that can be still categorized as ELMs but cannot be found in the online database using the exact keywords. Furthermore, as the term engineered living materials is newly employed, publications related to the field after 2018 do not use the term religiously. Nevertheless, Figure 2 depicts a rapid growth of this young field of research in the recent years, which demands a taxonomical organization to understand better what has been obtained and what may still be invented or created.

Among inspiring efforts for organizing this dawning field, Srubar III proposes a taxonomy for ELMs research, in which scale, design, organism type, material properties, and application are used as taxa. That study provides an excellent introduction to ELMs in general and highlights related research areas and trends, and a taxonomy for the ELMs research field is provided. Complementarily, in our study, we concentrate on a taxonomy for the actual engineered living materials. Hierarchically speaking, in our view, organism type should be the fundamental taxon, if a taxonomy for ELMs is developed.

Whether ELMs should be considered life or not and the related ethical implications are topics beyond the purpose of this study. However, we believe that it is plausible and even probable that, if research efforts deploy as expected, ELMs will have to be
considered living entities and incorporated into the taxonomy of life. Most reviews on ELMs have organized the field considering the HLM and bio-ELM areas and the types of materials used as scaffolds or abiotic scaffold for the case of HLMs. The European Union Commission also employed this approach in the recently launched EIC Pathfinder Challenge on Engineered Living Materials. Nevertheless, considering that the most relevant feature of living materials is that they are made of or include living cell colonies and micro-organisms, we consider that ELMs should be classified and presented differently, more related to life taxonomies than materials science disciplines.

To approach solving this need for classification, to the authors’ best knowledge, this study presents the first complete proposal of taxonomy for ELMs, in which life taxonomies and materials classifications are hybridized hierarchically. Once the proposed taxonomy is explained, its applicability is illustrated by classifying several examples of bio-ELMs and HLMs and its utility for guiding research in this field is analyzed. Finally, possible modifications and improvements are discussed and a call for collaboration is launched, as needed for progressing in such a complex and multidisciplinary field.

PROPOSED TAXONOMY

Rationale for the taxonomy proposal

If living materials are considered life, the higher rank taxa (domains and kingdoms) should take inspiration from life’s taxonomy. In fact, the most relevant and distinctive features of any ELM are the living constituents that make it alive. On the other hand, the lower rank taxa (classes and below) would depend on the material types used for the living materials’ “skeletons” or scaffold, abiotic in the case of HLMs, providing structural support to the living cell colonies. In this way, we propose merging life’s taxonomy with common materials classifications.

Even from a functional or an application perspective, cell type is the most fundamental feature for living materials and, therefore, should constitute the higher rank. For instance, the living material employed for a building cover, capable of gathering energy from sunlight, relies on being formed by prokaryotic or eukaryotic cells, whether their support is a carbon mesh, chainmail, or an interwoven glass fiber. At the same time, a living material, which can survive in remote locations and under extreme environmental conditions or enables energy or materials production essential for space colonization, emphasizes more on including extremophilic archaea or prokaryotes, compared with the choice of the scaffold material. To cite another example, self-contractile microbiobots can be made of hydrogels, soft elastomers, or 3D-printed mechanisms using a wide set of materials families for their abiotic scaffold, but their actual autonomous motion and life expectancy depend on the use of mammal cardiomyocytes and a related life-supporting extracellular matrix, biochemical factors, and nutrients. Changing the cardiomyocytes by electrically controlled musculoskeletal cells, or incorporating neural cells for future cognitive tasks, may affect the performance and potentials of such microbiobots much more than modifying the materials used as scaffold.

On the basis of the above, the following subsections present the taxonomy, from the higher rank to the lower rank taxa, before analyzing its viability and utility for classification and discovery purposes.

Domains, superkingdoms, and kingdoms inspired by life’s taxonomies

Our proposal for the higher rank taxa takes inspiration from the more widespread taxonomy for life, which uses three domains and six kingdoms (Woese’s system).
Accordingly, we propose archaeal, bacterial, and eukaryotic living materials as basic domains and complement them with an additional domain for those with synthetic cells as constituents (Figure 3). Besides, co-cultures of entities from different domains may prove viable in synthetic biology and lead to successful applications. In consequence, we add a cross-domain for living materials created by combining cells or living entities from diverse domains. The same principles apply to the kingdoms (archaebacterial, eubacterial, protist, fungal, vegetal, and animal) ELMs, to which we also add a kingdom for ELMs with artificial cells and a cross-kingdom.

Between the domains and kingdoms, we consider the possibility of two superkingdoms: bio-ELMs (made only of cells) and HLMs (combining cells with an abiotic structure). As mentioned earlier, the cell type of an ELM is the most important aspect in determining the functionalities of the ELM. Therefore, life taxa hold the highest ranking in the taxonomy, and we assign the classification of bio-ELM and HLM below that as superkingdoms. Furthermore, the concept of bio-ELM and HLM is already established and taken up by several researchers in their publications. Inclusion of these superkingdoms in the taxonomy will not only include already standard classification of ELMs but also consolidate any interesting and unexpected progress in either of the classifications. A similar revolution is now taking place in the related field of tissue engineering, in which scaffold-free approaches are gaining research attention after decades of scaffold-based developments, and synergies among both categories are being increasingly explored. Nonetheless, the long-term utility of such superkingdoms and a possible simplification of the taxonomy are discussed later in the manuscript.

Phyla, classes, families, and species inspired by materials classifications

Proposed phyla act as the bridge between the higher ranks, linked to the living entities, and the lower ranks, connected to the materials used as extracellular matrices or scaffold for the ELMs. Considering that life is divided into phyla depending on the spatial configuration of their cells and that all kinds of materials can also be grouped according to their topology, we propose phyla based on dimensional features for the living materials taxonomy, as illustrated in Figure 3.
Consequently, n-dimensional phyla lead to 0D, 1D, 2D, and 3D living materials. ELMs may be considered 2D if their depth is one order of magnitude smaller than their breadth and length. 1D materials make reference to those with a length at least one order of magnitude larger than their other two dimensions, and 0D refers to quasi-punctual configurations, usually a single cell or cell cluster with a fixed position in space. If the materials, apart from being alive, can move and interact with the environment through autonomous or controlled shape morphing or through purposely designed degradation, the living material can be considered “dynamic” ELMs. Dynamic refers to materials, in which their chassis or body may undergo a significant transformation or metamorphosis (bending, rolling, folding, deploying, or purposefully moving): dynamic phylum means reconfigurable body. Those living materials with multi-scale self-replicating morphology, recursive definition, or non-integer dimensions are fractal ELMs. Cross-phylum living materials and derived systems may combine, for example, 2D biofilms with 3D scaffolds or 0D cell clusters fixed upon specific regions of a biofilm, to cite some examples.

In our opinion, these dimension-derived phyla will be useful, as both living tissues and traditional non-living materials are classifiable through their dimensional features. Besides, the studies on biological materials, in many cases, describe and model tissues as 1D fibers working under traction, as 2D shells and meshes, or as 3D constructs. 2D and 3D classifications have been also proven useful for organizing another emergent field, metamaterials, into 3D metamaterials and quasi-2D metasurfaces. Furthermore, the dynamic phylum incorporates stimuli-responsive shape-morphing materials across dimensions, particularly corroborating the emerging field of 4D printing, which is also opening new horizons in materials science.22,23

Regarding the proposed classes for the taxonomy, the actual type of material used as extracellular matrices or abiotic scaffold is considered. This applies mainly to HLMs in our first taxonomy proposal but could be considered for all kinds of ELMs, if some minor modifications are included, as discussed later in the manuscript. The common materials classification depending on the chemical bonds and composition, which determine the mechanical, electrical, and thermal properties, is used here for the classes of living materials. This leads to metallic, ceramic, polymeric, carbon, and composite ELMs (here HLMs), which in short can be referred to as living metals, living ceramics, living polymers, living carbons, and living composites, respectively.

Families are organized by further dividing the classes of materials, used as extracellular matrices or scaffold, by employing the common subdivisions used in materials science. For example, living metals are divided into families, including living steels, living Cu alloys, and living Ni alloys, to name a few. The same applies to the families of other living materials classes, presented in detail in Figure 4. As for the species, an additional level of detail is expected. For instance, the living carbon nanotubes (CNTs) family can be further categorized into living single-walled CNTs (SWCNTs) and multi-walled CNTs (MWCNTs) and living thermoplastic polymer can include specific species of living polyactic acid (PLA), living polycaprolactone (PCL), or living poly(lactic-co-glycolic acid) (PLGA). The use of specific cell colonies from concrete living species may also lead to different species within the same family.

The proposed classes and families should also apply to bio-ELMs, as they depend on the extracellular matrices produced by the cellular organisms. For instance, bio-ELMs, where the primary extracellular matrix is polysaccharides or proteins, can be referred to as living polymers. In such cases, new ELM families may be
introduced, such as living polysaccharides or living proteins. Furthermore, several cellular organisms, mostly engineered bacterial strains, can produce gold or silver nanoparticles, which can be inducted to the living metals and alloys class and to the family of living precious metals. The bio-ELMs consisting of bio-mineralized extracellular matrices fall in the category of living ceramics. However, currently, the material classes for bio-ELMs are not as diverse as HLMs. Some types of materials cannot be produced by living entities as the extracellular matrix, for instance, carbon or carbide materials. This may change in the future with the progress in engineered cells or synthetic cells, which can produce new types of materials. In that case, the class and families of bio-ELMs will need modification, or perhaps a new taxonomical scheme for bio-ELMs needs to be introduced for these taxa.

Evidently, the species are too many for being enumerated here, and additional efforts are needed to completely detail the whole taxonomy and related nomenclature, as further discussed in the final sections. Before entering discussions and future proposals, the next section demonstrates the applicability and puts forward the utility of the proposed living materials taxonomy.

**APPLICABILITY AND UTILITY OF THE TAXONOMY**

**Classification of pioneering examples of living materials**

A total of 94 examples of ELMs have been categorized according to our taxonomy proposal to validate their applicability and utility, as summarized in Table 1. The following considerations are taken into account for constructing Table 1 according...
Table 1. Application of the taxonomy to a collection of engineered living materials described in recent literature

| Pioneering examples of engineered living materials (ELMs) | Classification according to proposed taxonomy |
|--------------------------------------------------------|-----------------------------------------------|
| **Described ELMs**                                      | **Domain** | **Superkingdom** | **Kingdom** | **Phylum** | **Class** | **Family** |
| Archaeal cultures for polymeric production28          | materials production | archaeal   | biological ELMs | archaebacterial ELMs | 3D ELMs | living polymers | living thermoplastics |
| Archaeal cultures for gold production24                | materials production | archaeal   | biological ELMs | archaebacterial ELMs | 3D ELMs | living metals | living precious metals |
| Archaeal cable-like structures upon biofilms29         | smart surfaces and structures | archaean    | hybrid living materials | archaebacterial ELMs | 1D/2D ELMs | living polymers | living elastomers |
| Cellulose produced by bacteria in culture29            | materials production | bacterial  | biological ELMs | eubacterial ELMs | 3D ELMs | living polymers | living thermoplastics |
| Cellulose produced by bacteria in culture31            | materials production | bacterial  | biological ELMs | eubacterial ELMs | 3D ELMs | living polymers | living thermoplastics |
| 3D-printed self-healing bacterial biofilms32            | materials production | bacterial  | biological ELMs | eubacterial ELMs | 3D ELMs | living polymers | living elastomers |
| 3D self-healing living material33                       | materials production | bacteria   | biological ELMs | eubacterial ELMs | 2D/3D ELMs | living polymers | living elastomers |
| Genetically programmable self-regenerating bacterial hydrogels34 | tissue engineering | bacterial  | biological ELMs | eubacterial ELMs | 3D ELMs | living polymers | living hydrogels |
| Bacterial-biominerlized inorganic substrates35          | materials production | bacterial  | biological ELMs | eubacterial ELMs | 3D ELMs | living ceramics | living concrete |
| Bacteria-generated curli nanofiber matrices for tissue repair36 | tissue engineering | bacterial  | biological ELMs | eubacterial ELMs | 3D/2D ELMs | living polymers | living elastomers |
| Bacteria-generated curli nanofiber biofilms for catalysis37 | biotechnology and bioprocessing | bacterial  | biological ELMs | eubacterial ELMs | 2D ELMs | living polymers | living elastomers |
| Extracellular matrix created by bacteria in culture38   | materials production | bacterial  | biological ELMs | eubacterial ELMs | 2D ELMs | living polymers | living hydrogels |
| Bacterial cultures for polymeric production37          | materials production | bacterial  | biological ELMs | eubacterial ELMs | 2D ELMs | living polymers | living thermoplastics |
| Bacterial cultures for polymeric production38          | materials production | bacterial  | biological ELMs | eubacterial ELMs | 2D ELMs | living polymers | living thermoplastics |
| Bacterial culture for producing stiff living materials39 | materials production | bacterial  | biological ELMs | eubacterial ELMs | 2D ELMs | living polymers | living thermoplastics |
| Bacterial biofilms for gold production34                | materials production | bacterial  | biological ELMs | eubacterial ELMs | 3D ELMs | living metals | living precious metals |
| Bacterial cultures for gold nanoparticle production35   | materials production | bacterial  | biological ELMs | eubacterial ELMs | 0D ELMs | living metals | living precious metals |
| Bacterial cultures for silver nanoparticle production36 | materials production | bacterial  | biological ELMs | eubacterial ELMs | 0D ELMs | living metals | living precious metals |
| Functional nano-objects in living engineered bacterial biofilms37 | biotechnology and bioprocessing | bacterial  | biological ELMs | eubacterial ELMs | cross-phyllum (0D on 2D) | living composites | living polymer matrix composite |
| Bacterial biofilms with nanoparticles and quantum dots38 | materials production | bacterial  | hybrid living materials | eubacterial ELMs | fractal | living metals | living precious metals |

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Table 1. Continued

| Described ELMs | Purpose/application field | Domain | Superkingdom | Kingdom | Phylum | Class | Family |
|----------------|---------------------------|--------|--------------|---------|--------|-------|--------|
| Sand-hydrogel scaffolds with photosynthetic cyanobacteria | smart buildings | bacterial | hybrid living materials | eubacterial ELMs | 3D ELMs | living composites | living polymer matrix composite |
| Bacteria cultured on polymeric scaffolds growing ceramics | materials production | bacterial | hybrid living materials | eubacterial ELMs | 3D ELMs | living composites | living ceramic matrix composite |
| Self-healing, bacterial-loaded concrete | smart buildings | bacterial | hybrid living materials | eubacterial ELMs | 3D ELMs | living ceramics | living concrete |
| Hydrogel hosting genetically programmed bacteria | smart surfaces and structures | bacterial | hybrid living materials | eubacterial ELMs | 3D ELMs | living polymers | living hydrogels |
| 3D bioprinted hydrogels with programmed bacteria | smart surfaces and structures | bacterial | hybrid living materials | eubacterial ELMs | 3D ELMs | living polymers | living hydrogels |
| 2D and 3D bioprinted hydrogels encapsulating bacteria | smart surfaces and structures | bacterial | hybrid living materials | eubacterial ELMs | 3D ELMs | living polymers | living hydrogels |
| Hydrogels with stiffness controlled by encapsulated bacteria | smart surfaces and structures | bacterial | hybrid living materials | eubacterial ELMs | 3D ELMs | living polymers | living hydrogels |
| Optoregulated hydrogels with encapsulated bacteria | biotechnology and bioprocessing | bacterial | hybrid living materials | eubacterial ELMs | 3D ELMs | living polymers | living hydrogels |
| Smart grippers with sensing bacteria | Robotics | bacterial | hybrid living materials | eubacterial ELMs | 3D ELMs | living polymers | living elastomers |
| Multi-layered polymeric biofilm with embedded bacteria | smart surfaces and structures | bacterial | hybrid living materials | eubacterial ELMs | 3D ELMs | living polymers | living elastomers |
| Bacteria grown on marine agar synthesize silk proteins | materials production | bacterial | hybrid living materials | eubacterial ELMs | 3D ELMs | living polymers | living elastomers |
| Silica gel particles with bacteria for enhanced biodegradation | biotechnology and bioprocessing | bacterial | hybrid living materials | eubacterial ELMs | 3D/0D ELMs | living ceramics | living silicon oxide |
| Printed biofilm on both sides of latex sheets | smart surfaces and structures | bacterial | hybrid living materials | eubacterial ELMs | dynamic ELMs | living polymers | living elastomers |
| Penicillin-producing living surfaces | biotechnology and bioprocessing | bacterial | hybrid living materials | eubacterial ELMs | 2D ELMs | living polymers | living hydrogels |
| 3D bioprinted photosynthetic cyanobacteria | energy production | bacterial | hybrid living materials | eubacterial ELMs | 2D ELMs | living polymers | living hydrogels |
| Polymeric films loaded with water-responsive bacterial spores | smart surfaces and structures | bacterial | hybrid living materials | eubacterial ELMs | 2D ELMs | living polymers | living elastomers |
| Biosensing alginate beads with bacterial colonies | smart surfaces and structures | bacterial | hybrid living materials | eubacterial ELMs | 2D ELMs | living polymers | living elastomers |
| Nanofibrous webs hosting bacteria | biotechnology and bioprocessing | bacterial | hybrid living materials | eubacterial ELMs | 2D ELMs | living polymers | living elastomers |

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Table 1. Continued

| Described ELMs                                                                 | Purpose/application field | Domain       | Superkingdom | Kingdom | Phylum       | Class       | Family          |
|--------------------------------------------------------------------------------|---------------------------|--------------|--------------|---------|--------------|-------------|-----------------|
| Layer-by-layer assembly of polymer nanotube and skin bacteria                  | biotechnology and bioprocessing | bacterial     | hybrid living materials | eubacterial ELMs | 3D ELMs     | living polymers | living thermoplastics |
| Bacteria-coated polypropylene for virus trapping                              | biotechnology and bioprocessing | bacterial     | hybrid living materials | eubacterial ELMs | 2D ELMs     | living polymers | living thermoplastics |
| Cyanobacterial film on graphite electrode                                      | energy production          | bacteria      | hybrid living materials | eubacterial ELMs | 2D ELMs     | living carbon   | living graphite |
| Bacteria patterned onto textiles and polymers                                  | smart surfaces and structures | bacterial     | hybrid living materials | eubacterial ELMs | 2D ELMs     | living polymers | living thermoplastics |
| Self-assembled graphene-bacterial biofilms                                     | smart surfaces and structures | bacterial     | hybrid living materials | eubacterial ELMs | 2D ELMs     | living carbons  | living graphene |
| Graphene biofilms with electroactive bacteria                                  | energy production          | bacterial     | hybrid living materials | eubacterial ELMs | 2D ELMs     | living carbons  | living graphene |
| Graphene-CNT biofilms with built-in bacteria                                  | energy production          | bacterial     | hybrid living materials | eubacterial ELMs | 2D ELMs     | living carbons  | other living carbons |
| Spray-dried polymer/bacteria microparticles for electrospinning                | materials production       | bacterial     | hybrid living materials | eubacterial ELMs | 2D/1D/0D ELMs | living polymers | living thermoplastics |
| Polymeric fibers and meshes loaded with bacteria                              | biotechnology and bioprocessing | bacterial     | hybrid living materials | eubacterial ELMs | 1D ELMs     | living polymers | living thermoplastics |
| Metal-organic framework (MOF)-encapsulated bacteria                            | biotechnology and bioprocessing | bacterial     | hybrid living materials | eubacterial ELMs | 0D ELMs     | living composites | other living composites |
| Biogenic gold nanoparticles with radiation-resistant bacteria                  | biotechnology and bioprocessing | bacterial     | hybrid living materials | eubacterial ELMs | 0D ELMs     | living metals   | living precious metals |
| C-dots synthesized by bacteria upon graphene oxide (GO) films                 | biotechnology and bioprocessing | bacterial     | hybrid living materials | eubacterial ELMs | 0D ELMs     | living carbons  | other living carbons |
| Multi-scale carbon structures encapsulating cells                             | tissue engineering         | eukaryotic    | hybrid living materials | animal ELMs     | fractal      | living carbons  | living glassy carbon |
| Microencapsulated mammalian cells in natural polymers                         | tissue engineering         | eukaryotic    | hybrid living materials | animal ELMs     | 3D ELMs     | living polymers | living elastomers |
| Self-contraction PDMS with cardiomyocytes                                      | robotics                   | eukaryotic    | hybrid living materials | animal ELMs     | 3D ELMs     | living polymers | living elastomers |
| Collagen structure with musculoskeletal tissue                                | robotics                   | eukaryotic    | hybrid living materials | animal ELMs     | 3D ELMs     | living polymers | living elastomers |
| Biological machines with musculoskeletal cells                                | robotics                   | eukaryotic    | hybrid living materials | animal ELMs     | 3D ELMs     | living polymers | living hydrogels |
| Micropatterned color hydrogels with cardiomyocytes                             | robotics                   | eukaryotic    | hybrid living materials | animal ELMs     | dynamic ELMs | living polymers | living hydrogels |
| Bioencapsulated animal cells in silica matrices                               | smart surfaces and structures | eukaryotic    | hybrid living materials | animal ELMs     | 3D ELMs     | living ceramics | living silica |
| Cardiomyocytes on elastomeric body with gold skeleton                         | robotics                   | eukaryotic    | hybrid living materials | animal ELMs     | 3D ELMs     | living composites | living polymer matrix composite |
| PDMS thin films with cardiomyocytes                                            | robotics                   | eukaryotic    | hybrid living materials | animal ELMs     | 2D ELMs     | living polymers | living elastomers |
| PDMS medusoids with living tissues                                            | robotics                   | eukaryotic    | hybrid living materials | animal ELMs     | 2D ELMs     | living polymers | living elastomers |

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| Described ELMs | Purpose/application field | Domain | Superkingdom | Kingdom | Phylum | Class | Family |
|----------------|--------------------------|--------|--------------|---------|--------|-------|--------|
| Musculoskeletal cells attached to hair | robotics | eukaryotic | hybrid living materials | animal ELMs | 1D ELMs | living polymers | living elastomers |
| 3D printed bio-ink containing cardiac tissue | smart surfaces and structures | eukaryotic | hybrid living materials | animal ELMs | dynamic ELMs | living polymers | living hydrogels |
| Cardiomyocytes with CNTs | robotics | eukaryotic | hybrid living materials | animal ELMs | dynamic ELMs | living carbon | living CNTs |
| Mycelium-based composites (fungi into organic substrate) | materials production | eukaryotic | hybrid living materials | fungal ELMs | 3D ELMs | living polymers | other living polymers (fibers) |
| Bioprinted bioniks with baker’s yeast | biotechnology and bioprocessing | eukaryotic | hybrid living materials | fungal ELMs | 3D ELMs | living polymers | living thermoplastics |
| Mycelium-based composites (fungi into inorganic structure) | smart buildings | eukaryotic | hybrid living materials | fungal ELMs | 3D ELMs | living polymers | living thermoplastics |
| Swelling-responsive hydrogels with yeast | smart surfaces and structures | eukaryotic | hybrid living materials | fungal ELMs | 3D ELMs | living polymers | living hydrogels |
| Bioprinted stimuli-responsive yeast | smart surfaces and structures | eukaryotic | hybrid living materials | fungal ELMs | dynamic ELMs | living polymers | living hydrogels |
| Yeast-CNT bionic nanocomposite | smart surfaces and structures | eukaryotic | hybrid living materials | fungal ELMs | 3D ELMs | living carbons | living CNTs |
| Yeast-graphene bionic nanocomposite | smart surfaces and structures | eukaryotic | hybrid living materials | fungal ELMs | 3D ELMs | living carbons | living graphene |
| Silica-alginate-fungi biocomposites | biotechnology and bioprocessing | eukaryotic | hybrid living materials | fungal ELMs | 3D ELMs | living composites | living polymer matrix composite |
| Polymeric layers with Penicillium roqueforti | smart surfaces and structures | eukaryotic | hybrid living materials | fungal ELMs | 2D ELMs | living polymers | living elastomers |
| Algae-laden hydrogel scaffolds | tissue engineering | eukaryotic | hybrid living materials | protist ELMs | 3D ELMs | living polymers | living hydrogels |
| 3D-printed corals hosting microalgae | energy production | eukaryotic | hybrid living materials | protist ELMs | 3D ELMs | living composites | living ceramic matrix composite |
| Bioprinted photosynthetic living materials | energy production | eukaryotic | hybrid living materials | protist ELMs | 2D ELMs | living composites | living polymer matrix composite |
| Trees interwoven with scaffolding structures | smart buildings | eukaryotic | hybrid living materials | vegetal ELMs | | fractal | living metals | living steel |
| Plant-cell-laden hydrogel scaffolds | biotechnology and bioprocessing | eukaryotic | hybrid living materials | vegetal ELMs | 3D ELMs | living polymers | living hydrogels |
| Plant cells in silica matrices | smart surfaces and structures | eukaryotic | hybrid living materials | vegetal ELMs | 3D ELMs | living ceramics | living silica |
| Bioencapsulated plant cells in silica matrices | smart surfaces and structures | eukaryotic | hybrid living materials | vegetal ELMs | 3D ELMs | living ceramics | living silica |
| Self-assembled DNA nanotubes and artificial cells | synthetic biology | synthetic cells | biological ELMs | artificial cells ELMs | 3D ELMs | living polymers | other living polymers |
| Photosynthetic membranes with synthetic chloroplasts | biotechnology and bioprocessing | synthetic cells | biological ELMs | artificial cells ELMs | 2D ELMs | living polymers | other living polymers |
| Protocellular models producing sugar and stimulating bacteria | biotechnology and bioprocessing | synthetic cells | biological ELMs | artificial cells ELMs | 0D ELMs | living polymers | other living polymers |
| Protocells for the synthesis of ATP | biotechnology and bioprocessing | synthetic cells | biological ELMs | artificial cells ELMs | | | |

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| Pioneering examples of engineered living materials (ELMs) | Classification according to proposed taxonomy |
|-----------------------------------------------|-----------------------------------------------|
| **Described ELMs** | **Purpose/application field** | **Domain** | **Superkingdom** | **Kingdom** | **Phylum** | **Class** | **Family** |
| Self-assembled bacterial cellulose capsule from co-cultured synthetic cells | materials production | cross-domain | biological ELMs | cross-kingdom ELMs | 3D ELMs | living polymers | other living polymer |
| Mushroom with cyanobacteria and graphene nanoribbons | energy production | cross-domain | hybrid living materials | cross-kingdom ELMs | fractal | living carbons | living graphene |
| Lichen-inspired latex with microalgae and cyanobacteria | biotechnology and bioprocessing | cross-domain | hybrid living materials | cross-kingdom ELMs | 3D ELMs | living polymers | living elastomers |
| Algae, yeast, and bacteria encapsulated in bioprintable hydrogel | smart surfaces and structures | cross-domain | hybrid living materials | cross-kingdom ELMs | 3D ELMs | living polymers | living elastomers |
| Synergic smart materials production by bacteria and yeast | materials production | cross-domain | hybrid living materials | cross-kingdom ELMs | 3D ELMs | living polymers | living thermoplastics |
| Bacterial nanocellulose scaffolds for endothelial cultures | tissue engineering | cross-domain | hybrid living materials | cross-kingdom ELMs | 3D ELMs | living polymers | living thermoplastics |
| Cell scaffolds with antibiotic-secreting bacteria | tissue engineering | cross-domain | hybrid living materials | cross-kingdom ELMs | 3D ELMs | living polymers | living hydrogels |
| Bacteria-laden gels for stem cell engineering | tissue engineering | cross-domain | hybrid living materials | cross-kingdom ELMs | 3D ELMs | living polymers | living hydrogels |
| Biomaterials with interactions among hMSCs and bacteria | tissue engineering | cross-domain | hybrid living materials | cross-kingdom ELMs | 2D ELMs | living polymers | living hydrogels |
| Hydrogel surfaces with bacteria to trigger cell adhesion | tissue engineering | cross-domain | hybrid living materials | cross-kingdom ELMs | 2D ELMs | living polymers | living hydrogels |
| Soft matter created by cooperation of microalgae and bacteria | materials production | cross-domain | hybrid living materials | cross-kingdom ELMs | 2D ELMs | living polymers | living thermoplastics |

hMSCs, human-bone-marrow-derived mesenchymal stem cells.
to our proposed taxonomy, together with our previously described rationale and taxonomic structure.

Within the superkingdom of bio-ELMs,\textsuperscript{115,116} we include the ELMs, which are made of only cells or cells and cell-generated extracellular matrices, without the employment of an external scaffold. When ELMs are constructed using scaffolding structures by seeding cells within them or through direct interaction and self-assembly of cells and biomaterials, we classify them as HLMs. In some cases, cells cultured in suspension (usually for materials-production purposes) are included as bio-ELMs, while cells cultured upon biofilms of biomaterials are HLMs.

We have not included tissue-engineering scaffolds as ELMs for different reasons. Although tissue-engineering scaffolds are historical precursors and relatives to ELMs, they are typically employed as structural elements, and the role of cells within them is to improve their biological performance. With literally thousands of examples of tissue-engineering scaffolds populating the fields of tissue engineering and biofabrication, they constitute a research area on their own. Besides, in the vast majority of cases, they lack most of the representative features of true ELMs, including intelligent responses, autonomous motion or interactions with the environment, self-sensing abilities, self-repair capabilities, and self-replication. In some exceptional cases, we have included tissue-engineering scaffolds in Table 1 due to their more apparent connection with ELMs or their pioneering examples of biohybrid combinations (i.e., new type of scaffold or innovative combinations of biological entities across different domains). In any case, the field of tissue engineering is progressively evolving into biofabrication, and the traditional structural scaffolds are now becoming smart scaffolding structures with living cells, in which case they may well become part of the living materials realm and can be perfectly classified according to the proposed taxonomy.

In addition, the term “artificial cell” or “synthetic cell” is emergent and can represent different entities, like integral biological imitators, cell-like structures with biological functions, engineered existing cells with modified functions, or inert biotemplated cell-like structures, to cite a few.\textsuperscript{117,118} In some cases, these so-called artificial cells are inert polymeric bubbles or chambers, which may be used for drug delivery or for distracting pathogens but cannot be considered true living entities or living materials because they lack the basic functions of life. Engineered cells, using common synthetic biology tools like mRNA or CRISPR, are sometimes called artificial cells. However, a human being receiving an mRNA-based vaccine or born from an engineered embryo to avoid a congenital disease would still be a human being. Applying a similar rationing scheme, an ELM based on edited (or engineered) animal cells would still be an “animal ELM,” and an ELM benefiting from modified bacteria should still be considered an “eubacterial ELM.” In consequence, our list of ELMs made of synthetic cells does not include biotemplated inert materials or engineered living cells employing state-of-the-art molecular biology and genetic engineering technologies. We opt for completely synthetic cells, in which artificially produced microstructural entities are loaded with synthetic genes or organelles for autonomously interacting with the environment and count with basic living features, like self-replication, self-sensing abilities, independent motion, and collective interactions. Although this decision importantly limits the current number of existing examples of ELMs made of true synthetic cells, we believe that it agrees with recent definitions from relevant research calls\textsuperscript{12} and studies\textsuperscript{119–121} and that several examples of ELMs with synthetic cells will be developed in the short term.
Considering the above, pioneering examples of ELMs classified according to the taxonomy are presented in the following pages (Table 1). Its utility is further analyzed in the next section.

**Finding gaps in the living materials portfolio and guiding research efforts**

Apart from helping to classify what already exists, taxonomies can be applied to analyze what could exist, especially in a field connected to synthetic biology. This will further help propose new research directions. After classifying the selected pioneering examples of ELMs according to the proposed taxonomy, the most populated domains, kingdoms, and classes are analyzed and summarized in Figures 5 and 6.

![Figure 5. Distribution of ELMs based on different taxa](image)

Engineered living materials by (A) domain, (B) kingdom, and (C) class, ordered by number of examples found in the literature search according to Table 1.

According to the gathered examples and data, bacterial ELMs account for 50% of examples of living materials, while eukaryotic ELMs correspond to 30.9% and archaeal ELMs to 3.2%. The industrial relevance of bacterial cultures for white, green, and blue biotechnologies is probably responsible for this prevalence, while several examples of eukaryotic ELMs are connected to fields like tissue engineering and biohybrid robotics. Up to now, the potential of archaea has not been fully exploited, probably due to their less studied nature. Their extremophilic properties may find relevant applications linked to space exploration and developing resources.
and raw materials in remote and extremely harsh environments. ELMs with completely synthetic artificial cells, with 4.3% of examples, are still underrepresented, considering their extraordinary potentials. We assume that this situation will significantly change soon. Interestingly, a remarkable 11.7% of studies deal with cross-domain ELMs, in which bacterial and eukaryotic cells co-exist and perform symbiotic functions.

Regarding the employment of an abiotic scaffold or not, HLMs constitute 75% of the analyzed examples, while bio-ELMs without synthetic scaffolding structures account for the remaining 25%. Within HLMs, most scaffolds (67%) are polymeric, followed by carbon-based materials (10.6%), metals and alloys (7.4%), composites (8.5%), and ceramics (6.4%). The distribution of the different ELM kingdoms and classes is presented in Figure 6. Considering polymers, the interesting properties of hydrogels for cell-culture applications make them a usual choice (30% within polymers), despite their very poor mechanical properties. Elastomeric materials like polydimethylsiloxane (PDMS) are also employed similarly to that of hydrogels, although their improved properties do not allow for truly high-performance applications.

The different application fields for the developed ELMs are also considered for their industrial and social interest, even if the application itself has not been employed as a taxon. The function of the ELMs that determine the application should be considered as a taxon. However, the current application fields are quite diverse. Furthermore, as it is an emerging field, numerous applications are expected to emerge in the future. Therefore, we believe it is too early for a classification considering function or application as a taxon. However, this is subject to further debate and discussion. Nevertheless, we attempted to categorize different broader application areas based on Table 1. The distribution is presented in Figure 7. Materials production acquires the top position with 24.2% of all the applications, followed by smart surfaces and structures with 22.1%. The top position of materials production is due
to the high interest in bio-ELMs using living cells, particularly bacteria, for the synthesis of extracellular materials, such as cellulose nanofibers or gold and silver nanoparticles. In comparison, HLMs account for smart surfaces and structures due to the synergistic activities of living cells and the abiotic scaffold. However, there are growing interests in other fields, including biotechnology, bioprocessing, and tissue engineering. One of the reasons is the processability of the living entities using additive manufacturing technologies, either by directly 3D printing or through assembly with a 3D-printed scaffold. The fields of bio-robotics and energy production are also emerging slowly, utilizing unique functions of specific cell lines.

**DISCUSSION ABOUT POSSIBLE MODIFICATIONS AND ALTERNATIVES TO THE TAXONOMY**

The proposed taxonomy is useful for hierarchically classifying the emergent field of ELMs in a univocal manner and for finding unexplored combinations of living kingdoms and materials classes to orient research efforts in the field. However, some questions for debate arise, which will need consensual responses. Further developments in ELMs, especially regarding bio-ELMs, may lead to modifications and additions to this initial taxonomy proposal. Some relevant issues that we have already detected are discussed here, with the aim of initiating fruitful debate with colleagues.

**On the utility of the bio-ELMs and HLMs superkingdoms**

Arguably, the common division of ELMs into bio-ELMs and HLMs, with their respective bottom-up and top-down approaches, derives from the different initial methods and techniques used by the pioneering communities of biologists (usually applying bottom-up tools) and engineers (typically resorting to top-down resources). In a way, although widespread, this division is more procedural and historical than linked to the essential features of ELMs. Therefore, it could be progressively abandoned, and the superkingdoms could be omitted from the taxonomy.

In fact, bio-ELMs, if successfully developed to achieve long-lasting living constructs, will generate their own extracellular matrices (as in normal living organisms). These extracellular matrices can perform exactly the same function as the abiotic scaffold in HLMs. Following this rationale, the superkingdoms could be further modified to introduce new superkingdoms. For examples, another superkingdom, “living biomaterials,” could be introduced, taking into account the cell-produced biological scaffold (e.g., proteins, polysaccharides, and mineralized tissues, to name a few). New classes and families could be further introduced as well, based on the new superkingdom.
Alternatively, future research direction could see a unification of these two super-kingdoms, where the differences between bio-ELMs and HLMs could be considered reconcilable by the research community. In that case, the superkingdoms could be eliminated and a new taxonomy would be needed.

**On the completeness of the taxonomy**
On purpose, we have not yet used the intermediate and lower rank taxa of “order” and “genus” for this first approach to the taxonomy, as it will need to be completed. We understand that the ELMs field is nascent and that unforeseen methods, techniques, combinations, and synergies between engineering and biology will lead to a plethora of multi-cellular synthetic creations, for which more complex classification schemes with more taxa will be needed. If the effect of using different cell types from the same kingdom leads to clearly different ELMs, the addition of orders within the proposed families may prove useful. Even some taxa may see their ranks modified and thus enable the incorporation of new taxa, changing the hierarchy. Genus may also be applied to complement the families, especially for the more populated materials families, like polymers and composites. Anyhow, we believe that the global scheme is valid and robust, and it can be adapted and updated as the research in this field progresses and new ELMs appear.

**On virus and prions in living materials**
Some life taxonomies have included non-cellular domains, like Prionobiota (for prions) and Virusobiota (for viruses) and related kingdoms. However, it is generally accepted that viruses and prions do not constitute living entities. Accordingly, they have not been proposed as domains or kingdoms for the taxonomy of living materials.

Notwithstanding this consensus, it is also true that the incorporation of prions and viruses to living materials may be transformative and lead to unexpected new entities, functions, and applications. For example, in the field of biomaterials and tissue engineering, lentiviral vectors have been used in cultured cells, with which artificial muscles have been bioengineered, for enhancing angiogenesis and bioactivity. In parallel, implants coated with viral vectors encoding for osteogenic genes are being studied for enhanced bone formation in complex large-bone and cranial repairs, to cite some examples.

It is foreseeable that viruses will find applications within ELMs, co-cultured or incorporated together with other cell types. Despite this possibility, we believe that the taxonomy is prepared for these and similar circumstances without the need for adding new domains or kingdoms. Even if including viruses or prions, the living materials would be classified according to their cellular constituents. Besides, the cross-domain and cross-kingdoms options can help integrate the more complex living materials, as would be the case of multi-cellular chimeras functionalized with viruses or prions.

Arguably, this may change in the future due to the progressive blend of frontiers between the living and non-living, to which the field of ELMs also adds new unknowns and debates. May it be the case that prions and viruses become considered living entities, the proposed taxonomy could also be updated by adding additional domains and kingdoms.

**CONCLUDING REMARKS**
To summarize, we attempted to classify the emerging field of ELMs, taking inspiration from life taxonomies and materials classifications, particularly for HLMs. The
field is still in its “infant” stage and will require a workforce to mature in the next decades to address several global challenges of the 21st century and beyond. The proposed taxonomy will be useful for categorically employing the muscle and brain power in this field. The proposed classification will still need reformulations as the field grows over time. However, with this taxonomy proposal, we hope to provide a good start for a hierarchical classification of ELMs and to promote fruitful debate leading to a more consensual, complete, univocal, and long-lasting taxonomy for living materials.

ACKNOWLEDGMENTS

J.G.K. and M.I. acknowledge support from the Deutsche Forschungsgemeinschaft under Germany’s Excellence Strategy via the Excellence Cluster 3D Matter Made to Order (EXC-2082/1-390761711). All the authors thank the Karlsruhe Institute of Technology and Universidad Politécnica de Madrid for their support in facilitating a safe and healthy work environment during the adverse period of the coronavirus disease 2019 (COVID-19) pandemic. Figure 1A was prepared using biorender.com.

AUTHOR CONTRIBUTIONS

Conceptualization, A.D.L. and M.I.; methodology, A.D.L.; investigation, A.D.L., J.G.K., and M.I.; funding acquisition, J.G.K.; writing—original draft, A.D.L. and M.I.; writing—review & editing, A.D.L., J.G.K., and M.I.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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