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## Engineered living composite materials

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### ABSTRACT

Since the inception of fibre-reinforced composite materials, they have been widely acknowledged for their unparalleled weight-to-performance ratio. Nonetheless, concerns are escalating regarding the environmental impact of these materials amidst global warming and pollution. This perspective explores a ground-breaking shift towards harnessing living organisms to produce composite materials. Living composites not only offer sustainable, carbon-capturing alternatives but also afford an unprecedented level of control over shape and anisotropy. Recent advancements in biology, particularly genetic engineering and sequencing, have provided extraordinary control over living organisms. Coupled with ever-evolving additive manufacturing techniques, these breakthroughs enable the construction of engineered living materials from the ground up. Here, we explore the key factors propelling the emergence of engineered living materials for structural applications and delve into the capabilities of living organisms that can be harnessed for creating functional materials, including harvesting energy, forming structures, sensing/adapting, growing and remodelling. Incorporating living organisms can revolutionise manufacturing for renewable and sustainable composite materials, unlocking previously unattainable functionalities.

### 1. Introduction

Since the conception of fibre-reinforced composite materials in the 1950s, their advancements have been focused on processing and reliability. Whereas fibre-reinforced composites continue to be the benchmark in weight-to-performance ratio, concerns are rising about the environmental costs versus the benefits of such materials [1,2]. In a world where the effects of global heating and environmental pollution are becoming ever more prevalent [3], most materials we use today must be re-evaluated. Materials such as steel, concrete, and plastics are inseparably linked to global heating and environmental pollution; their production requires a high input of energy and fossil-based resources; and, at the end of their life, they are challenging to recycle [4–6]. Conversely, the structural materials found in Nature are formed with minimal energy input from renewable sources and, because of their place in a complex ecosystem, can often easily be reused by another organism, incorporating them into the short carbon cycle of our planet [7,8].

For inspiration, structural engineers have looked at these biomaterials for centuries, especially since the original advent of fibre-reinforced composite materials [9–11]. They have been inspired by

the intricate structure of bird feathers, bone, wood, and insect exoskeletons; which exhibit the elegant interplay between fibres, polymers, and their microstructural architecture. These unique natural materials have been mimicked by combining traditional resources to make composite structures lighter, stiffer, and tougher [12]. However, producing such materials with a living organism has proven difficult since living organisms rely on complex biochemical processes, multi-level manufacturing mechanisms, and tight spatiotemporal control, which have been multi-objectively optimised over 3.8 billion years. For humankind to recreate the exquisite structural materials found in Nature, we must have a deep understanding of the processes underlying the formation of these structures and, to some extent, understand their optimisation objectives.

Only in the past decades have we gained sufficient control over living organisms to endeavour to create the materials found in Nature in the laboratory. Recent breakthroughs in biology, such as next-generation sequencing, CRISPR genetic engineering, and cloning techniques, have given more control over living organisms than ever before [13]. This, in combination with advances in additive manufacturing techniques, has created unprecedented possibilities to create intricate bio-based structural materials from the bottom up. This will enable us to transition from

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developing biologically inspired materials to biologically produced materials. Such structural materials, which exploit the capabilities of living organisms, we can call engineered living composite materials.

This perspective explores the exciting advancements in the field of engineered living composite materials. Firstly, we discuss the recent advances in biology and manufacturing which have enabled the field, after which we will look at the key advances subdivided into four categories: 1) how living organisms can be used to harvest energy, 2) produce structural material, 3) sense and adapt, and 4) grow and remodel. We will see that engineered living composite materials can mitigate the environmental problems of traditional composite materials and surpass traditional composites in properties, creating entirely new functional composite materials with features that could only be dreamed of a few decades ago.

## 2. Key enabling advances

For centuries, engineers have turned to Nature for inspiration in improving the performance of materials and structures. From the early designs of Leonardo Da Vinci [11] to the construction of modern aeroplanes, engineers have sought to capture the exquisite complexity of biological designs. One of the most famous examples is the winglet on an aeroplane, which draws inspiration from the curled feathers found on birds of prey, effectively controlling wingtip vortices and thus reducing energy consumption during flight [14]. Many more examples can be found in Nature: the shells of bivalves like clams and mussels are incredibly tough and stiff [15,16], the dactyl club of a mantis shrimp absorbs energy when it strikes prey, and the complex microstructure of human bone has reinforcement in fibre direction and increased density along the main load-bearing lines [17]. To fulfil their unique purpose, each of these natural structures relies on the complexity of their microstructures, bringing together their constituent parts [18].

For many years, a strong desire has been to replicate the intricate microstructures in natural structural materials. However, reproducing the precisely controlled cellular processes and organisation within a tissue has proven to be a significant challenge. For example, researchers have been trying to produce nacre, the strong and tough pearl brick-and-mortar structure which makes up the shell of bivalves, *in vitro* in many ways [19]. While many excellent materials have been developed in the process, they have yet to succeed in reproducing the nacre found in Nature with the same mechanical properties, low environmental footprint, and ease of manufacturing [19,20].

The same is true for spider silk, a highly sought-after material because of its incredible strength and toughness. For a long time, the only means to obtain spider silk was to 'milk' a spider. Because of advances made in the past decades, spider silk can, in the present day, not only be made without spiders but also in large quantities and with designer properties [21].

To obtain the tight spatiotemporal control essential for forming the intricate microstructures of such materials, one must possess the tools to control the organisms, cells, and molecules that form these structures. In the past two decades, many tools that enable the fabrication of such microstructured biomaterials have been discovered and thus have enabled the field of engineered living composite materials. Biology has witnessed several crucial breakthroughs in genetic engineering and bioinformatics techniques, which gave unprecedented control over biological processes.

### 2.1. Genetic engineering

Genetic engineering has had a tremendous impact, stretching far beyond the field of biology. To genetically engineer an organism, one requires two elements: a means to assemble pieces of DNA into constructs and a tool to incorporate the genetic construct into the genome of the organism of interest. A coding sequence is needed to engineer an organism functionally: the gene and its regulatory sequences. Often, this

consists of a promotor, which controls the expression of the gene; the gene itself, a piece of coding DNA (cDNA); and a terminator, which ensures the readout of a particular gene is appropriately ended. The entire construct of the promotor-gene-terminator is called an expression cassette. One or more cassettes are inserted in a plasmid, a piece of circular DNA which can be easily multiplied by a bacterium. This plasmid is then incorporated into a target organism directly or in a vector, which can insert the DNA into the target organism's genome.

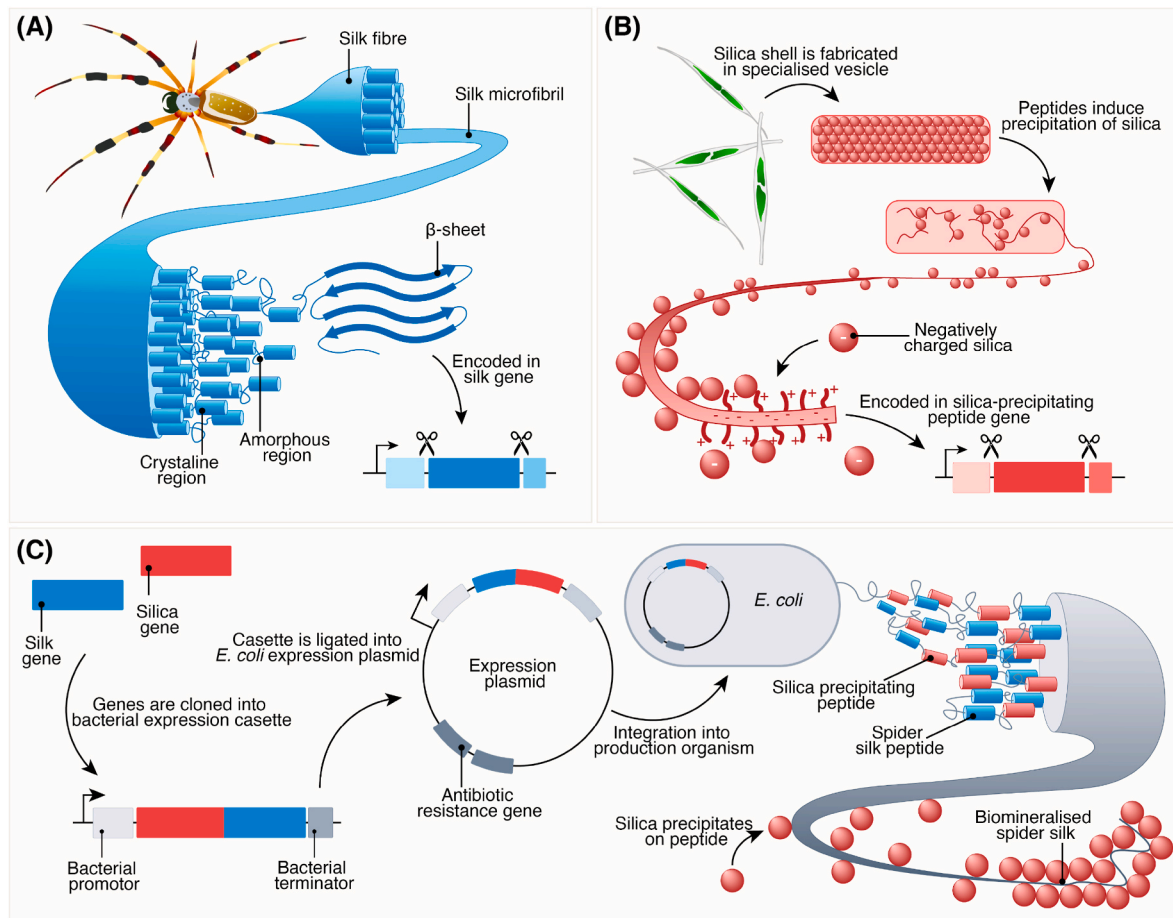
Commercial cloning methods such as 'Gateway Cloning' [22], 'Gibson Assembly' [23] and 'Golden Gate Cloning' [24] have made the process of making such plasmids much faster and more cost-effective. Additionally, for common model organisms, there are genetic toolkits available [25–29], simplifying the selection of promoters and terminators, which also come with all required chemicals in one package. Next to that, the price of synthesising novel pieces of DNA has drastically decreased. This dramatically reduces the time spent on promotor selection and synthesis. Alongside synthesis, precisely engineering all the sequences to be compatible with each other has become rapid. While engineering an organism would comprise several years of work a few decades ago, it can now be performed in a matter of weeks.

With techniques to create genetic constructs, we also need a method to alter the genetic information of an organism and introduce new genes. Many mechanisms to achieve this exist, but none are as ground-breaking as the CRISPR (clustered regularly interspaced palindromic repeats) system. First discovered in 2012 by the groups of Emmanuelle Charpentier and Jennifer A. Doudna [30], for which they received the Nobel Prize in chemistry in 2020, the technique uses the immune system of Bacteria and Archaea. In essence, the CRISPR-Cas9 mechanism is a pair of guided molecular scissors.

The CRISPR guide-RNA targets specific sequences of nucleotides and recruits the Cas9 to induce a double-strand break. This works exceptionally well for making mutants of genes. Before CRISPR, random mutagenesis was used to target genes, which had to be found in a pool of mutants with hundreds to thousands of other mutations. With CRISPR, one only needs a guide RNA to introduce a cut in a precisely determined DNA location. Next to this, when introducing a cut in the DNA together with a carefully designed sequence, the cellular DNA repair mechanisms will introduce the sequence of interest on the location of the break [31], thus effectively introducing a gene for *de novo* expression in a host organism.

We can consider fusion proteins to see how modern biological techniques can drastically improve the mechanical properties of materials. These are engineered for a specific application by fusing proteins from distinct organisms together to form proteins that do not occur naturally. An exciting example of how these genetic engineering techniques are applied to improve the mechanical properties of a material can be found in the biomineralisation of spider silk. These fibres are renowned for their extreme toughness and tensile strength (around 500 (kN•m)/kg) [32], and silica is known for its hardness (around 500 HV) and stiffness (around 30 MN•m/kg). Researchers have combined the silica-precipitating domains of a protein found in *Cylindrotheca fusiformis*, a marine microalga, and the domain which makes up dragline silk from the spider *Trichonephila clavipes* to make a composite material which excels in strength and toughness as can be seen in Fig. 1 [33–35].

Recent endeavours have extended the flexibility of fusion protein engineering even further. Researchers designed genetically encoded spider silk click chemistry, resulting in a platform that can incorporate many different functionalities into the spider silk, such as fluorescence, increased cell attachment, and enzymes [35]. On top of that, the structures could be predicted using the AlphaFold2 algorithm, resulting in a relatively straightforward means to go from design to protein material. This demonstrates the possibilities that recent advances in bioengineering bring when designing novel bio-based composite materials. This beautifully illustrates how genetic engineering and protein prediction can be used to improve the qualities of biomaterials such as spider silk.



**Fig. 1.** A demonstration of how modern genetic engineering techniques can create novel structural materials. The gene from the spider *Trichonephila clavipes* encoding a polypeptide in spider silk was fused with a silica-precipitating peptide from the marine microalgae *Cylindrotheca fusiformis* to produce a fusion protein. The novel fusion protein is produced in *E. coli* [33–35].

This is only one example of the myriad of endeavours that leverage disruptive advances in bioengineering to create complex products, such as precisely tuning the mechanical properties of structural polypeptides and even creating fusion-proteins, a protein complex with domains originating from two or more different organisms. This enables the realisation of proteins with novel catalytic or structural properties that do not exist in Nature [36].

## 2.2. Genetic sequencing

Another essential enabling factor for efficient genetic engineering has been the incredible advances in genetic sequencing technologies. Around the turn of the millennium, sequencing the human genome was a decade-long effort from a large consortium called the Human Genome Sequencing Consortium [33]. However, in the present day, it can be performed in a matter of hours or days.

Next-generation sequencing relies on cutting the DNA into small fragments in the order of a few hundred base pairs in length, sequentially sequenced in a highly parallel manner. These short reads are assembled into a complete genome by aligning the overlap between the small reads using an algorithm such as the 'Basic Local Alignment Search Tool' (BLAST) [37]. Another new sequencing technology involves threading DNA strands through nanopores, allowing for real-time analysis of the electrical resistance as individual nucleotides pass through [38]. This approach enables, with a slight compromise on the accuracy, the use of minimal amounts of DNA, and one can hold the complete system in the palm of a hand, making it ideal e.g. medical diagnosis in remote locations.

One can see that next-generation sequencing generates copious amounts of data and thus dramatically benefits from massive increases in computing power over the past few decades. This increase in computing power not only enables the processing of large amounts of genetic data but has also advanced the possibility of computationally predicting structure and property relations [39]. Advances in AI have made it possible to make predictions about protein structure and function from the underlying genetic sequences. The advances in biology in the past decade have provided roadmaps and toolboxes to transform living organisms with efficiencies and timescales, which have never been possible before.

## 2.3. Additive manufacturing

Manufacturing the intricate structures found in Nature with traditional manufacturing methods is challenging since biological materials consist of intricate hierarchical microstructures, heterogeneity and difficult-to-make shapes. Fortunately, recent advances in additive manufacturing are enabling the shaping freedom needed to match the complexity found in biological matter. New additive manufacturing techniques have enabled the creation of biomimetic structures with continuous carbon fibres along the load-bearing lines. However, the formation of genuinely biobased structures relies on embedding living cells within a structure. The most common way to achieve this is by embedding living cells in a hydrogel and subsequently 3D printing the hydrogel. The process of 3D printing with viscoelastic materials is called direct ink writing and lies at the core of engineered living composite materials [40] due to its ability to handle a range of soft matter inks,



which lend themselves well to the proliferation of organisms [41,42].

In the direct ink writing process, one needs an ink that can sustain a living organism and have the rheological properties that enable printing. The key to reliable direct ink writing is formulating a visco-elastic ink that is elastic enough,  $\sim 1$  kPa, to retain its shape at rest but is liquid enough,  $< 1$  kPa s to flow through a printing nozzle [43]. This can be achieved by designing a shear-thinning hydrogel, which yields at the shear rates the ink experiences in the printing nozzle, which are in the order of  $10\text{ s}^{-1}$ , and by having an ink with a quick recovery rate of  $< 1$  s that, after deposited on the sample, returns to a solid state [44]. The biocompatibility of such ink highly depends on the organism which must survive in the ink, which is commonly achieved by dissolving the required nutrients in the aqueous phase of the hydrogel [45]. These nutrients will not only allow the organism in the ink to grow but will also have a direct impact on the mechanical properties of the printed structure. An example of this dependence was shown in the study of Gantenbein et al. [41], where increasing the malt extract of 3D-printed mycelium hydrogels by 5 % decreases the stiffness of the printed structures from 25 kPa to 15 kPa approximately, depicting the sensitivity of these living composites. This study is a clear example that when all the above criteria are met, one can 3D print intricate structures with living organisms.

Next to advances in manufacturing techniques, the advent of topology optimisation has enabled engineers to replicate the evolutionary process that honed structural biological materials [46]. The optimal placement of the material is algorithmically determined by subjecting a part to a specific load case in simulation [47]. This can significantly improve the mechanical performance and reduce the material used for parts, especially with anisotropic materials, where, for example, the best orientation of a fibre can be determined to take on the load [48]. Thus, we have the tools to design and manufacture the biomaterials found in nature and have the means to optimise them for our specific application.

Advances in genetic engineering, genetic sequencing, additive manufacturing, and computational modelling have thus given us the tools required to construct engineered living composite materials. In the remaining part of this review, we focus on how these advances have been used to create engineered living composite materials. We have identified four essential skills of life which can be exploited to produce a new generation of composites: harvesting energy, forming structures, sensing and adapting, and growing and remodelling.

### 3. Nature's skills harnessed for engineered living materials

#### 3.1. Harvesting energy

Processes such as the production of carbon fibres and the forming and working of metals have two things in common: firstly, these processes require extremely high energy input, and secondly, they often require fossil-based resources. This starkly contrasts with how living organisms construct their materials, for which they can harvest the energy needed directly from sunlight, feed on other living organisms, or even directly harvest energy from inorganic compounds. Respectively, these energy-gathering methods are commonly subdivided into autotroph, heterotroph, and chemotroph. To see how the energy-harvesting capabilities of life can be used in forming composite materials, we will take a closer look at each of these three categories.

##### 3.1.1. Autotrophs

One can argue that at the basis of every food web on planet Earth, there are organisms which can harvest energy from sunlight, such as plants, microalgae, and cyanobacteria. In this process, called photosynthesis – thought to have evolved 2.0–2.7 billion years ago [49] – the energy of a photon is converted to chemical energy through highly complex biochemical pathways. Remarkably, the process happens at nearly 100 % efficiency using quantum superposition [50–52]. This sets up a proton gradient, which is subsequently converted to energy carriers

for downstream use, such as forming living structural matter like wood in trees [53]. During the photosynthesis process, the carbon from carbon dioxide is converted to useful carbons for the organism, and oxygen is released. Photosynthesis is the dominant mechanism by which atmospheric carbon is sequestered; even only marine autotrophs are responsible for 50 % of all carbon fixation and 71 % of all carbon storage on the planet [54].

Photosynthesis can be used to control the microstructure of a material. An example of this can be found in the work of Yu et al. [55]. In this work, chloroplasts extracted from a spinach leaf are embedded in a 3D-printed polymeric hydrogel matrix, carefully designed to crosslink in glucose. Upon irradiation of the sample, the chloroplasts perform photosynthesis, and the glucose produced in this process crosslinks the hydrogel *in situ* (see Fig. 2B). In samples containing chloroplasts, an impressive increase in Young's modulus (1.5 MPa–8.4 MPa), tensile strength (2.2 MPa–7 MPa), and fracture energy (2–6.5 kJ/m<sup>2</sup>) is observed (Fig. 2D). An even higher increase of the tensile strength can be achieved in the samples after pre-stretching them, resulting in a strength value of nearly 12 MPa. The mechanical properties were also dependent on the chloroplast concentration in the polymer and the illumination period, depicting the sensitivity of these living composites and the tunability opportunities. Furthermore, with this novel approach, the local properties can also be controlled by masking the light, resulting in samples with stiffness gradients that guide a crack through a determined path (Fig. 2E). Finally, the researchers showed the self-healing capabilities of their living composites by healing a damaged experimental propeller structure (Fig. 2F) and showing its ability to, in fact, propel a remotely controlled boat.

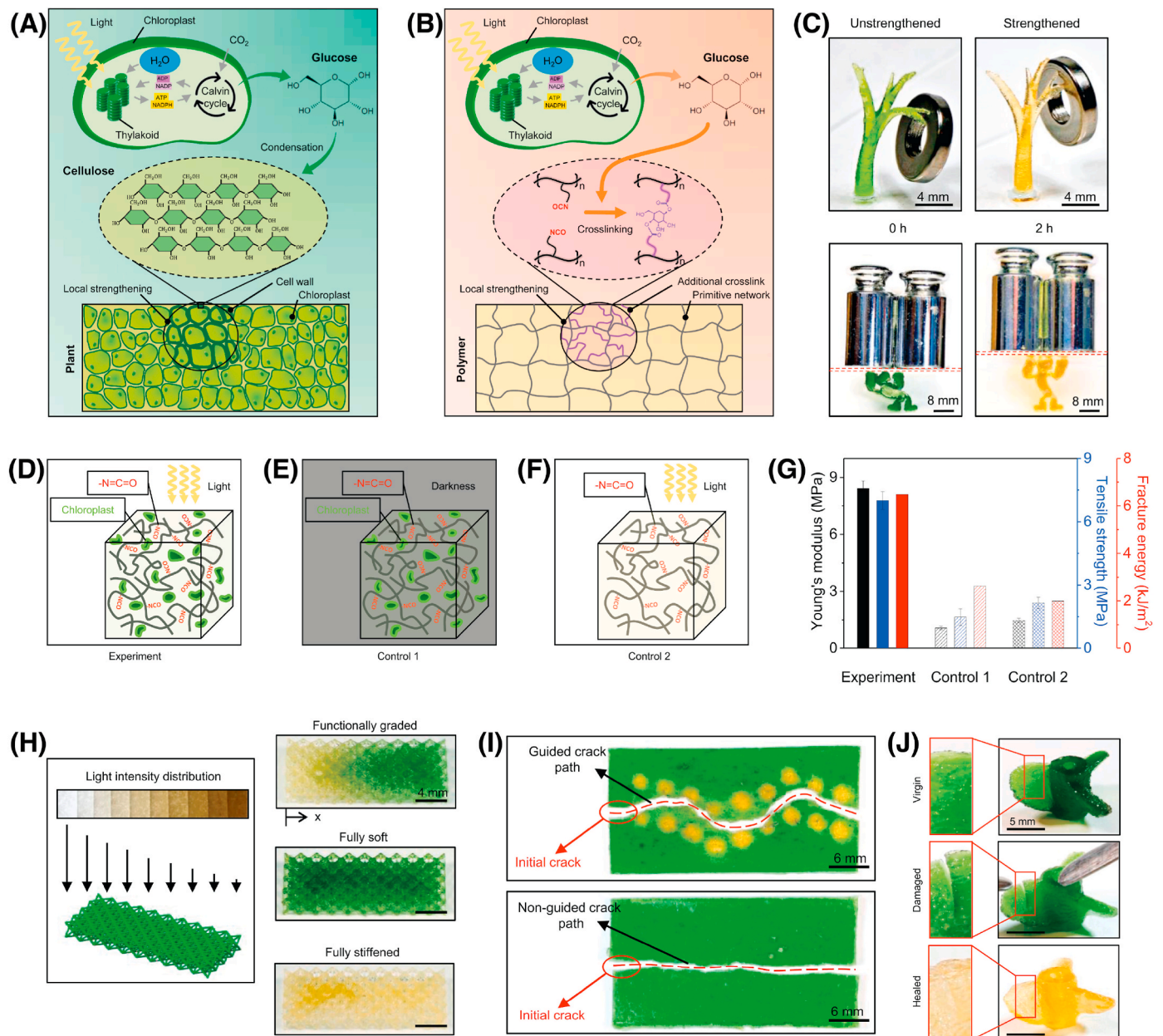
Using autotrophic organisms to produce engineered living composite materials, we can build structural materials with energy directly harvested from sunlight. Further, the energy produced by autotrophic organisms can fuel new functionalities in a material. One example of this is algae-based photovoltaics, which directly converts the power generated by photosynthesis into electrical energy [56]. Light can thus provide a renewable way to incorporate electrical functionalities into materials.

Conversely, electricity can be used to increase the efficiency and rate of carbon fixation. In microbial electrosynthesis, autotrophic pure- and mixed cultures convert carbon dioxide to useful chemical compounds, such as acetic or butyric acid, by using externally supplied electrons [57–59]. This technique is gaining more and more interest since yields are increasing because of the development of novel bio-electrodes with high surface areas and compatibility with microorganisms [60]. Microbial electrosynthesis could provide a technique to utilise surplus energy from renewable sources. When wind and sunlight are abundant, they can be used to capture CO<sub>2</sub>, produce hydrogen, and synthesise carbon chemical compounds for later use. These can be microbially converted back to energy when wind and sunlight are lacking [61].

Many obstacles still exist before autotrophic organisms can be used to harvest energy to make engineered living composite materials. Questions such as: how can we increase the efficiency of photosynthesis? And how do microorganisms behave, survive, and thrive within a material such as a hydrogel? Have only partially been answered [62]. To increase the impact that autotrophic engineered living materials (ELMs) can have, research has focussed on improving the efficiency of photosynthesis [63–66] and creating a suitable microenvironment that enables the organism's prolonged survival and creates a high surface area for gas exchange [67–69].

##### 3.1.2. Chemotrophs

To examine how a living organism could be used to harvest energy and form materials in remote places where other life would not be able to thrive, we will look at chemotrophs. These organisms obtain energy by oxidising or reducing chemical compounds in their environment. *Shewanella*, a marine bacterium, is known to use extracellular electron sinks, metal oxides, and ions as an energy source. This opens the possibility of generating electrical power since it can be directly harvested



**Fig. 2.** (A) In a plant, glucose is condensed to form cellulose, the key structural material of a plant. (B) The chloroplasts from spinach leaves can be used to crosslink, and thus mechanically strengthen, a hydrogel structure under UV radiation. (C) Demonstration of the uncrosslinked and crosslinked hydrogel and a clear improved gel stiffness. (D–F) Samples for mechanical tests and its control experiments. (G) Young's modulus, tensile strength, and fracture energy of samples with embedded chloroplasts after 4 h of illumination and 4 h of darkness (experiment), samples with embedded chloroplasts after 8 h of darkness (control 1), and samples without chloroplasts after 4 h of illumination and 4 h of darkness (control 2). (H) A gradient of light can be used to change the material's stiffness in a continuous manner. (I) Crack paths of samples with and without guided crack path. (J) Three-dimensional experimental propeller structure at the damaged and healed state. Adapted from Yu et al. (2021) [55].

from the organism's metabolism. The ability to extract this electrical power strongly depends on the electrodes used to interface with the organism. Researchers have demonstrated that introducing silver nanoparticles into and around the cell membrane results in charge-extracting capabilities several orders of magnitude greater than some of the inanimate counterparts [70], thus significantly increasing the feasibility of such microbial fuel cells.

To highlight the tantalising applications for such organisms from a structural viewpoint, we look at recent research endeavours that assess the possibilities for microbes to be used for 'space mining' [71]. For terraforming exoplanets, flying every building block to the destination is impossible, but one could take a small vial of bacteria. *Shewanella* can

use a wide range of solid and dissolved electron acceptors as an energy source and thus reduce  $\text{Fe}^{3+}$  from the lunar or Martian rock, secreting the product  $\text{Fe}^{2+}$ , which precipitates in the form of magnetite and can be magnetically concentrated. This is thus a straightforward way in which iron can be refined from the local rock, which can be further processed for construction. By using organisms in this way, one, in essence, has a self-replicating biochemical micro-factory, which can be easily deployed on a remote location such as the moon's surface or Mars. While these applications in space may seem far-fetched, in the present day, such microbial fuel cells are already being explored as a power source for remote sensing equipment and telecommunication on Earth [72,73].

### 3.1.3. Heterotrophs

Heterotrophic organisms feed upon other organisms or the products they make. There is a copious amount of energy stored within substances that traditional industries would label as waste; microorganisms could upcycle such streams into functional structural materials. What techniques have been developed to use the metabolic capacity of living organisms to create valuable compounds and even structural materials?

One elegant example is the bacterial production of polyhydroxyalkanoates, a type of thermoplastic polymer, from a wide range of waste streams such as municipal wastewater [74,75], wastewater from the sugar industry [76], agricultural waste [77], landfill leachate [78], and even the waste gas from steel factories and biomass gasification [79–82]. Researchers use mixed microbial cultures to perform this conversion on a cost-effective and large scale. Microbial mixed cultures are created by imposing artificial selection to obtain a culture enriched in cells that can convert. These cultures are incredibly robust, which overcomes the need for sterile feedstock and bioreactors, making them extremely cheap and versatile systems for large-scale applications.

While mixed microbial culture fermentation is ideal for converting large bulks of waste streams at a low price, it is relatively limited in the complexity of the fermentation products. Genetically engineered organisms can produce more complex chemical compounds in a process called precision fermentation. Such advanced fermentation can produce pharmaceuticals, flavours and fragrances, antibiotics, biofuels, and polymers. Precision fermentation opens a wide range of possibilities from a structural point of view since we have the tools to tune the genetic instructions and, thus, the structural properties of the materials found in living organisms precisely.

We can take collagen as an example. Collagen is a structural protein which serves as the extracellular matrix throughout the human body and other mammals. It is commonly found in cartilage, bones, tendons, ligaments, and the skin. Collagen is an essential component for tissue engineering, yet it is still extracted from animal sources such as pigs and cows. Collagen from a recombinant yeast platform can yield unprecedented possibilities to create biopolymers with designer properties [83] whilst moving away from animal products.

In the case of collagen, it has been demonstrated that both the amino acid sequence and the post-translational modifications can be tuned to influence its superstructure and, thus, mechanical properties [84–86]. Such recombinant collagens have been demonstrated to be extremely useful for constructing synthetic scaffolds for tissue engineering [87], bone regeneration [88], and wound dressing [89]. Collagen has been demonstrated to be very 3D-printable and easily chemically functionalised to, for example, include UV cross-linkable moieties [87,90], which makes it a promising candidate for engineered living composites grown from tissues.

This example highlights how synthetic biology can enhance the control and quality of engineered living composite materials. The remarkable efficiency of living organisms in producing structural materials is awe-inspiring. If we compare the mechanical properties of natural materials to common engineering materials today, we see that natural materials can compete with traditional engineering materials in many applications (see Table 1). Especially if one takes into account the environmental impact linked to the production of traditional materials, an even stronger case is made for natural materials.

When we compare the mechanical properties of natural materials to those of traditional engineering materials and account for the environmental impact of production, natural materials far surpass their counterparts. This can be visualised by dividing the material's mechanical properties by the carbon dioxide emitted in the production, as seen in Fig. 3. This stark contrast underscores the potential of natural and living materials to revolutionise manufacturing, offering engineers the means to construct with significantly reduced carbon dioxide emissions. In the following sections, we delve into the intricate processes by which living organisms create these structural materials and how researchers have leveraged these mechanisms to develop engineered living composite

**Table 1**

Summary of some of the materials presented in this review, divided into ELMs, bio-inspired or bio-produced materials. Compared with the properties of common engineering materials. The mechanical properties presented correspond to the highest values found in the different studies. *E*: Young's modulus,  $\sigma_U$ : ultimate strength,  $\delta$ : damping loss factor, *G*: fracture energy, *CO*<sub>2</sub>: CO<sub>2</sub> footprint.

Material	Mechanical properties	Remarks	Main challenges
<b>Engineered living materials</b>			
Hydrogel polymer with embedded chloroplasts [91,92]	<i>E</i> : 8.4 MPa $\sigma_U$ : 12 MPa <i>G</i> : 6.5 kJ/m <sup>2</sup>	Self-healing, stiffness gradient, and crack guiding	Maintaining long-term living states, increasing mechanical properties, and more complex material models
Mycelium composites [41]	<i>E</i> : 670 kPa $\sigma_U$ : 57 kPa	Self-healing, hydrophobic, and adaptive structures	Limited structural integrity and nutrient delivery
Cement with encapsulated bacteria [93]	$\sigma_U$ : 65 MPa <sup>a</sup>	Self-healing	Costs, bacteria safeguarding, and nutrition requirements
Engineered bacterial biofilm [94]	<i>E</i> : 1 GPa $\sigma_U$ : 18 MPa	Water processable biodegradable bioplastic	Low yields, limited shapability
<b>Bioinspired or bio-produced materials</b>			
Nacre-like composites [95]	<i>E</i> : 180 MPa $\sigma_U$ : 380 MPa $\delta$ : 0.03	Strong and tough structures, surpassing properties observed in natural nacre	Scalability
Bacterial cellulose and phenolic resin composites [96]	<i>E</i> : 28 GPa $\sigma_U$ : 140 MPa <sup>b</sup>	Biomimetic bacterial cellulose	Performance, processing, and costs
<b>Partially Natural materials</b>			
Hydrogel with cellulose nanocrystals [97]	<i>E</i> : 1.6 GPa $\sigma_U$ : 50 MPa	Shear-induced alignment, reinforcement in printing direction, and a high degree of shaping freedom	Limited structural integrity
Infiltrated wood [98]	<i>E</i> : 70 MPa $\sigma_U$ : 600 MPa	Mechanical interlocking of the densified wood cells	Limited shaping freedom
<b>100 % Natural materials</b>			
Wood [99]	<i>E</i> : 20 GPa $\sigma_U$ : 100 MPa	Adaptability to external mechanical stresses and hierarchical structures	–
Nacre [95]	<i>E</i> : 70 MPa $\sigma_U$ : 170 MPa $\delta$ : 0.01	Strong and tough structures	–
Bone [99]	<i>E</i> : 30 GPa $\sigma_U$ : 300 MPa	Remodelling, adaptability to mechanical stresses, and mechanosensory function	–
<b>Typical engineering materials [99]</b>			
Aluminium	<i>E</i> : 70 GPa $\sigma_U$ : 2200 MPa <i>CO</i> <sub>2</sub> : 12.8 kg/kg	–	–
Steel	<i>E</i> : 210 GPa $\sigma_U$ : 550 MPa <i>CO</i> <sub>2</sub> : 5.4 kg/kg	–	–
CFRP	<i>E</i> : 150 GPa $\sigma_U$ : 1050 MPa <i>CO</i> <sub>2</sub> : 18.3 kg/kg	–	–
GFRP	<i>E</i> : 28 GPa $\sigma_U$ : 280 MPa	–	–

(continued on next page)



**Table 1** (continued)

Material	Mechanical properties	Remarks	Main challenges
Flax/epoxy composites	$CO_2$ : 8.3 kg/kg $E$ : 39 GPa $\sigma_U$ : 1050 MPa	–	–
Concrete	$CO_2$ : 3.0 kg/kg $E$ : 38 GPa $\sigma_U$ : 60 MPa <sup>a</sup> $CO_2$ : 0.49 kg/kg	–	–

<sup>a</sup> Compressive.<sup>b</sup> Bending.

materials.

### 3.2. Forming materials & structures

Engineered living composite materials can provide novel solutions for two common problems plaguing composite engineers: how to create hierarchically optimised structures and how to create circular and sustainable materials.

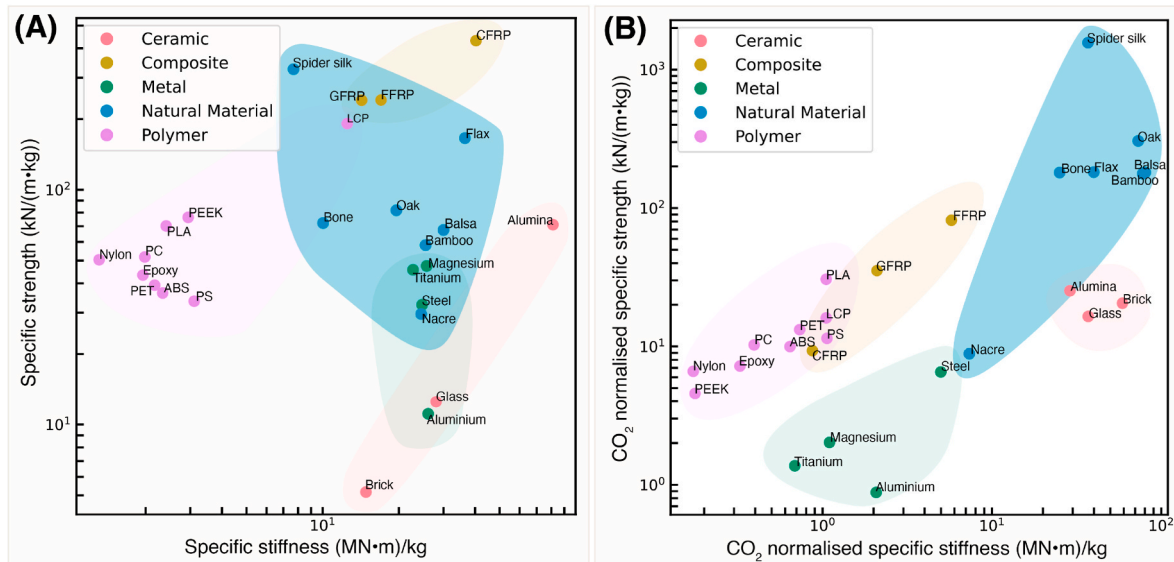
Let us take the example of a tree. Its mechanical properties rely on an intricate hierarchical structure to obtain its mechanical properties: wood [100]. If we zoom in on a piece of timber, we first see that the grain structure of the wood, i.e. the directionality of the xylem cells, is oriented to carry the load of the tree most efficiently. At branching points, we can see that the grain is continuous on the bottom side, and at the top side in the knee of the branch, it is oriented in a swirling pattern to prevent tear-out [101]. If we zoom in even further, we can observe that the individual xylem cell also forms an intricate composite material [100] (see Fig. 4a).

The secondary cell wall, which forms the major structural component of wood, is a composite of, for the most significant part, three biopolymers: cellulose, hemicellulose, and lignin [102]. These constituents come together in a structure analogous to a fibre-reinforced polymer composite. Cellulose is the most abundant biomacromolecule on earth, consisting of  $\beta(1-4)$  linked D-glucose units. In plants, cellulose

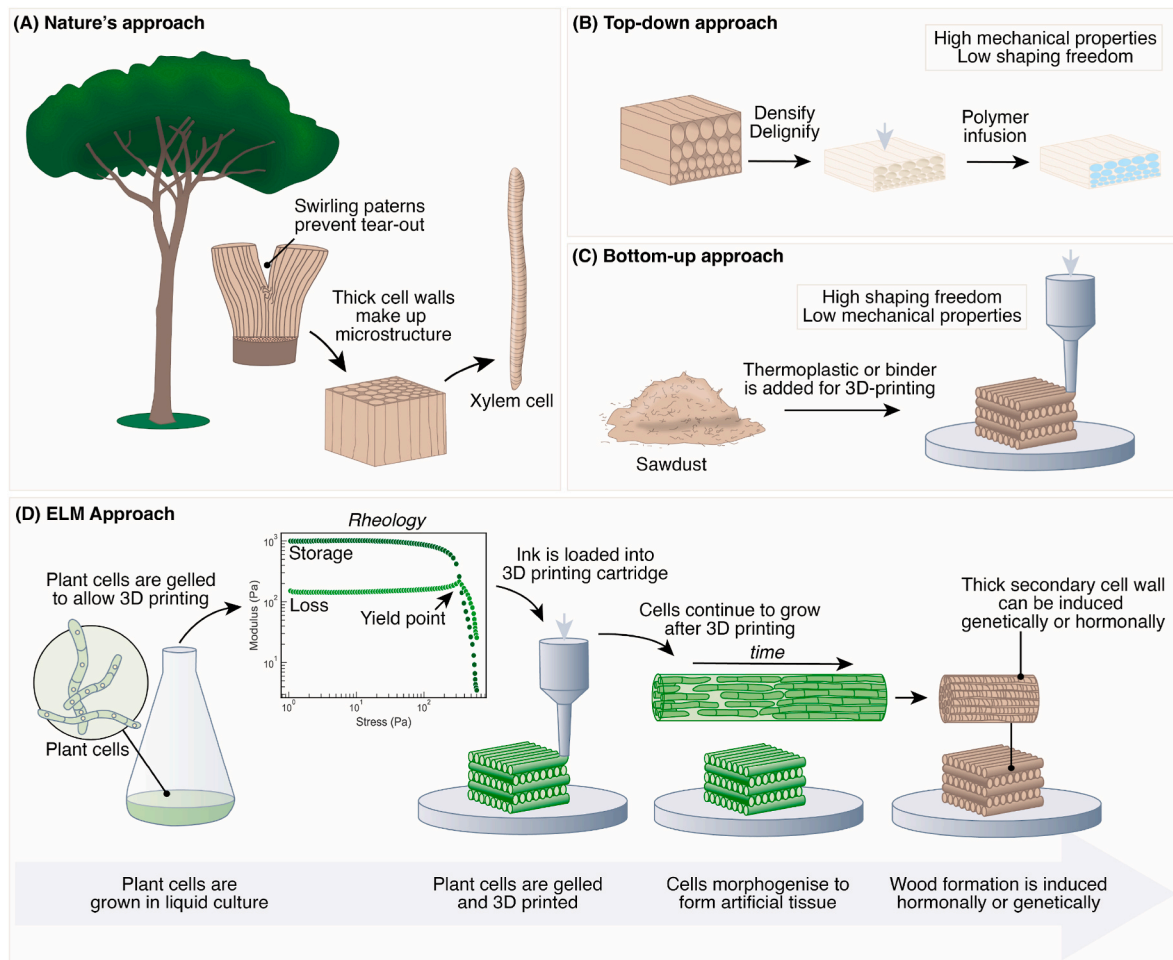
is formed by cellulose synthase complexes embedded in the cytoplasmic membrane [103]. Almost directly after synthesis, the molecular polysaccharide chains combine to form high-tensile-strength semi-crystalline nanofibrils with a diameter of about 2.5 nm [104]. These fibrils comprise crystalline cellulose that can reach tensile modulus up to 145–165 GPa [105] and strength up to 10 GPa. They are embedded in a matrix of manoglucan and xylan hemicelluloses combined with lignin, which are stabilised through intra- and intermolecular hydrogen bonding. Between the different layers of the secondary cell wall – the  $S_1$ ,  $S_2$ , and  $S_3$  – the cellulose microfibrils are offset by a certain angle [106], effectively making a cross-laminate radial structure where the primary mechanical performance is afforded through the  $S_2$  layer [100].

Wood has been the preferred building material for millennia [107] and was even used to construct aeroplanes well into the 20th century [108]. However, it has gradually been surpassed by steel, concrete, and plastics because these are easier to shape, more homogeneous and predictable in structure, and have higher mechanical properties. In efforts to let wood compete with modern engineering materials, many scientific endeavours have focused on improving the mechanical properties of wood.

Top-down approaches such as compression [98], chemical treatment [109], and infiltration with polymers and resins [110] have resulted in wood materials with remarkable mechanical performance and improved shaping into planar structural geometries [111] (see Fig. 4b). For example, matrix-infiltrated wood scaffolds reveal tensile values up to 70 GPa in stiffness and 600 MPa in strength due to mechanical interlocking between 70 vol% densified wood cells [98]. In contrast, non-infiltrated wood can reach a maximum stiffness of 20 GPa and strength of 100 MPa [99]. These mechanical properties of infiltrated wood are, in fact, comparable to those of aluminium, which has a stiffness of 70 GPa and a strength of 550 MPa [99]. In contrast to top-down approaches, bottom-up approaches that deconstruct and reassemble the wood structure could provide more structural homogeneity and shaping freedom [97,112,113]. For example, in a study by Siqueira et al. [97], composites consisting of cellulose nanocrystals were produced by direct ink writing. Shear-induced alignment of the nanocrystals yielded composites with enhanced mechanical properties in the printing direction, with stiffness and strength values of approximately 1.6 GPa and 50 MPa, respectively. Although it is fascinating that a natural material originating from wood can provide structures with programmable



**Fig. 3.** Ashby diagrams displaying the (A) specific strength in kN/(m·kg) and specific stiffness in (MN·m)/kg, and (B) the specific strength in kN/(m·kg) and specific stiffness in (MN·m)/kg normalised with respect to the carbon dioxide equivalents emitted during the primary production of the material. Natural materials outperform traditional engineering materials if it comes to strength and stiffness per kilogram of material per kilogram of carbon dioxide emitted. Properties obtained from the Materials Universe database.



**Fig. 4.** (A) Nature's approach to the formation of wood as a structural material, xylem cells with a thick secondary cell wall make up the tissue, and the directionality of the fibres determines the macroscale properties. (B) Top-down approaches are mainly centred on densifying wood and infusing it with polymers and resins to increase mechanical performance, but shaping freedom remains limited. (C) Bottom-up approaches focus on disrupting wood's microstructure and binding it with polymer or resin; this enables greater shaping freedom, but mechanical performance is limited. (D) An ELM approach to creating wood with high shaping freedom would be to culture plant cells *in vitro*, 3D printing them in a gelled state, and controlling the growth and morphogenesis of these cells into xylem cells.

reinforcement, retaining the mechanical performance of the original wood structure has proven difficult (see Fig. 4c).

To address the inherent limitations of both bottom-up and top-down approaches in enhancing wood properties, researchers have explored 3D printing techniques involving living plant cells. This involves the printing of plant cells into a desired shape, followed by inducing their differentiation into xylem cells [114,115]. Despite these efforts, no successful attempts have yielded a material with structure and mechanical properties comparable to native wood. The challenge persists in comprehending how to assemble individual plant cells into artificial plant tissues effectively. Extensive research has delved into understanding the control of xylogenesis—the natural process of wood formation [116,117]; however, much of this research still must be applied in an engineered living materials context (see Fig. 4d). However, if one could form wood starting from a single cell harvested from a bioreactor, it would enable full control over the micro- and macrostructure of wood. This would enable new avenues of manufacturing with wood. One could additionally manufacture topology-optimised wood structures and genetically change the composition of the cell walls. Such advancements would revolutionise manufacturing with wood since it would become faster, easier to form, and possibly stronger than traditional wood.

Other efforts have focussed on trying to valorise the copious amounts of plant waste generated by society. Since lignocellulosic waste is so abundantly available – wood scraps [118], agricultural waste [119], and

even herbivore manure [120] – research has focussed on using the broken-down plant-derived cellulose to create materials with new functionalities. A common technique is to oxidise cellulose partially. Since the crystalline region is highly packed, and thus sterically less accessible, the amorphous regions of the cellulose will be oxidised first. The product comes in many different forms, depending on the size, but is generally called cellulose nanocrystals. Such nanocelluloses possess some interesting properties: extremely high tensile strengths of 7.5 GPa, Young's modulus of 100–140 GPa, large surface areas of 150–250 m<sup>2</sup>/g, and a range of interesting optical and electrical properties [121].

Because of the vast, interesting properties of cellulose nanocrystals, they have been used in diverse applications. For example, cellulose nanocrystals have been used in biobased water filters for the removal of heavy metals because of their relative stability and high surface area [122,123]. Its remarkable mechanical properties have led to the use of cellulose nanocrystals to reinforce thermoplastics. The mere addition of 1 % in weight of sisal cellulose nanocrystals increased the tensile strength of a thermoplastic by 84 % (from 22 to 42 MPa) and the Young's modulus by 63 % (2.1 GPa–3.4 GPa) [124]. Other research has utilised the dimensions and crystallinity of cellulose nanocrystals to create structural colours, which can be found on the exoskeleton of beetles or certain berry varieties. Because of the high crystallinity, small size and high aspect ratio, cellulose nanocrystals self-assemble in optically active chiral nematic films as a solvent slowly evaporates [125].



The seemingly endless possibilities of cellulose nanocrystals do not come without drawbacks. The conversion of plant-derived cellulose to nanocrystals is a process that uses harsh chemicals and conditions. Because of this, much research has focussed on bacteria as an alternative purer source of cellulose. While bacterial cellulose is produced in a relatively similar way as plant cellulose is made, by cellulose synthase complexes in its cytoplasmic membrane, bacterial cellulose is without hemicelluloses or lignin in the supramolecular structure. It is more crystalline at 70–80 % and produced in ribbons of 30–100 nm in width [126].

As a demonstration of the excellent mechanical properties of bacterial cellulose, we can look at the work of Nakagaito et al. They demonstrated that a composite made from bacterial cellulose and phenolic resin has a 1.5 times higher Young's modulus at 28 GPa than the composite made with plant-derived cellulose fibrils [96]. Because of this wide range of interesting properties, bacterial cellulose has also been used in various applications in biomineralised bacterial cellulose [127] or as a reinforcement of polylactic acid thermoplastics [128]. Bacterial cellulose can serve as an excellent biocompatible scaffold for the growth of other cells and microorganisms such as bone [129], cartilage [130], and microbial sensors [131].

Despite the abundance of material and use cases, cellulose poses quite a challenge if we want to make future-proof structural materials. Deepening our understanding of how we can harness, for example, the self-assembly properties of bacterial cellulose or other biophysics or biochemistry-based approaches to create intricate microstructures could pose a way to overcome this trade-off. As an extension of this, more research into the mechanisms which govern the formation of the wood microstructure could provide us with means to control plant cells or microorganisms to develop microstructures bottom-up in an autonomous fashion.

Some first endeavours have been made to use CRISPR genetic editing of Aspen wood's DNA to better suit sustainable fibre production [132]. Flax plants have been engineered to increase the mechanical properties of the fibre in various ways [133]: for example, by decreasing the lignin contents [134], or by incorporating *de novo* synthesis of polymers such as PHA [135]. However, examples of improving the mechanical properties of plants by genetic engineering are still in their infancy. While tuning these structural proteins has been relatively successful, genetic engineering to the present day still needs development to architect the mineral structure, carbohydrate structure, and supramolecular interactions in general. As well as leading to more sustainable materials, this will open the possibility of exploiting phenomena such as stick-slip interactions, which may introduce tremendous resilience to structural materials [136]. Coupling this behaviour with highly structured architecture, like those found in hierarchical materials, such as a nacreous material in molluscs, could be a breakthrough.

Let us again zoom in on nacre, since there is much to learn from studying how nacre is formed in a bivalve and why it is as strong and tough as it is. While consisting of around 95 % of brittle calcium carbonate in the form of aragonite crystal structures, the resulting material is exceptionally stiff, tough, and fracture resistant. The material consists of sub-micron-thick platelets, whose growth is guided by an organic scaffold carefully deposited by epithelial cells [137]. Through biomineralisation, the organic template forms into aragonite platelets [138, 139]. The platelets are stacked in a brick-and-mortar structure and encapsulated in proteins and polysaccharide-based hydrogel polymer layers as thin as 30 nm [140].

Via the biomineralisation processes, platelets nucleate into layers and grow laterally until they impinge on one another. This results in exquisite hexagonal close-packed arrangements of platelets within each layer. In layers, platelets are staggered relative to each other but also closely packed such that any variation in thickness dovetails into the platelets above and below. Nucleation of this growth process also produces complex platelet-platelet interfaces through mineral nano-asperities and mineral bridges connecting platelets together.

Fluctuations in the molluscs' nutrition and environment result in structures commonly referred to as growth bands [141], biopolymer-rich layers about 25  $\mu\text{m}$  thick subdivide the brick-and-mortar structure at approximately 300  $\mu\text{m}$  thick intervals and, analogous to the annual growth rings on a tree trunk, grow seasonally to result in a multiscale material structure. This fascinating hierarchical arrangement gives nacre an elastic modulus of approximately 70 MPa while maintaining a damping loss factor of 0.01, making it stiff but fracture-resistant [95]. To put these properties into context, Aluminium has the same modulus. Still, it presents a damping loss factor of at least one order of magnitude lower than nacre, portraying the typical antagonism that many conventional engineering materials exhibit instead of natural materials.

Studying natural materials' structure and mechanical behaviour at multiple length scales has enabled a better understanding of the design principles underlying the combined strength and toughness of biological composites. Strength and toughness are often reconciled by providing intrinsic and extrinsic toughening mechanisms at small and large length scales, respectively. This increases the toughness since it can deflect a crack, and thus substantially more energy is absorbed [137–139,142].

Fabrication of these intricate hierarchical structures is achievable through techniques such as ice-templating [143,144] or directed colloidal assembly [145]. A specific method involves magnetically assembling  $\text{TiO}_2$ -coated alumina platelets, forming brick-and-mortar structures [146]. The subsequent step involves hot-pressing at varying temperatures, resulting in nacre-like scaffolds exhibiting different levels of mineral nano-interconnectivity.

During hot pressing, mineral contacts between alumina platelets are established due to the partial sintering of the titania coating. The manipulation of mineral nano-interconnectivity is key to tailoring the scaffold properties. Creating denser scaffolds with increased fractions of mineral bridges and platelet clusters achieves nacre-like composites with unprecedented specific strength, stiffness, and fracture toughness. Notably, these materials have surpassed the mechanical properties of natural nacre and demonstrated remarkable stiffness, strength, and damping loss factor. These properties reached values up to 180 GPa [95], 380 MPa [16], and 0.03, respectively, originating from alumina platelets with approximately 1 GPa strength.

These findings provide valuable insights into the critical role of submicron mineral bridges in the mechanical behaviour of nacre. Additionally, they offer robust guidelines for the manufacturing of high-performance and sustainable materials inspired by nacre, paving the way for advancements in material science.

While producing materials with properties far superior to nacre, the batch process makes production energetically intensive and slow. An approach to achieve more scalability could be through bacterially produced nacre-inspired composites, which uses the self-assembling properties of highly crystalline bacterial cellulose [127]. The bacterially produced cellulose is mineralised by the bacterium *S. pasteurii*, and upon drying, the cellulose and calcium carbonate composite undergoes a self-assembly process. This makes the mineralisation process a one-pot process, greatly reducing processing times and increasing the ease of manufacturing.

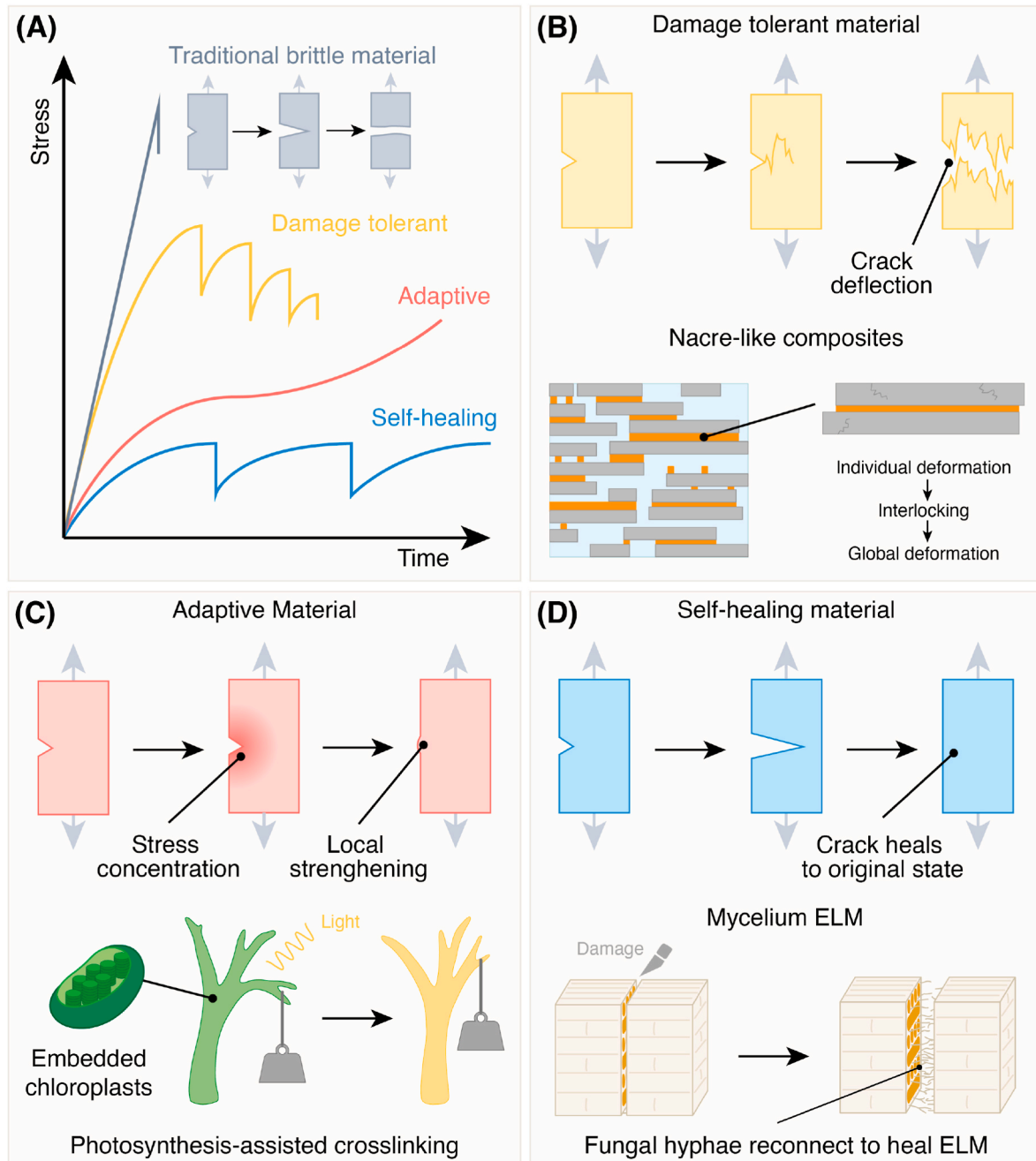
The versatility of the *E. coli* platform can be extended even further: for example, one research endeavour engineered *E. coli* to produce protein nanofibers, which has been shown to be directly useable as a structural component in a 3D-printing ink for ELMs [147]. Further, it is possible to control the production of such structural elements by an external stimulus. For example, researchers have modified *E. coli* to produce mussel foot protein and amyloid protein, usually produced by mussels, to glue themselves to a substrate [148]. The genetic code was controlled by a light-inducible promoter. In essence, it creates a living glue system in which the glue production could be triggered by illumination.

We have seen how the materials found in nature surpass traditional engineering materials in terms of mechanical performance (see Fig. 5).

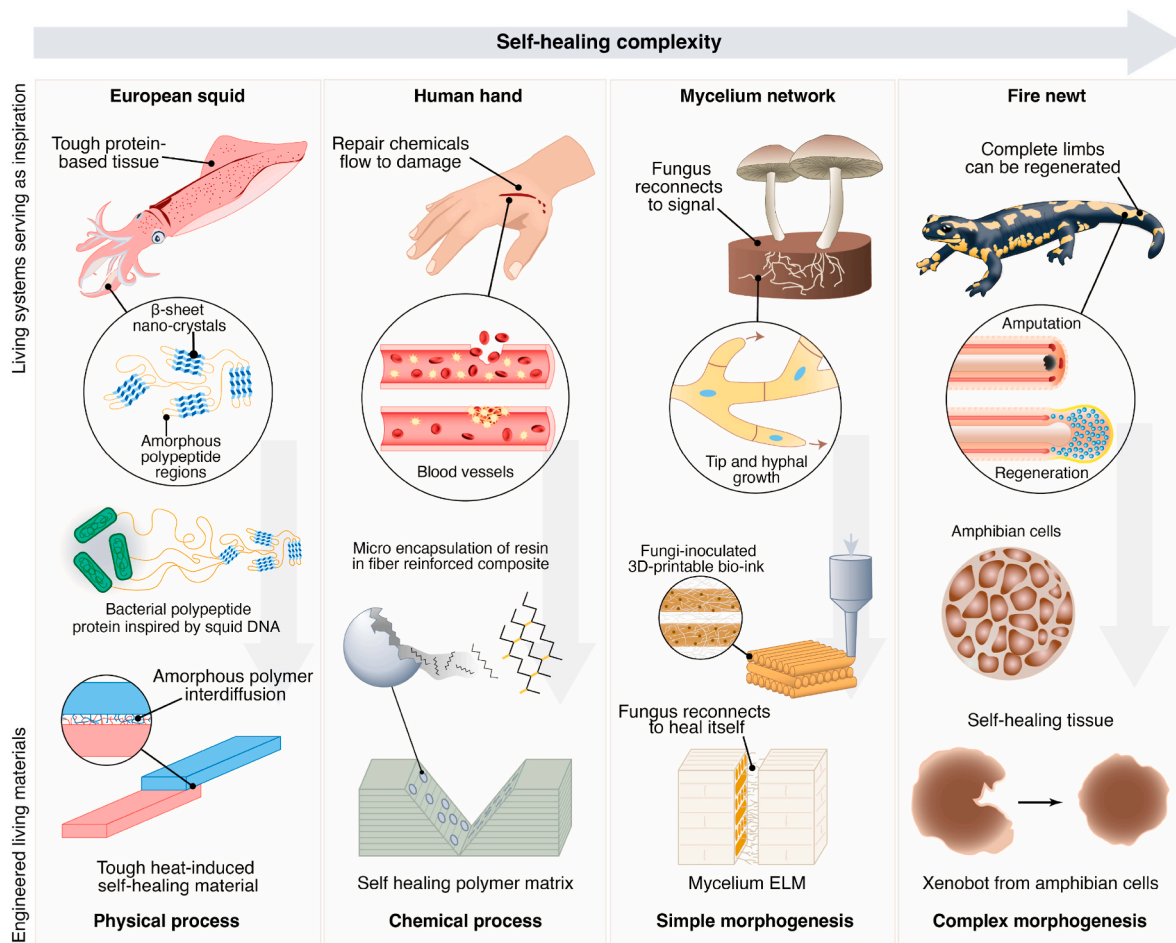
Materials such as nacre and wood are more damage tolerant than traditional engineering materials such as steel because of their hierarchical microstructure. Next, materials such as wood and bone adapt to their load case, creating topology-optimised structures with excellent weight-to-performance ratios. To extend the possibilities of structural ELMs even further, these materials can be improved by changing the constituents to stronger, tougher, stiffer synthetic compounds than available in nature whilst maintaining the living and responsive capabilities of the organism, such as their ability to sense and adapt (see Fig. 6).

### 3.3. Sense & adapt

One of the most striking skills of life is the ability to sense the environment and adapt to it. If we look, for example, at a seedling, it will sense the direction of the sunlight on its structure and will bend to increase the amount of light available, termed phototropism [149]. In this process, auxins on the dark side of the plant structure trigger the activation of proton pumps, resulting in a reduction of pH within the cells. The acidification of the cell wall initiates the activation of expansin enzymes [150]. Expansins disrupt hydrogen bonds within the cell wall structure, rendering the cell walls less rigid [151].



**Fig. 5.** (A) Stress-strain diagram of traditional brittle engineering materials, compared with three key properties exhibited by many biological materials: damage tolerant, adaptive, and self-healing, traditional engineering materials such as fibre-reinforced polymers fail shortly after the initial crack is formed, and quickly fails critically. (B) Damage-tolerant materials such as nacre and wood rely on a microstructure to deflect cracks, and thus absorb a higher amount of energy. This is being replicated in materials such as nacre-like composites. (C) Living materials such as bone and wood adapt to a particular load case and locally strengthen itself, an ELM which demonstrates adaptability under the influence of light has been demonstrated by Yu et al. [91] (E) Living materials can produce new material to self-heal defects, restoring the material to the original mechanical properties, a great demonstration is the self-healing mycelium material of Gantenbein et al. [41].



**Fig. 6.** Living organisms that self-heal and the ELMs that have been inspired by them. There are multiple levels at which self-healing can take place. The work by Pena-Francesch et al. demonstrates a microbially produced polypeptide which is produced from the DNA of a squid; the material self-heals within seconds [176]. Microcapsules with uncured resin have been added to fibre-reinforced polymers to replicate the self-healing of cuts in a human hand. These capsules rupture when a crack is formed, effectively healing the composite through a chemical reaction [168]. In Nature, a fungus grows and reconnects with itself to signal and transport nutrients. This process has been utilised to create self-healing structures which can recover its mechanical properties fully, as long as nutrients last [41]. One of the most complex examples of self-healing behaviour can be found in amphibians such as the fire newt. Amphibian cells have been explanted to become autonomous xenobots, exhibiting locomotion, morphogenesis, and self-healing behaviour [182].

Furthermore, the heightened activity of proton pumps facilitates the influx of more solutes into the plant cells on the shaded side, thereby increasing the osmotic gradient between the symplast and apoplast of these cells. Consequently, water moves into the cells following its osmotic gradient, leading to an elevation in turgor pressure. The combination of reduced cell wall strength and increased turgor pressure, surpassing a yield threshold, induces cell swelling, generating the mechanical pressure necessary for driving the phototropic movement of the plant. Ultimately, this leads to a cell elongation, which bends the plant towards light.

Wood species adapt their microstructure according to the mechanical stresses to develop and use prestress. Trees develop reaction wood to adapt to mechanical stresses and maintain structural integrity [152]. In gymnosperms (conifers), compression wood typically forms on the lower side of branches or leaning stems [153]. Compression wood has higher lignin content and lower cellulose content than normal wood. Lignin provides rigidity and strength to the cell walls, helping the wood withstand compression forces. It is common in gymnosperms (conifers) and is essential for their structural stability.

Conversely, tension wood usually forms on the upper side of branches or stems. It responds to tension forces, such as wind, causing the tree to lean or sway. Tension wood has a higher cellulose content and fewer lignin deposits [154]. This is because, in wood, the modulus and

tensile strength are inversely proportional to the lignin content and directly proportional to the cellulose content [155]. The cellulose fibres are oriented in a way that enhances tensile strength. Tension wood is more prevalent in angiosperms (hardwood trees). Compression and tension wood formation allow trees to optimise their structure to withstand specific mechanical stresses. These adaptations help trees maintain their form and integrity in various environmental conditions. The different properties of compression and tension wood also impact its mechanical properties, making it more suitable for its specific role within the tree.

Another example of a material that constantly adapts is bone. Osteocytes and osteoblasts are two types of cells involved in the dynamic process of bone remodelling, essential for maintaining bone integrity and adapting to mechanical stresses [156]. Osteocytes are mature bone cells that are embedded within the bone matrix. They have a mechanosensory function, detecting mechanical strains and stresses experienced by the bone. Osteocytes communicate with osteoblasts and osteoclasts (cells involved in bone resorption) to regulate bone remodelling in response to mechanical signals [157]. They release signalling molecules, such as sclerostin, which inhibits bone formation, and RANKL (Receptor Activator of Nuclear Factor  $\kappa$ B Ligand), which regulates bone resorption. Osteocytes are connected to each other and to the bone surface by tiny channels called canaliculi. Through this

lacunocanalicular network, osteocytes exchange nutrients, waste products, and signalling molecules, contributing to the coordination of bone remodelling activities [158].

Bone remodelling involves a continuous cycle of resorption and formation. Osteoclasts resorb old or damaged bone tissue, creating a cavity. Osteoblasts then fill this cavity by depositing a new bone matrix. Signals from osteocytes and other regulatory factors orchestrate the balance between bone formation and resorption to maintain bone homeostasis and respond to mechanical demands. Wolff's Law, proposed by German anatomist and surgeon Julius Wolff in the 19th century, states that bone structure adapts to the mechanical stresses placed upon it [159]. Specifically, Wolff's Law asserts that bone tissue will remodel and become stronger in response to the demands and pressures imposed on it. This principle highlights the dynamic nature of bone, emphasising its ability to adjust and optimise its structure based on the functional forces it experiences.

This demonstrates the adaptability of structural materials found in life, which could be exploited in the engineering realm today [160]. Recent advances that use living organisms' sensing and adapting capabilities to create engineered living composite materials that adapt to their environment have increased.

An organism can respond to a certain stimulus by introducing a reporter gene, which, in essence, is a promotor known to be activated by a specific stimulus, and a gene under its control, such as one which produces a fluorescent pigment dye. For example, the engineered fungus *Aspergillus niger* with a genetic modification was designed to produce melanin in the presence of the sugar xylose [161]. These features find applications in detecting heavy metals in water streams [156–158], and making optical bacterium-based materials, which, for example, can be used in autonomously shading windows [162]. To increase the longevity of such materials, sporulating bacteria such as *Bacillus subtilis* have been explored to increase shelf life [163] since the bacteria can produce extremely durable spores which can survive in the harshest environments. When rewetted, the spores regenerate the engineered bacteria, and the sensors reactivate.

Co-cultured organisms have also been used to produce the structural material, such as bacterial cellulose produced by *Komagataeibacter rhaeticus* and a sensory organism, *Saccharomyces cerevisiae*, in a one-pot process [131]. These researchers especially demonstrated the platform's versatility, demonstrating that this synthetic bacterial cellulose platform shows enzymatic functionalisation, photo activity, and tuning mechanical properties. Whether it is temperature [162], chemicals [131,164], light, ultrasound [165], etc., there is a myriad of possibilities to use organisms to create sensors by genetic engineering.

Rivera-Tarazona et al. [166] reported a material from engineered *Saccharomyces cerevisiae*, which proliferate upon irradiation with UV light. After proliferation, the material can be up to 400 % thicker in the irradiated areas in comparison with areas kept in the dark. This opens the possibility of creating materials which can morph and adapt in shape after the initial production. When such morphing living or non-living materials are produced employing additive manufacturing, it is commonly referred to as 4D printing. An even more striking example of a material can be found in the work of Lin et al., in which an artificial muscle is made with nano fibrillar hydrogels, which can be mechanically trained [167].

Life exhibits remarkable adaptability, demonstrated by phenomena such as phototropism in seedlings, where auxins trigger proton pump activation, leading to changes in cell structure and turgor pressure, ultimately causing the plant to bend towards light. Similarly, trees adapt to mechanical stresses through the development of compression wood on the lower side and tension wood on the upper side, optimising their microstructure for strength and flexibility. In bone remodelling, osteocytes act as mechanosensory, communicating with osteoblasts and osteoclasts to maintain bone integrity by adjusting its structure in response to mechanical stresses, as Wolff's Law describes. These natural adaptations inspire engineering approaches, such as using genetically modified

organisms to create living composite materials with responsive capabilities, offering potential applications in various fields, including sensing and adaptive materials.

### 3.4. Growth & remodelling

A universal problem with classical engineering materials such as steel, concrete, and carbon-fibre-reinforced composite materials is that after the production of a part, the properties degrade. This stands in sharp contrast to living materials. When a human bone breaks, a complex orchestration of muscle contractions results in shielding when possible, swelling to immobilise the joint and time for the bone to regrow and reform within a few weeks.

A yet more stunning example from Nature is observing that plants can be completely regrown from only a tiny cutting. When a plant cutting is taken, it often includes meristematic tissues, allowing for the formation of new organs. The meristematic cells in the cutting undergo rapid cell division and differentiation. Cell division leads to the formation of new cells, while differentiation involves specialising cells into specific types, such as root cells, stem cells, and leaf cells. When placed in a suitable growing medium, roots can develop from the meristematic tissues at the base of the cutting. Root formation is crucial for the cutting to establish itself as a new, independent plant. Simultaneously, shoots or new stems can develop from the meristematic tissues at the upper portion of the cutting. These shoots grow into new branches and leaves, creating a complete plant structure. One of the essential factors that enables such self-healing and regenerative behaviour is the fact that every single cell in an organism contains the information and machinery needed to produce the whole organism, the DNA and the enzymes.

There are many ways in which a self-healing material can be made. Still, there are some essential differences between advances in the last decade in composite engineering and ELMs. Classically, many endeavours to make fibre-reinforced composites self-healing rely on encapsulating a healing agent within the composite. When the composite is damaged, the microcapsule cracks, releasing the healing agent into the matrix [168]. An example is self-healing epoxy, which has microencapsulation of low-viscosity monomers; when the encapsulation breaks, the monomer encounters a solid catalyst in the vicinity [169]. The novelty of this system is embedding shape-memory wires throughout the epoxy, which can be activated to pull together the crack and spread the curing resin.

A broad range of results has been achieved in terms of recovery of mechanical properties. For example, Jin et al. [170] achieved a 111 % fracture toughness recovery, Kesslet et al. a strength recovery of 75 % [171] and Jones et al. [172] a fatigue life extension of 2000 %. These results were heavily dependent on control factors such as encapsulation volume, healing temperature, and healing time. There are many variants of this principle, e.g. with vasculatures [173] or hollow fibres [174]. One particularly interesting application of this principle is the encapsulation of live bacteria for self-healing concrete [93]. Upon cracking, bacteria react with the calcium lactate incorporated in the concrete, producing calcium carbonate that blocks cracks and micro-pores [175]. Healed concrete can present an increase in compressive strength of 42 % and flexural strength of 72 %. Nonetheless, significant developments in terms of costs, bacteria safeguarding, and nutrition need to take place for this new approach towards sustainable concrete to be industrially implemented. In general, and while they provide exciting ways to increase the lifespan of traditional engineering composites, the autonomy and efficiency of such self-healing mechanisms relying on encapsulation are far from the mechanisms found in living materials.

We can also see how a non-living system can self-heal when the molecular structure itself is inspired by nature. A squid's tough and elastic tissue possesses these interesting mechanical properties because it consists of nano-crystal-forming  $\beta$ -sheets linked by amorphous polypeptide regions. Researchers have synthetically produced the polypeptides in the squid *Loligo vulgaris* by engineering an *E. coli* platform.



This opened the possibility to assess the effect of different lengths of tandem repeats, the factor enabling  $\beta$ -sheet formation, and different molecular weights in general. Not only did these researchers create a material which surpasses the mechanical properties (native cohesion 10 kPa to a variable cohesion of 0–75 kPa) of the *Loligo vulgaris*, but they also succeeded in creating a material which self-heals on a timescale of several seconds [176]. This feat is rarely observed in Nature, and beautifully demonstrates how synthetic biology can be used to precisely tune the materials found in nature to form precisely engineered living composite materials.

The self-healing material described above relies on physical and chemical processes to heal, which requires close contact between two sides of a defect. If we want to form new material to fill more significant defects or bridge gaps, we must look at how organisms and cells in an organism grow, form new material, and self-heal defects in their tissue. One tantalising example of this living self-healing behaviour is that of fungal hyphae networks. Mycelium forms a continuous web of hyphae through which nutrients and signals are transported. When such a network is damaged, it will grow to form a continuous network once again. Let us look at how this inherent tendency of mycelium networks to grow out and reconnect with themselves when confronted with damage.

An exciting demonstration of the self-healing capabilities of mycelium can be found in the work of Gantenbein et al. [41] In this work, the researchers succeeded in creating a mycelium-inoculated 3D-printable hydrogel. The fully colonised hydrogel was demonstrated to work as a soft hydrophobic skin on robotic grippers and other robots. Of particular interest is the fact that the researchers have demonstrated that when severed, the mycelium could regrow and self-heal, even increasing the stiffness and strength of the original structure. Structures with initial stiffness and strength values of approximately 100 kPa and 17 kPa had an increase in these mechanical properties, presenting values of 670 kPa and 57 kPa respectively after one healing cycle with a duration of 6 days. These healed structures even fractured for the second time in a different location. This self-healing capability, however, is possible for as long as nutrients are available.

The eventual depletion of nutrients for the organism remains a recurring theme in producing engineered living composite materials. The longevity of engineered living composites, such as the mycelium composite in the work of Gantenbein et al. [41] can be significantly improved by including vasculature within the structure to allow fresh nutrients flow; however, this requires novel 3D-printing techniques to print such core-shell structures. Another possibility is to include autotrophic organisms within the structure and create synthetic symbiotic relationships. Some first endeavours to create these synthetic fungal autotroph symbiotes have been undertaken [177,178], creating a synthetic lichen, an extremely hardy and long-lived symbiotic culture.

One of the most striking examples from nature, in which heterogeneous and complex structures are formed, can be found in amphibians. Newts regenerate limbs through a process called epimorphic regeneration. After limb amputation, specialised cells at the injury site dedifferentiate, proliferate, and then re-differentiate into various cell types, guided by essential signalling pathways like Wnt and FGF [179,180]. These signalling pathways can: find the place of damage; through gradients, set the coordinates in which the new limb will be generated; and trigger proliferation and differentiation pathways for cells which will make up the regenerated limb.

The underlying reason that self-healing is so central to living systems and so difficult to achieve in traditional engineering composites, is the same reason why humans can get cancer and aeroplanes do not [181]. It has to do with the fact that aeroplanes are made through design and top-down instruction and shaping of inanimate matter. On the other hand, living matter is made up of cells, which can process inputs and determine its optimum output for a particular parameter space. This autonomy of cells within a macrostructure makes it possible to locally sense, grow, and thus self-heal. However, one can also imagine that

when a cell has autonomy, it can develop into a mutation instead of serving the greater good of the macrostructure.

To see how the autonomy of cells can be used for morphing and self-healing purposes, we can look at the work of Blackiston et al., where they made explants of the frog *Xenopus laevis* to live as autonomous robots [182]. The researchers tried to observe what a lump of cells would do when removed from the main body. Will these cells die? Will these cells start to navigate their influence sphere? It turns out that these ‘xenobots’ started to exhibit coordinated locomotion. Next to that, the cells could self-organise and self-heal. Whether it is the steering of tree growth to make living architectural elements on a large scale [183], or the use of bacteria to introduce microstructured elements on a small 3D print [184], it is the cellularity and multilevel autonomy of living materials which enables them to locally sense, adapt, and create.

There are many ways in which ELMs could self-heal. Whether it is by the production of precisely tuned polymers which can restore covalent or noncovalent bonding [185], organisms which induce crystal precipitation to fill voids with solid material, or the organism itself growing out to bridge a gap and regrow a structure, in all these cases the material possesses properties which can extend the lifetime and increase the robustness of an engineering material. One of the most significant challenges is maintaining the viability of organisms for the lifetime of a part. Future endeavours to enable truly long-lived self-healing materials must focus on maintaining this viability within a material. Many paths towards this goal have already been paved. For example, one can incorporate vasculature within a structure to supply the organism with fresh nutrients or use the spore-forming capabilities of many organisms, such as bacteria or fungi. However, much more work is needed to develop toolkits to engineer with shaping, vasculature, sporulation, etc, to enable the creation of long-living engineered materials.

#### 4. Conclusions & future prospects

The efficacy of life in energy utilisation, the cellular structuring of materials, and the adaptive and self-repairing traits of living organisms collectively position ELMs as having distinct advantages over conventional engineering materials. Advancements in biology, particularly in DNA sequencing and genetic engineering tools like CRISPR, have been instrumental in propelling the development of ELMs. The capacity to imbue desired properties into well-adapted organisms opens diverse possibilities, spanning the creation of novel compounds to the integration of sensory capabilities. Additionally, additive manufacturing, mainly through 3D printing, plays a vital role in replicating intricate bio-inspired forms and microstructures, facilitating the tailoring of ELMs to specific needs.

While ELMs show immense promise in bringing revolutionary new functionalities to composite materials, challenges persist. These challenges include demonstrating lower ecological impacts, meeting reliability and performance benchmarks akin to traditional materials, ensuring enduring durability, and navigating societal apprehensions surrounding genetic engineering and microorganisms. We highlight the necessity for new research to comprehend biological processes, optimise manufacturing techniques, and find an equilibrium between mechanical properties, degradability and durability across varied applications. We believe that capturing the responsive power of biological matter is key to developing reliable, durable, and sustainable materials and structures. ELMs could solve environmental concerns and surpass traditional composites in properties. This foresees a paradigm shift towards functional composite materials with boundless potential, marking a new era in materials science and engineering.

#### CRedit authorship contribution statement

**I.H.M.S. Nettersheim:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **N.S. Guevara Sotelo:** Writing – review & editing, Investigation, Formal



analysis. **J.C. Verdonk**: Writing – review & editing, Investigation. **K. Masania**: Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Funding acquisition, Formal analysis, Conceptualization.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

No data was used for the research described in the article.

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