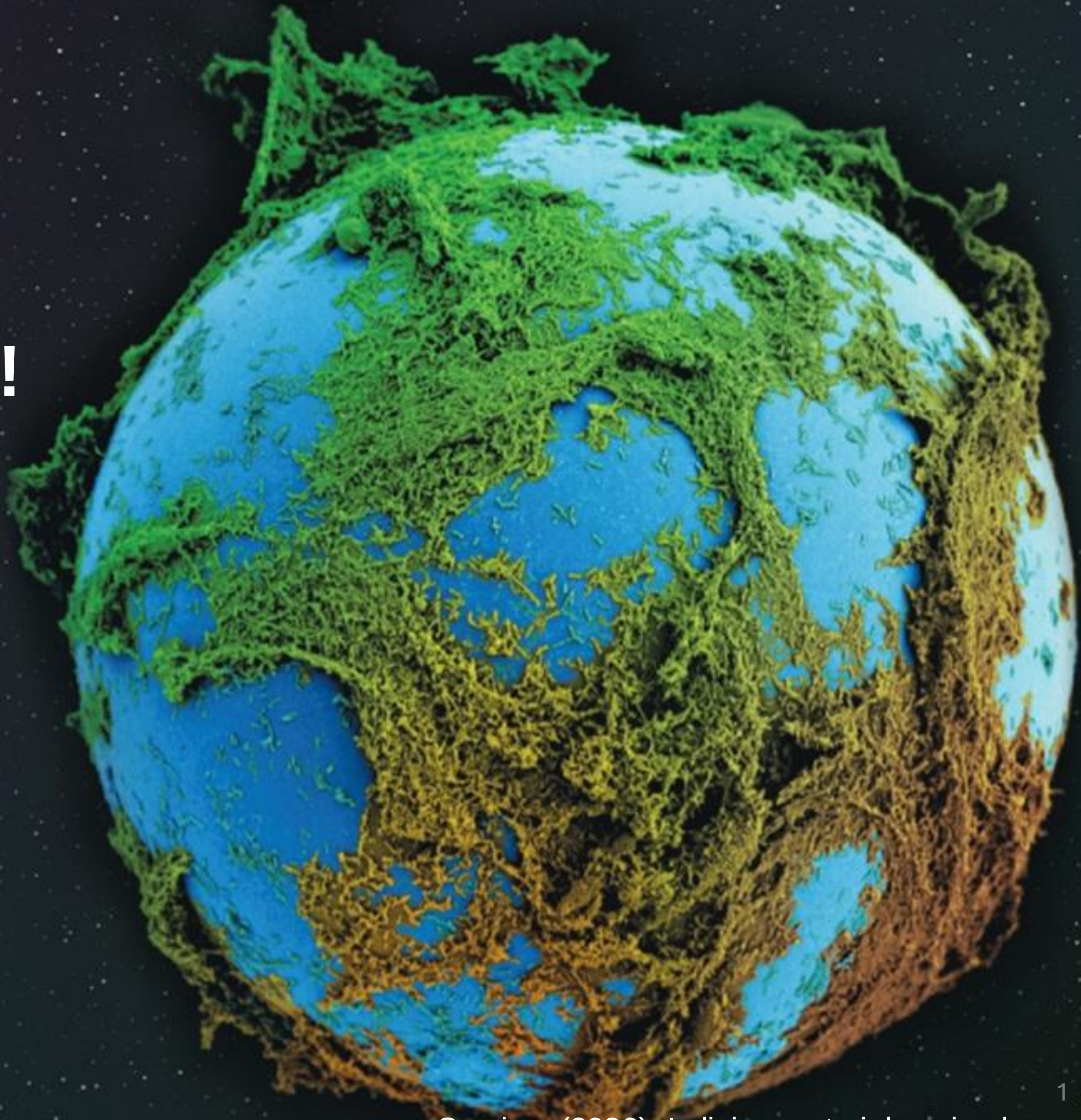
Living materials:
How materials come alive!



Service, (2020). In living materials, microbes are makers

Course Overview

Introduction
- Overview
- Historical Perspective

Challenges
- Human Health
- Environment

Solutions
- Old Polymers
- Emerging Concepts
- Attempts w/Impact

Applications
- Food and Ag
- Electronics and Optics
- Healthcare

Future
- What do we need?
- How do we get there?

Lecture 9-10

Introduction
- Overview
- Historical Perspective

Challenges
- Human Health
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Solutions
- Old Polymers
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Applications
- Food and Ag
- Electronics and Optics
- Healthcare

Future
- What do we need?
- How do we get there?

1. The need

2. Introduction to living materials

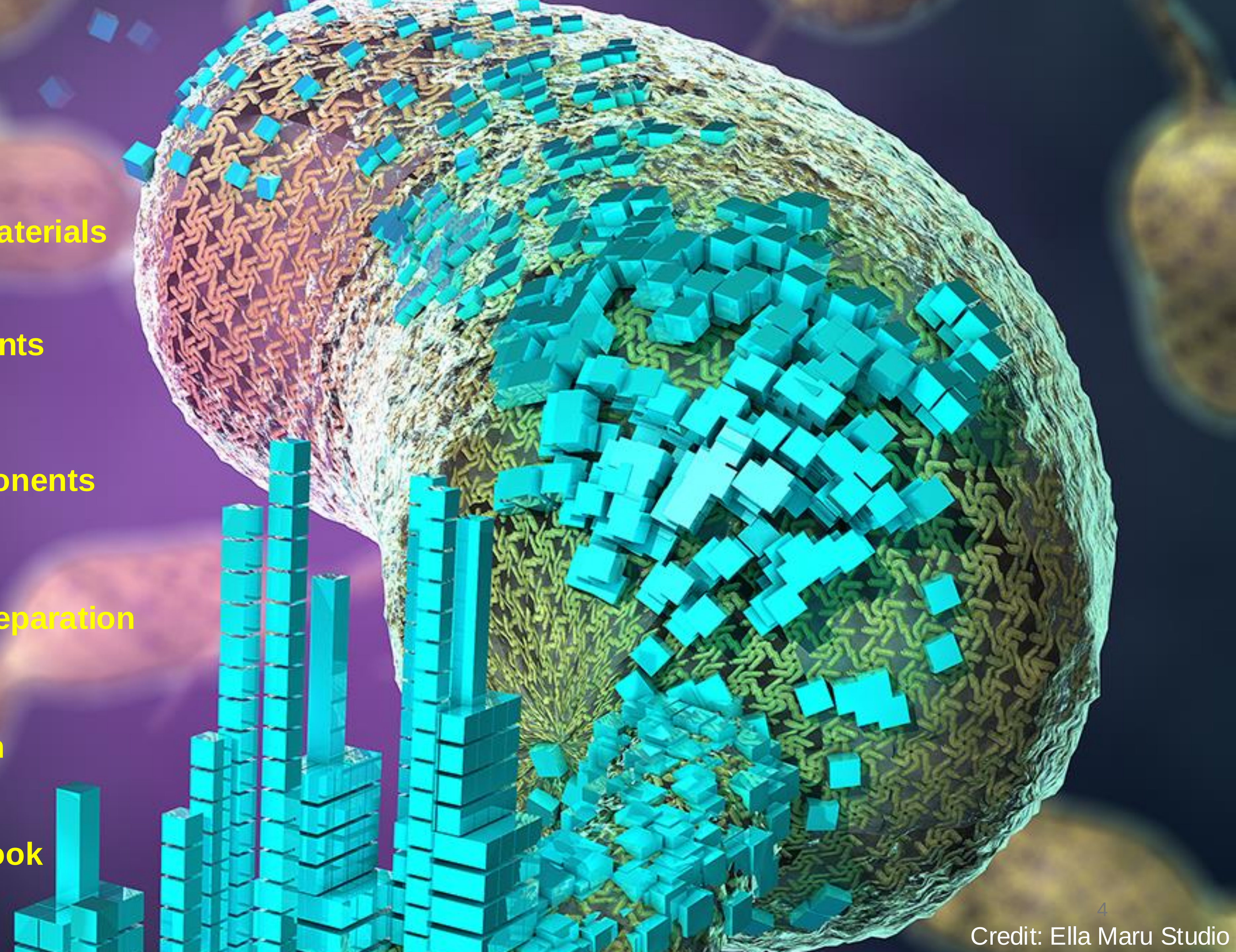
3. Living components

4. Non-living components

5. Living materials preparation

6. Example/Research

7. Limitations and outlook

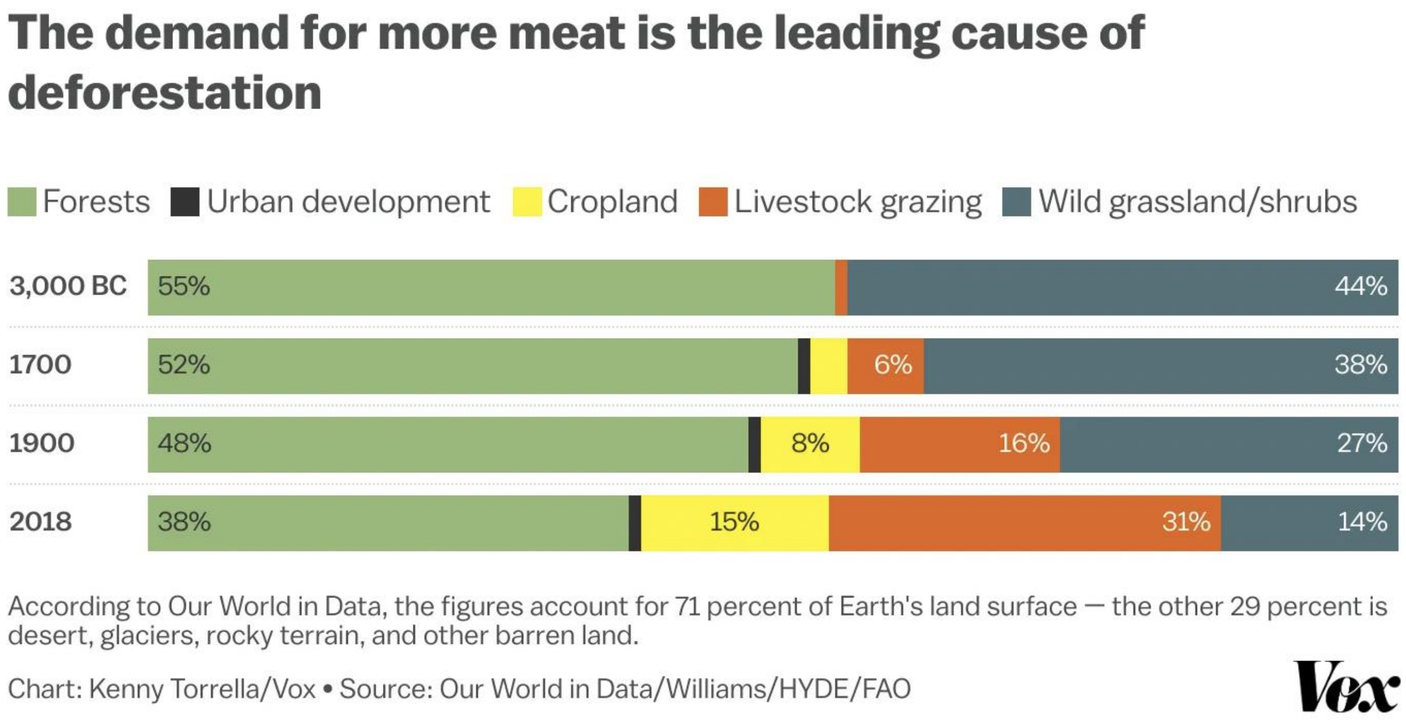


Credit: Ella Maru Studio

Food choices and consumption affect global climate and deplete resources

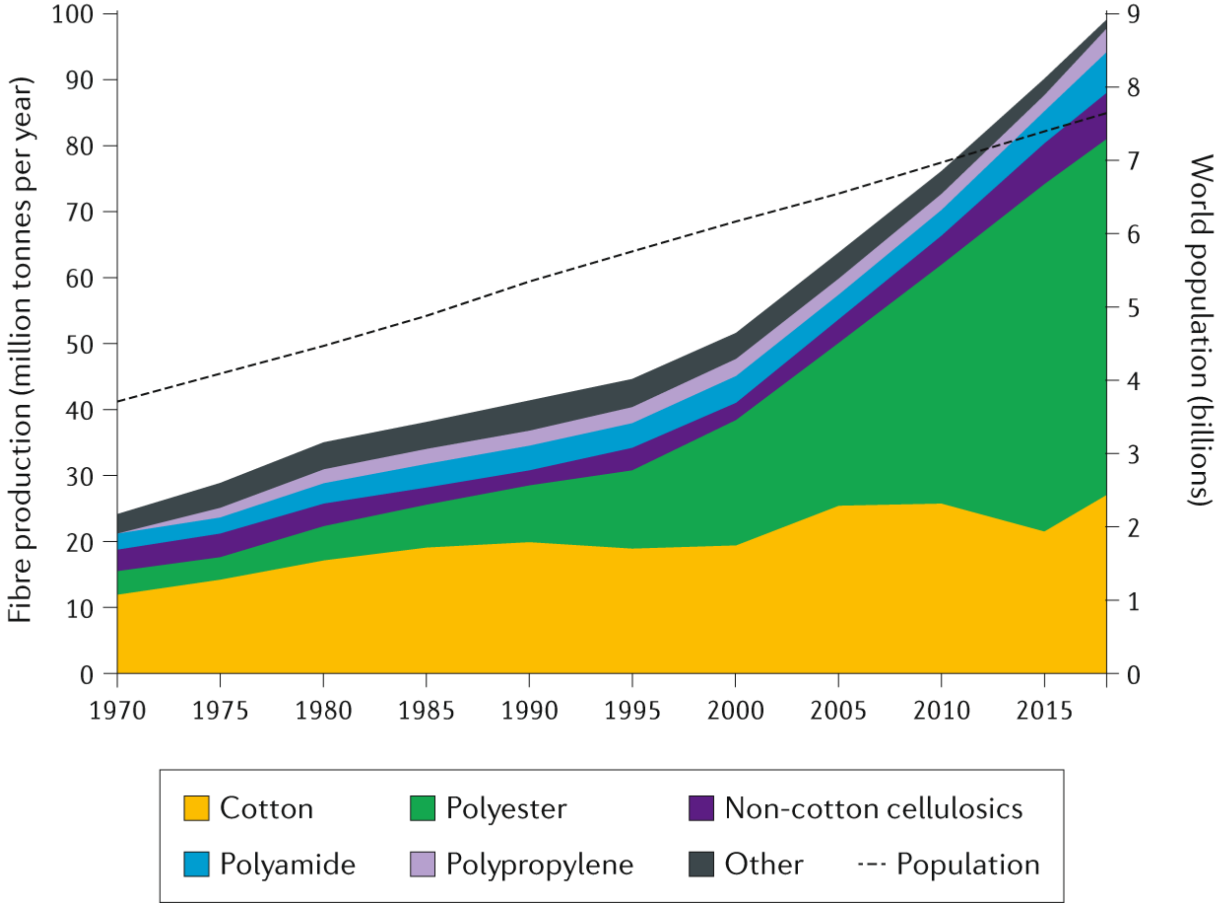
- Over **88 billion land animals** are raised and slaughtered for food production every year

- The animal agricultural industry is responsible for **>16.5%** global greenhouse gas emissions



Getty

Fiber production is increasing exponentially due to population increase AND ***overconsumption***



❖ Each production step has an environmental impact due to water, material, chemical and energy use

Niinimäki, Peters, Dahlbo, Perry, Rissanen, & Gwilt (2020). The environmental price of fast fashion. *Nature Reviews Earth & Environment*

Construction is the largest contributor to landfill waste and climate change


TODAY'S CLIMATE

Concrete is Worse for the Climate Than Flying. Why Aren't More People Talking About It?

Our twice-a-week dive into the most pressing news related to our rapidly warming world.

By Kristoffer Tigue
June 24, 2022

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Why Big Business Could Be the Real Winner at COP28

Protesting at UN Climate Talks Is Becoming Increasingly Difficult, Activists Say

At COP28, More and More Scientists Say Overshooting 1.5

- Cement industry is responsible for ~ **8%** of planet-warming carbon dioxide emissions— **more than global carbon emissions from aviation**

- If the cement industry were a country, it would be the third-largest emitter of carbon dioxide in the world, after the U.S. and China.

Miller & Moore, (2020). Climate and health damages from global concrete production. *Nature Climate Change*

Depleting resources

Global climate change

Waste generation

Naturally derived

Sustainably sourced

Degradable (*Bio*degradable)

Next generation advanced materials
with “smart” functional properties that surpass existing capabilities,

sense environmental cues,

ability to dynamically switch between different material states/ adaptation

self-maintenance

cells can be considered as nanomaterial factories that

constantly sense their environment,

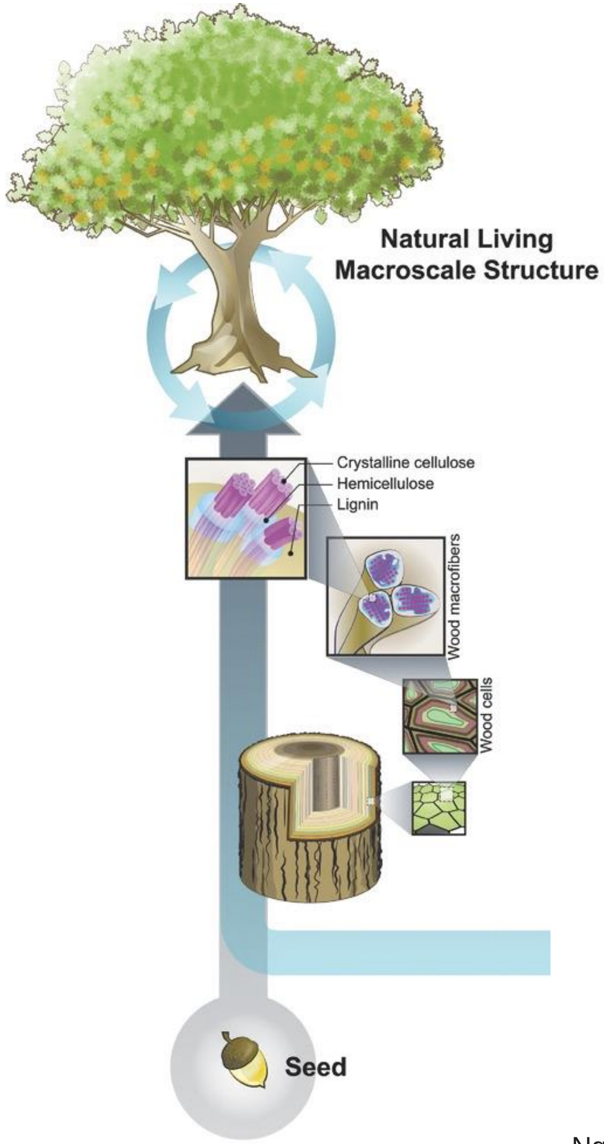
draw from a plethora of energy sources and form simplistic molecular building blocks,

organize these molecules into new structurally and functionally more complex materials, and

maintain these materials over time.

Solution: Look to nature for inspiration about how to build the future of materials

Trees: Ultimate inspiration for the living materials



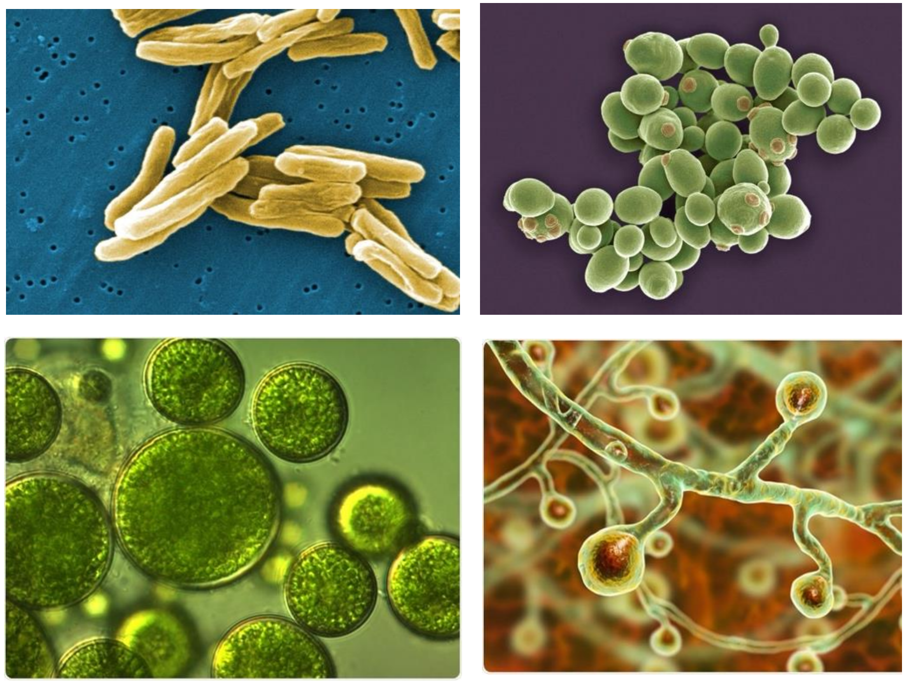
Maintenance and Renewal	Self-repair
Self-organization/ assembly	Self-assembly
Morphogenesis	Growth/ synthesis
Sunlight Water Energy/ carbon sources Nutrients	Responsiveness

Nguyen, P. Q., Courchesne, N. M. D., Duraj-Thatte, A., Praveschotinunt, P., & Joshi, N. S. (2018).. *Advanced Materials*, *30*(19), 1704847.

Living materials are composites made of living and non-living components

Living Material

Living



Non-living

Biotic source

Abiotic source

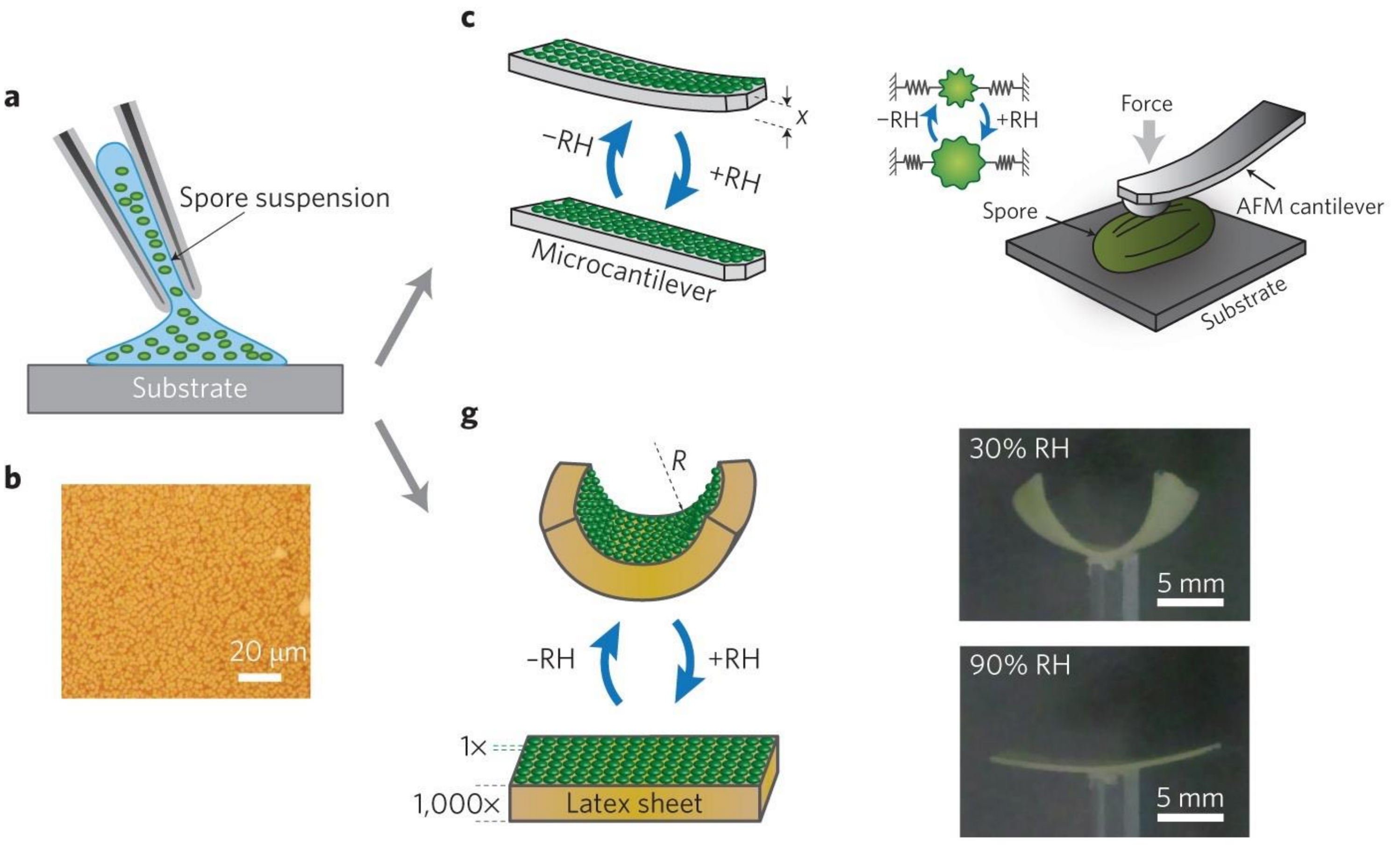
Bio-hybrid materials

A biologically derived component and a synthetic component.

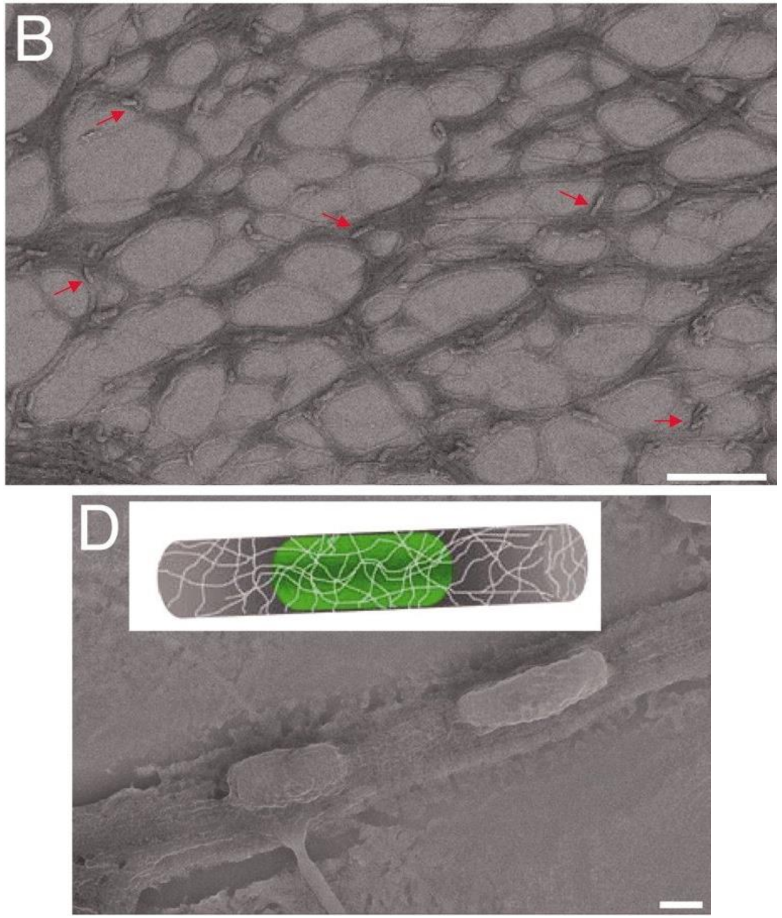
The biological component may be purified biomolecules, such as proteins or DNA, or living cells.

The synthetic component could be organic or inorganic polymers, minerals, ceramics, or even metals.

Bio-hybrid materials (examples)



Stimuli-responsive materials and nanogenerators



A bacterium encapsulated in the cross-linked FDMA fibers.

Chen, X., Mahadevan, L., Driks, A., & Sahin, O. (2014). Bacillus spores as building blocks for stimuli-responsive materials and nanogenerators. *Nature nanotechnology*, *9*(2)

Liu, Y., Rafailovich, M. H., Malal, R., Cohn, D., & Chidambaram, D. (2009). Engineering of bio-hybrid materials by electrospinning polymer-microbe fibers. *Proceedings of the National Academy of Sciences*, *106*(34)

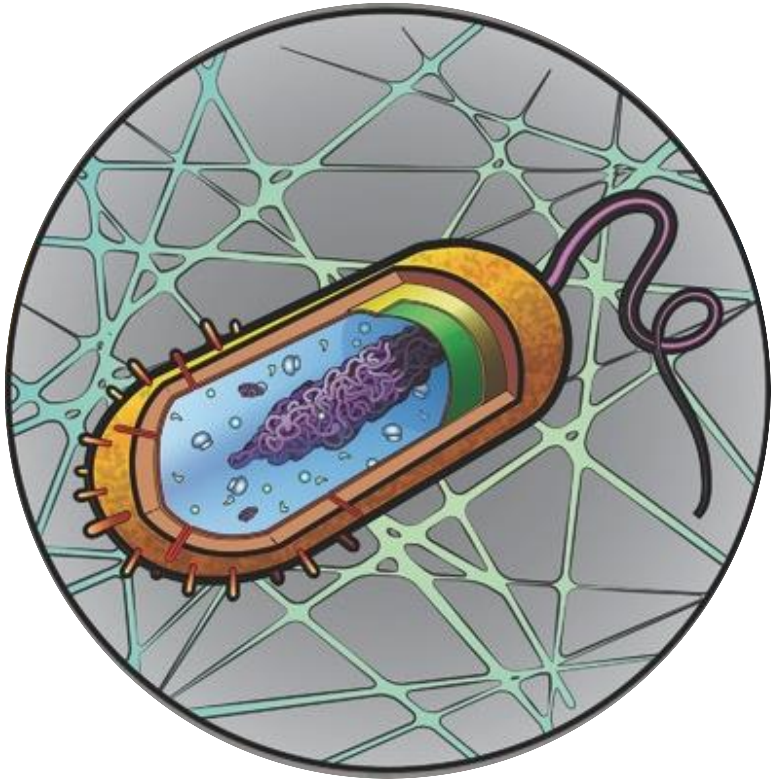
Biohybrid materials v/s Living materials

Bio-hybrids

Bio-component: cell-derived or includes living cells, is just one element of the engineered structure and does not actively create or modulate the bulk structure of the material (less biological system)

Living materials

the living cells act as materials factories, drawing upon energy feedstocks from their environment to create biopolymeric building blocks and direct the formation of, or maintain, the desired material



Nguyen, P. Q., Courchesne, N. M. D., Duraj-Thatte, A., Praveschotinunt, P., & Joshi, N. S. (2018). Engineered living materials: prospects and challenges for using biological systems to direct the assembly of smart materials. *Advanced Materials*, *30*(19), 1704847.

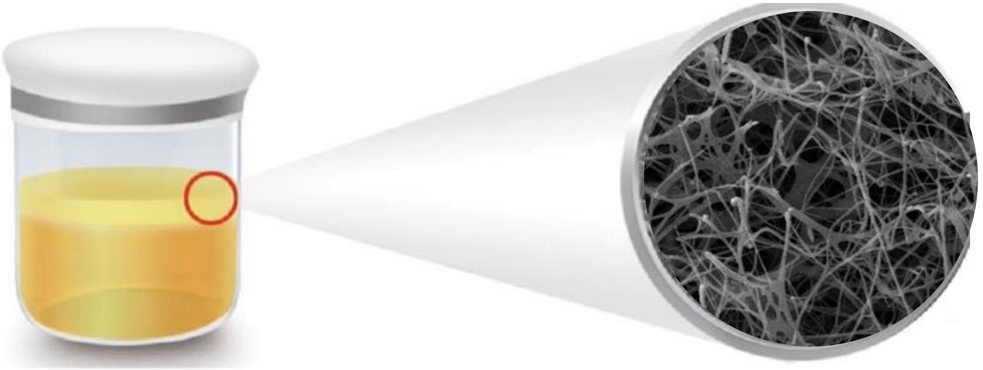
Living materials for sustainability

https://vimeo.com/327830046

Organisms can bio-synthesize **specific metabolites** and **biopolymers** for survival

Beta carotene, biosynthesized by algae

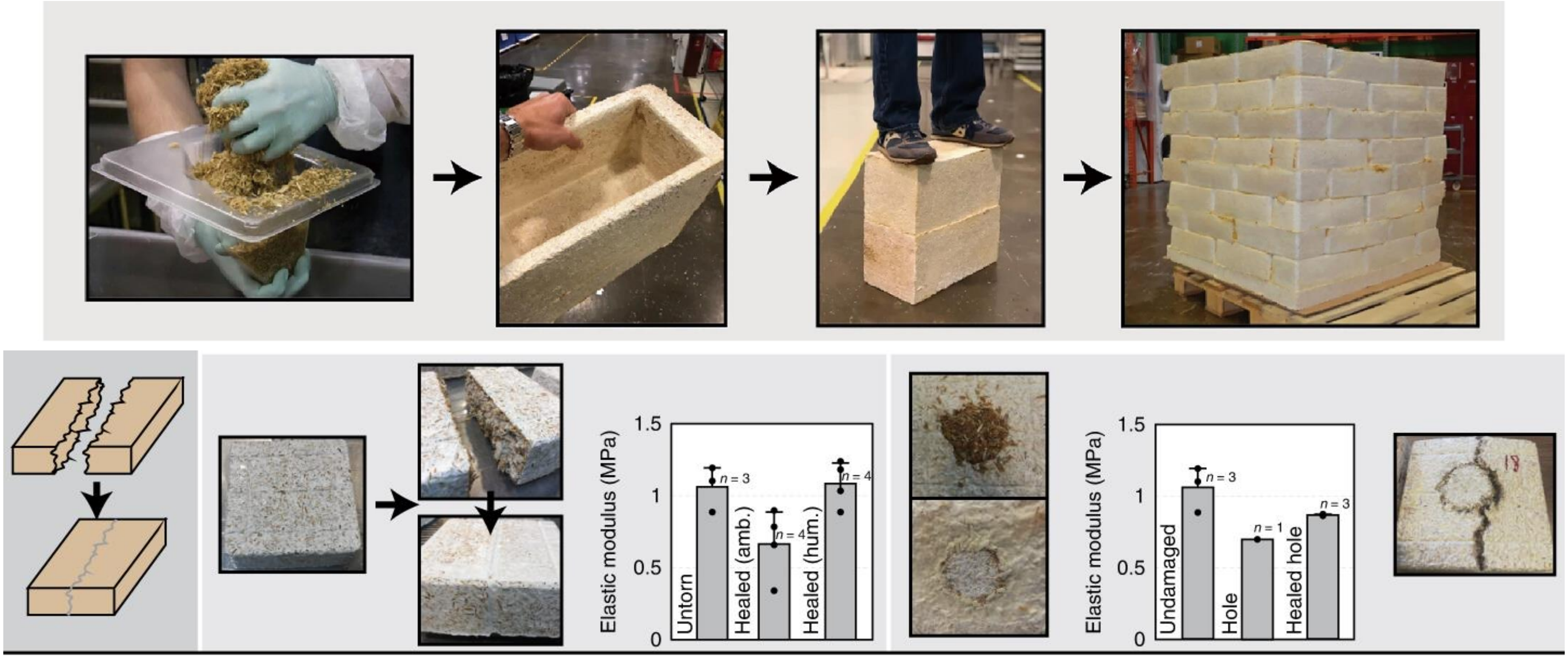
Examples:
- Pigments
- Structural/functional polymers (polysaccharides/ lignin/nucleic acids/ proteins)
-Metabolites
-energy components (diesel and ethanol)



Fibre structural organization in cellulose film

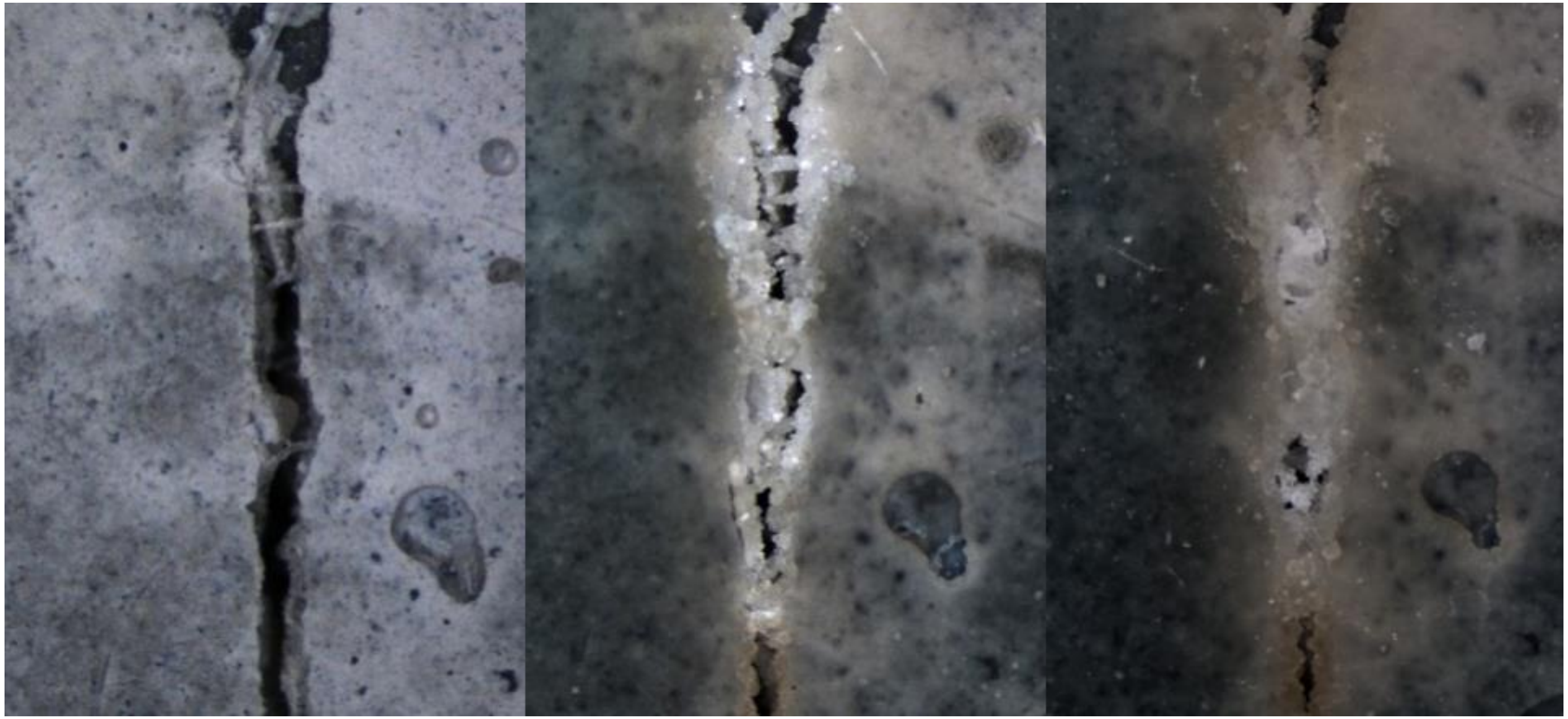
Living organisms can **grow** and **reproduce** in programmable shapes

Geoderma growth in the molds to make bio-bricks



McBee, R. M., Lucht, M., Mukhitov, N., Richardson, M., Srinivasan, T., Meng, D., ... & Wang, H. H. (2022). Engineering living and regenerative fungal–bacterial biocomposite structures. *Nature Materials*

Organisms can **self-repair damage and restore** functions



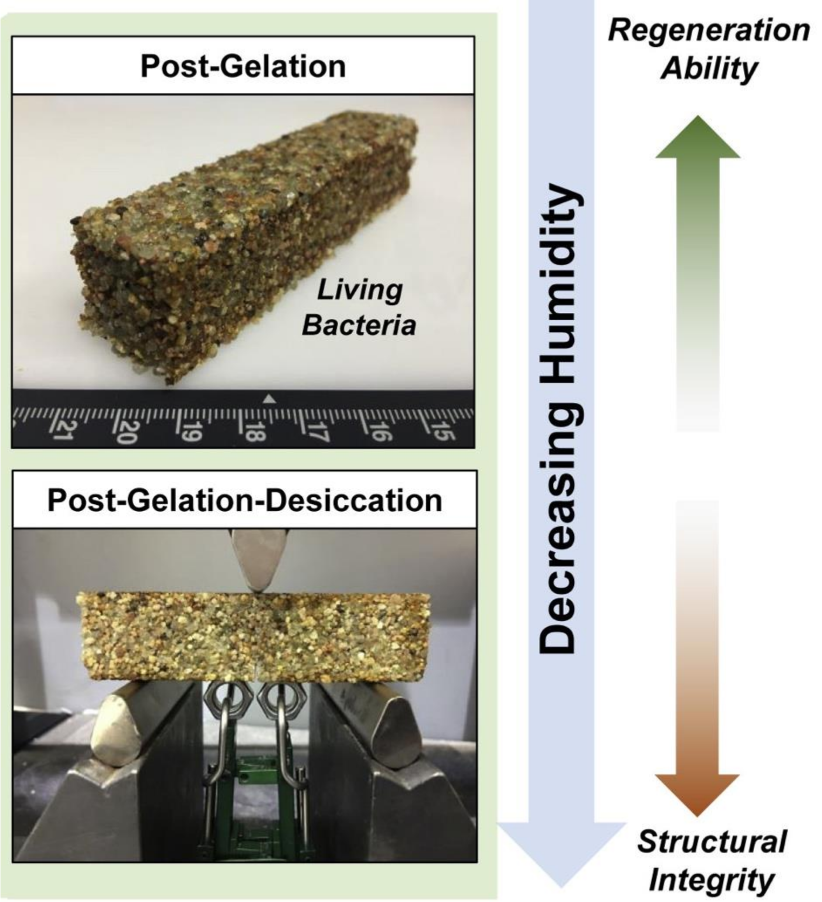
Bacteria embedded in the concrete produce limestone that repair cracks

Image Courtesy of Delft University

Cells and biopolymers can **self-organize** into ordered structures

Turing patterns occur in nature due to self-organization of the cells

Organisms **perceive** the external environmental stimuli and **respond**



Responsiveness in the living systems can help design living materials adaptable to external stimuli (pH, temperature, humidity, damage, etc)

Heveran, Williams, Qiu, Artier, Hubler, Cook, & Srubar (2020). Biomineralization and successive regeneration of engineered living building materials. *Matter*

Materials with living components have properties which enable the new age applications

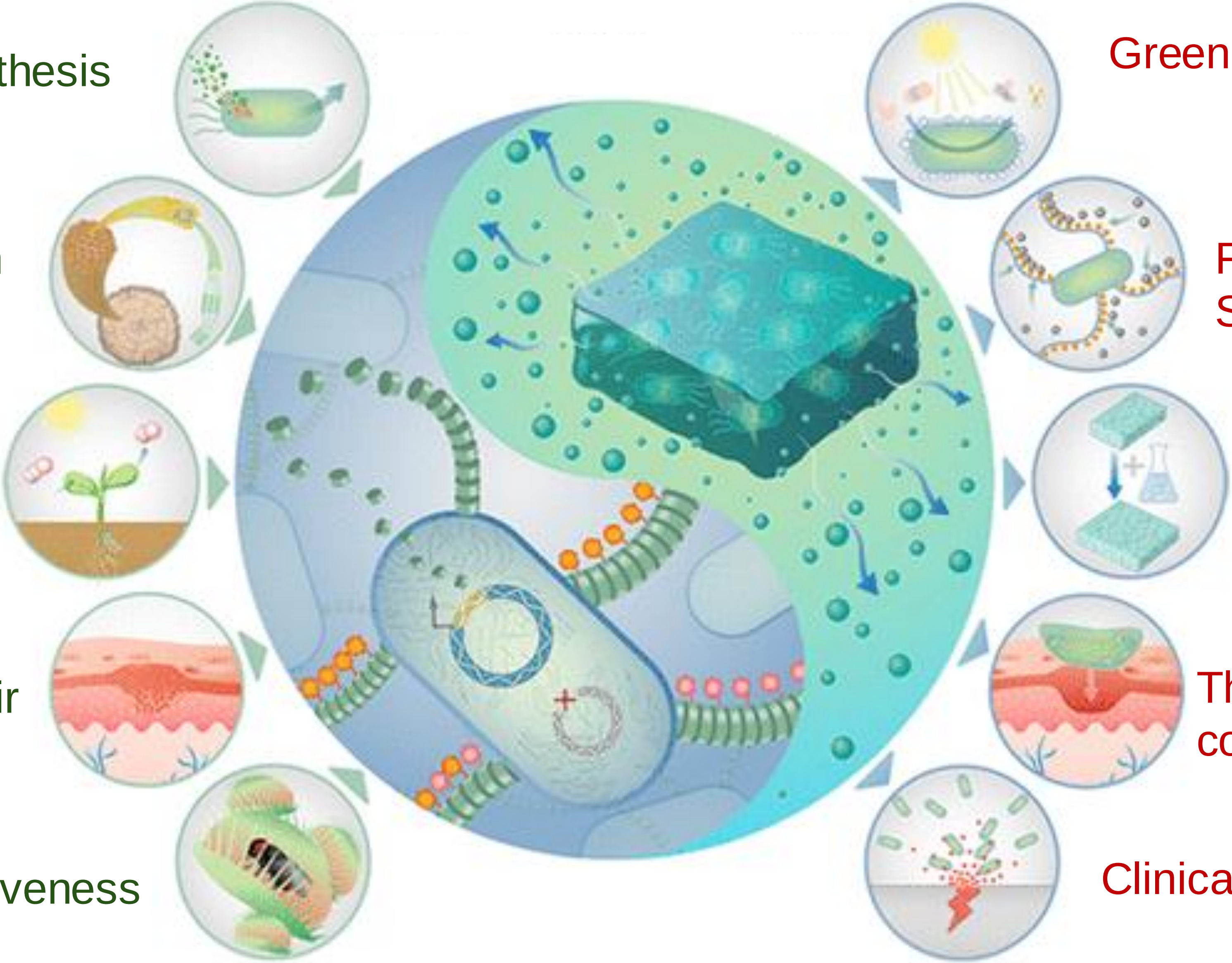
Biosynthesis

Self-organization

Self-growth

Self-repair

Responsiveness



Green energy/polymers

Functionalized materials/ SCOBY

Bio-brick

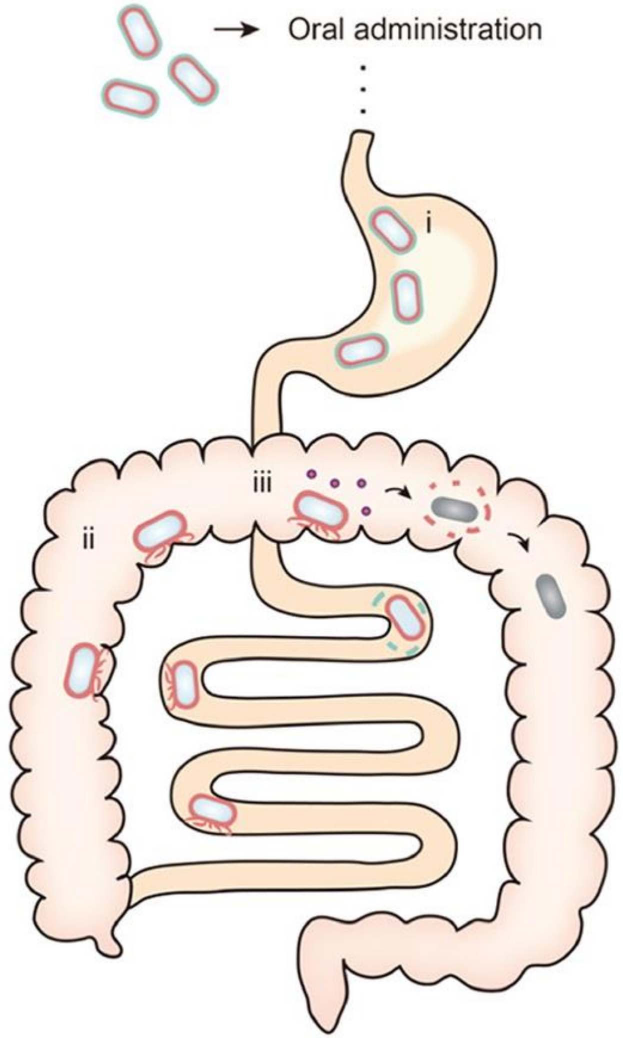
Therapeutic treatment/ construction

Clinical monitoring/ remediation

An, Wang, Huang, Wang, Liu, Xun, Zhong (2022). Engineered living materials for sustainability. *Chemical Reviews*

Living cells suffer limitations when on their own

- Lack of support and direction for growth
- More susceptible to abiotic factors (pH, temperature, salinity, humidity, etc)
- Weak mechanical strength in comparison to synthetic counterparts
- Less stability for longer duration (low shelf-life)

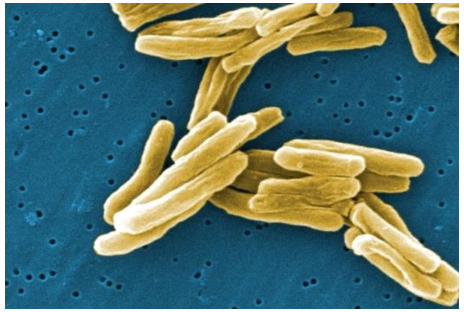
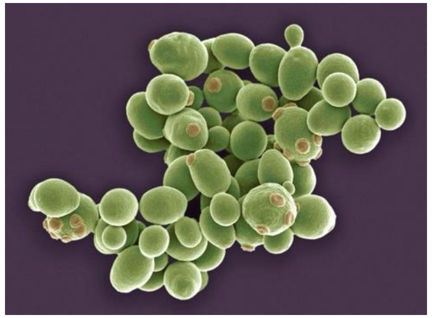
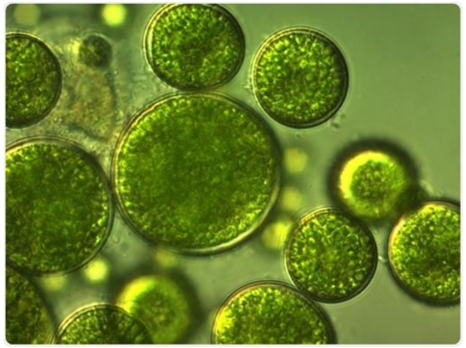


Liu, Li, Wang, Ding, Lee, Hu, Biomaterials Coating for On-Demand Bacteria Delivery: Selective Release, Adhesion, and Detachment. *Nano Today* **2021**

Living materials are composites made of living and non-living components

Living Material

Living


Non-living

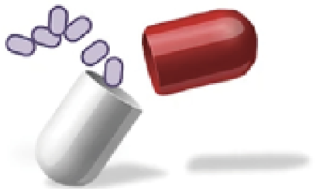
Biotic source

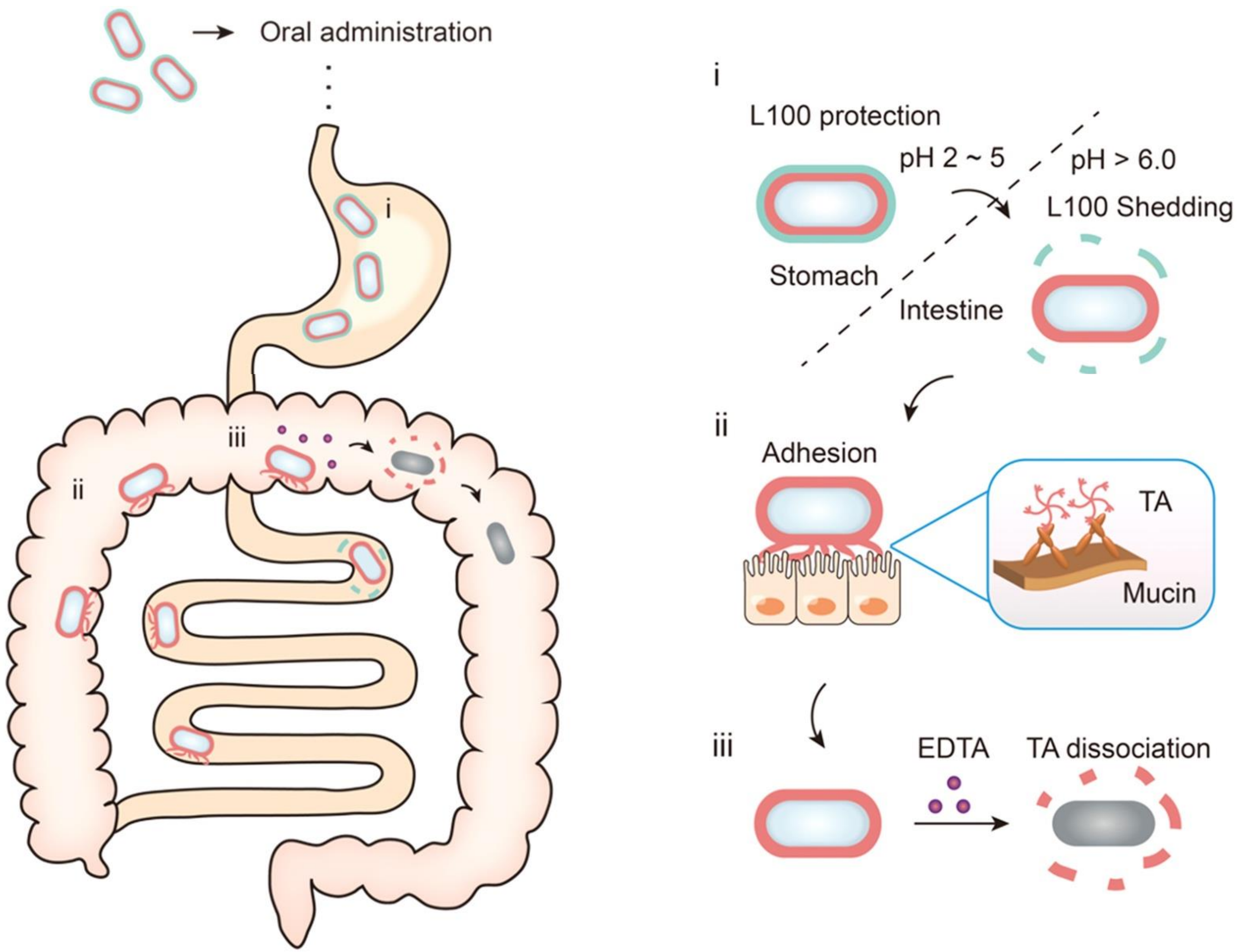


Abiotic source



Materials Coating for on-demand bacteria delivery





(i) Eudragit L100 polymer protects the living cells (*E.coli*) against low pH stomach environment

(ii) Tannic acid increases the retention of probiotics in the intestinal tract

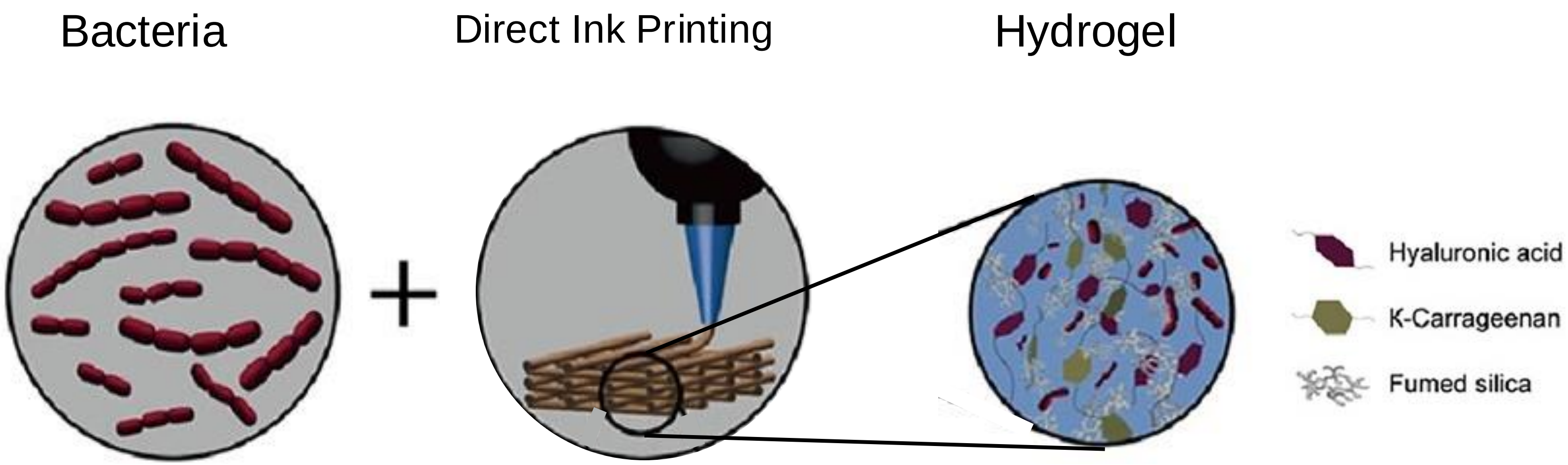
(iii) Ethylenediaminetetraacetic acid (EDTA) enables the on-demand removal of TA coatings from probiotics

Non-living components must meet certain criteria to sustain living materials

Requirements:

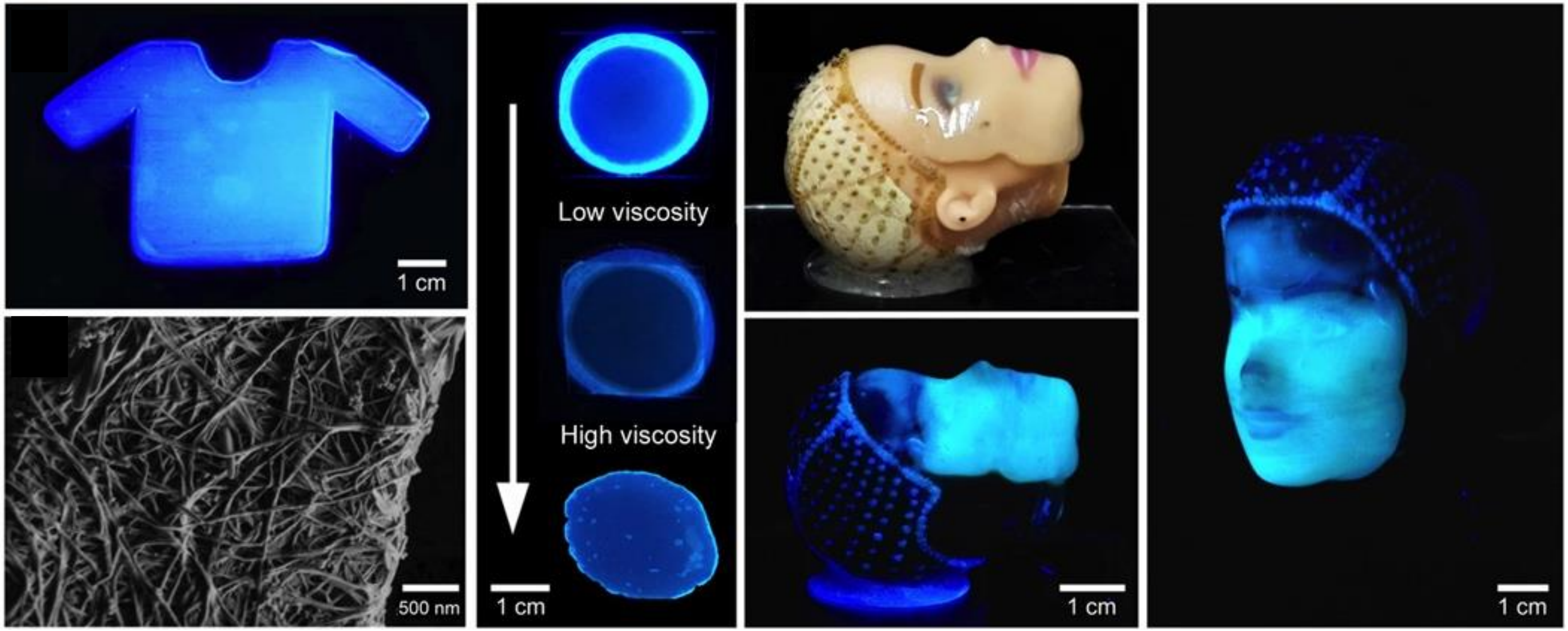
- Biocompatibility
- Cell adhesion
- Mechanical strength
- Oxygen permeability (aerobes)
- Low cost
- Easy availability
- Controlled Biodegradability
- Responsive
- Easy to prepare

- Functional living ink (Flink) made by combining HA and κ-carrageenan as natural viscoelastic gel components
- Ink blended with *Acetobacter xylinum* capable of producing medically relevant cellulose



Schaffner, Rühs, Coulter, Kilcher, Studart, 3D Printing of Bacteria into Functional Complex Materials. *Sci. Adv.* **2017**,

In situ formation of bacterial cellulose by *A. xylinum* in different shapes



High viscosity= less oxygen

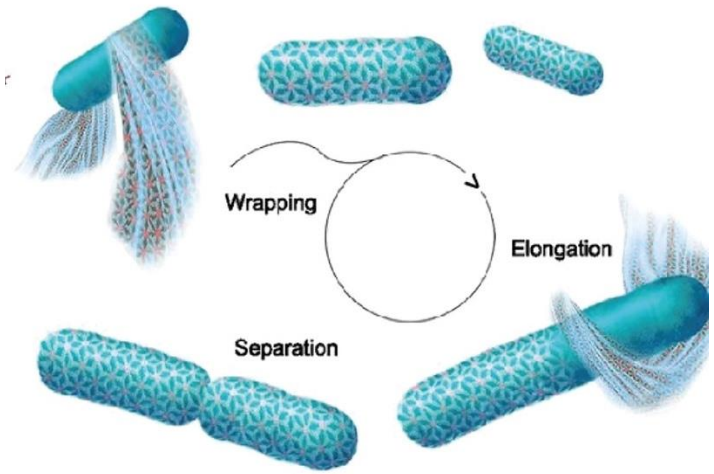
Non-living components must meet certain criteria to sustain living materials

Requirements:

- Biocompatibility
- Cell adhesion
- Mechanical strength
- Oxygen permeability
- Low cost
- Easy availability
- Controlled Biodegradability
- Responsive
- Easy to prepare

- Applied Metal organic framework (MOF) to protect the physiological activity of strictly anaerobic bacteria, *Morella thermoaceticabacteria*, in the presence of oxygen

MOF wrapping during life cycle

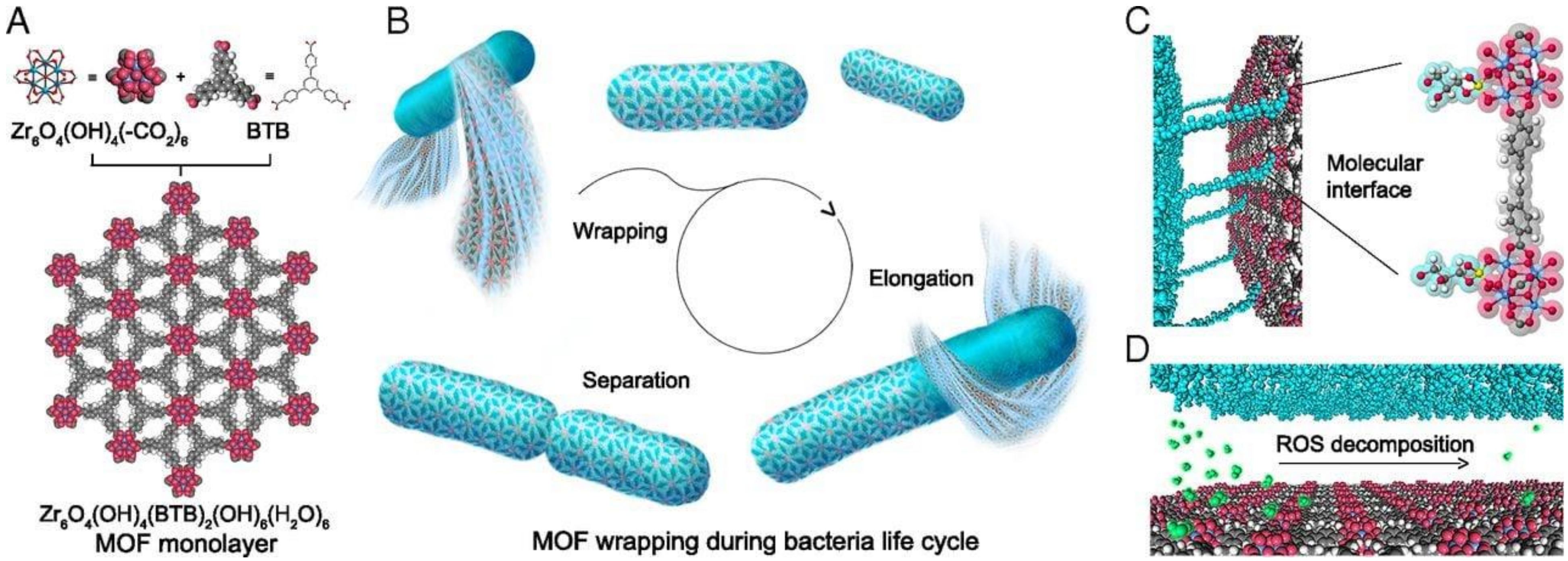


Ji, Zhang, Liu, Yaghi, Yang, Cytoprotective Metal-Organic Frameworks for Anaerobic Bacteria. *Proc. Natl. Acad. Sci. U.S.A.* **2018**

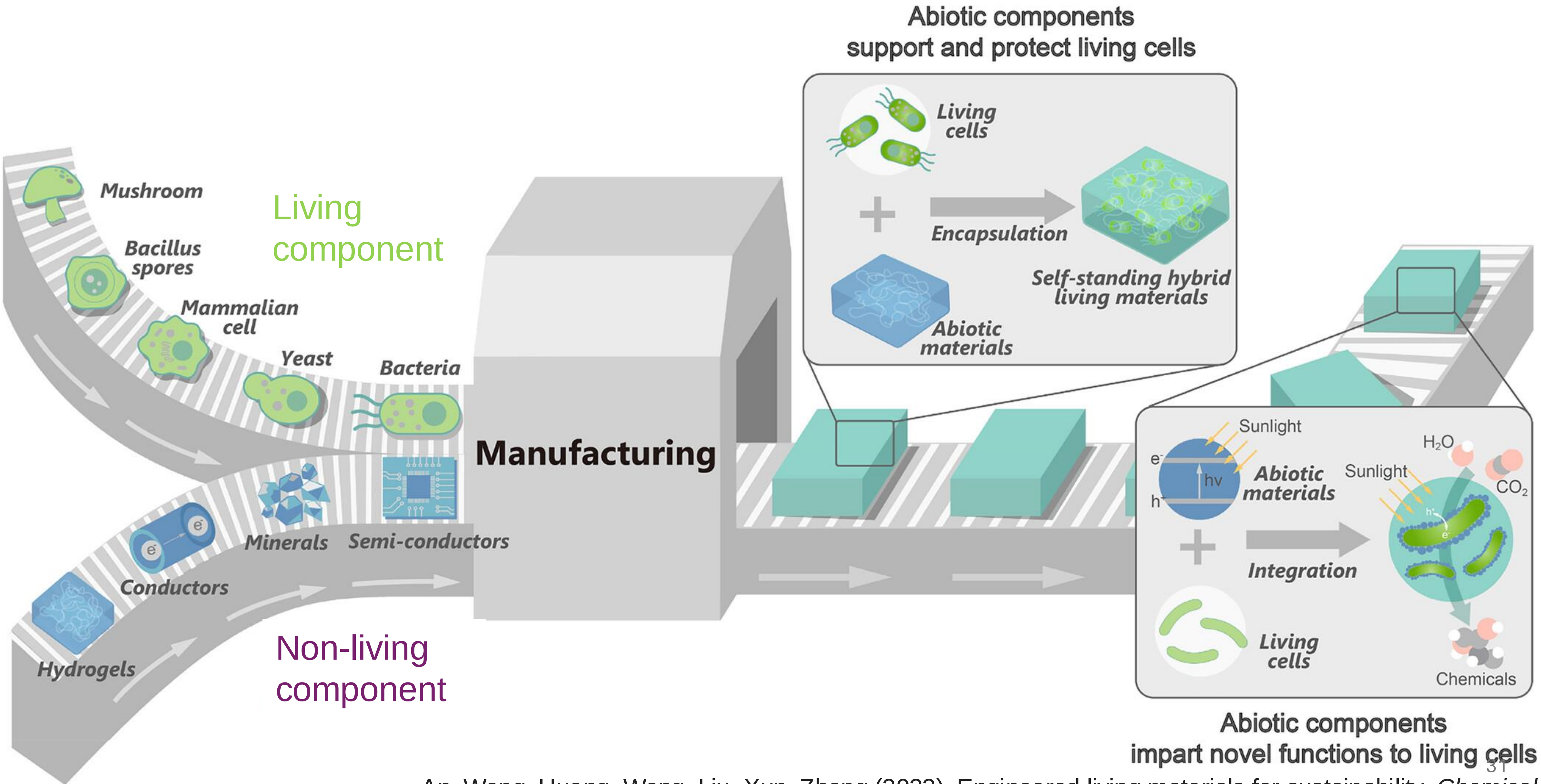
Semiconductor-sensitized anaerobic bacteria (*Moorella thermoacetica*) with a monolayer MOF, CO_2 was converted to acetate twice as long as that observed without such wrapping.

The fact the bacteria we report here were wrapped with only 1–2-nm MOF layer and the bonds at the bacteria–MOF interface are dynamic, leads to facile reproduction and maintains protection against oxidative stress.

It is worth noting that the excess MOF in the culture media can wrap over newly grown cell surfaces to pass on this protection over generations of anaerobes.

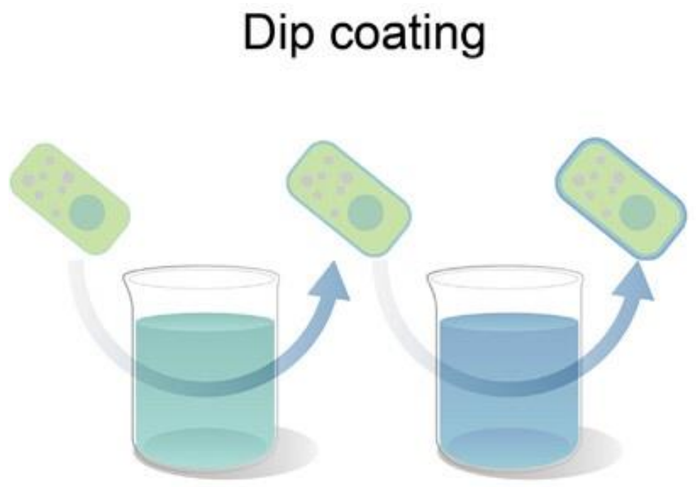


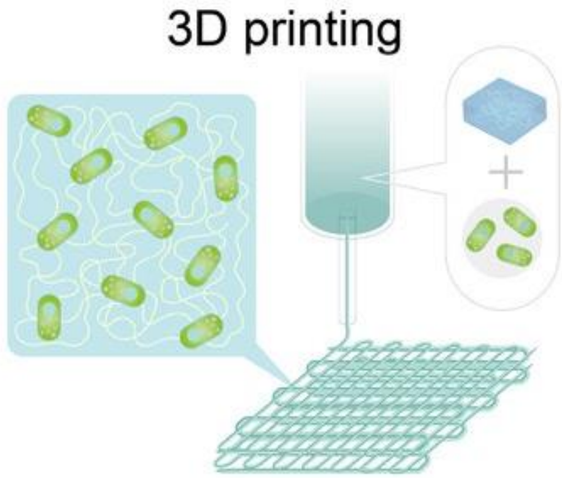
The manufacturing of the living material determines which functionalities are conferred

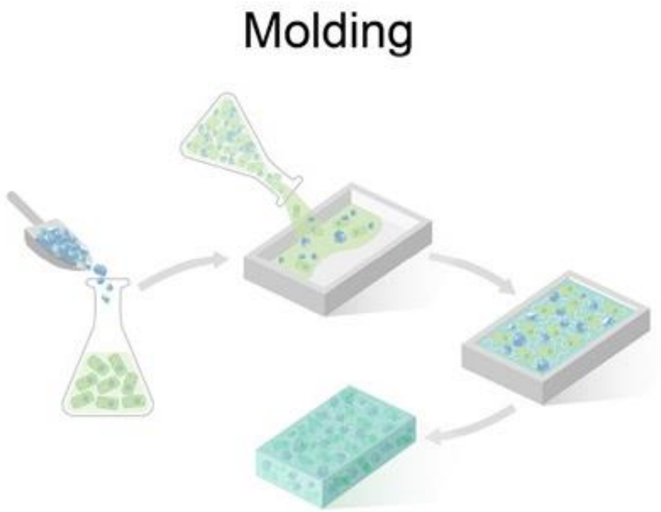


An, Wang, Huang, Wang, Liu, Xun, Zhong (2022). Engineered living materials for sustainability. *Chemical Reviews*

Many manufacturing techniques can be used to facilitating the production of hybrid living materials







- Dip-coating is a **surface modification** method
- Living cells are immersed in a non-living solution
- Coatings attach firmly to the substrate via **covalent** or **noncovalent** forces

- Nozzle or inkjet head deposits **bioink** (living + non-living material) on substrate
- Uses **soft ink** ingredients such as hyaluronic acid, silk fibroin, gelatin, etc

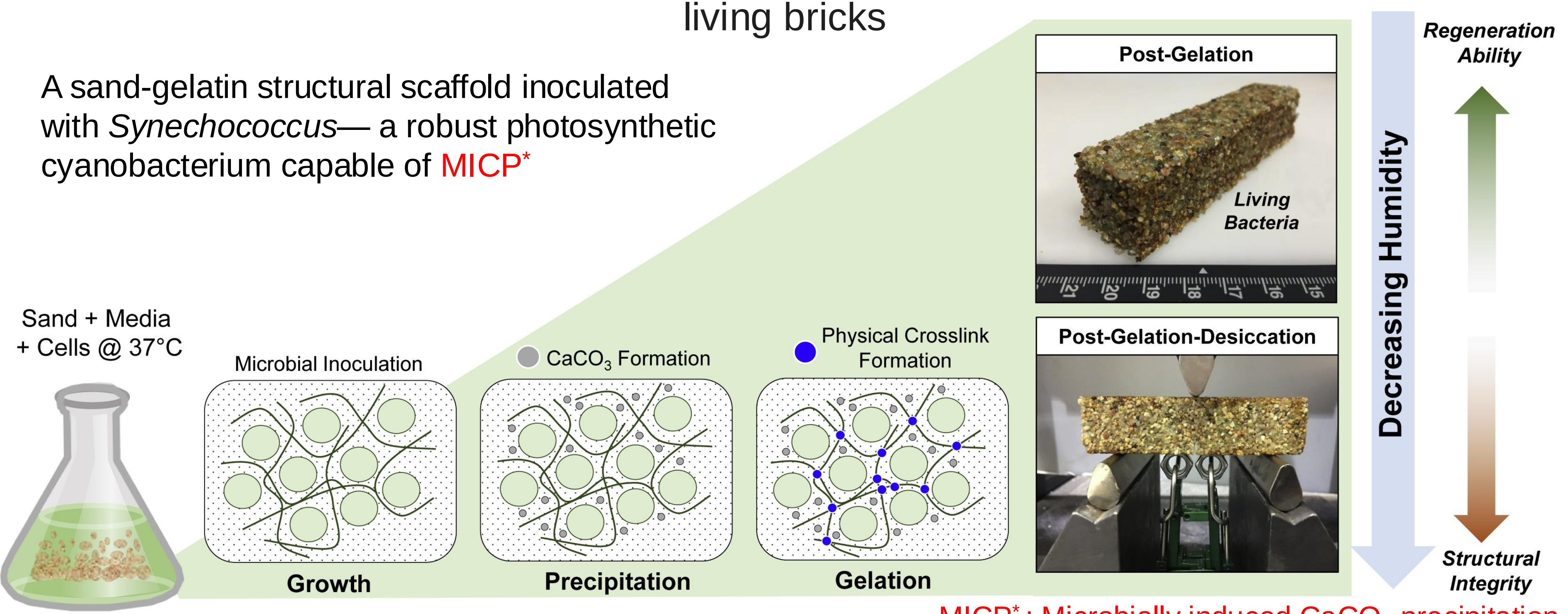
- Shaping polymer gels, plastics, glass, and ceramics, using **predesigned cavity containers**
- Preferred approach for **large architectures** for mass production

An, Wang, Huang, Wang, Liu, Xun, Zhong (2022). Engineered living materials for sustainability. *Chemical Reviews*

Living building materials

Example: Sand mixed with $CaCO_3$-producing bacteria form responsive, regenerating, living bricks

A sand-gelatin structural scaffold inoculated with *Synechococcus*— a robust photosynthetic cyanobacterium capable of MICP*



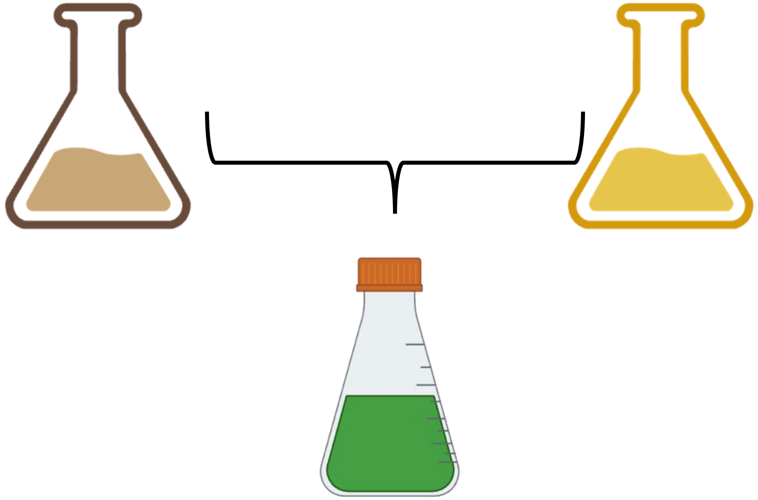
MICP*: Microbially induced $CaCO_3$ precipitation

Heveran, Williams, Qiu, Artier, Hubler, Cook, & Srubar (2020). Biomineralization and successive regeneration of engineered living building materials. *Matter*

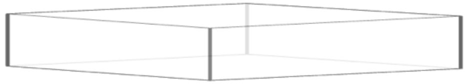
Preparation of living building materials (LBMs)

Abiotic media (50 mL)
(Minimal nutrients/ gelatin)
(High temperature)

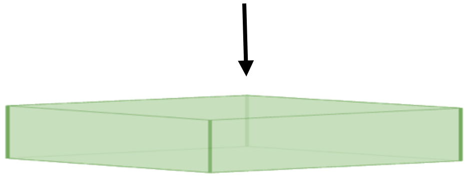
Synechococcus
(Photosynthetic cyanobacterium)



Mix with sand
(for 6 hours at 37 degrees, suitable for the cyanobacterium and gelatin is liquid)



Pour in a mould with the volume ~ 50 mL



Bio-brick stored at low temperature (~4°C)

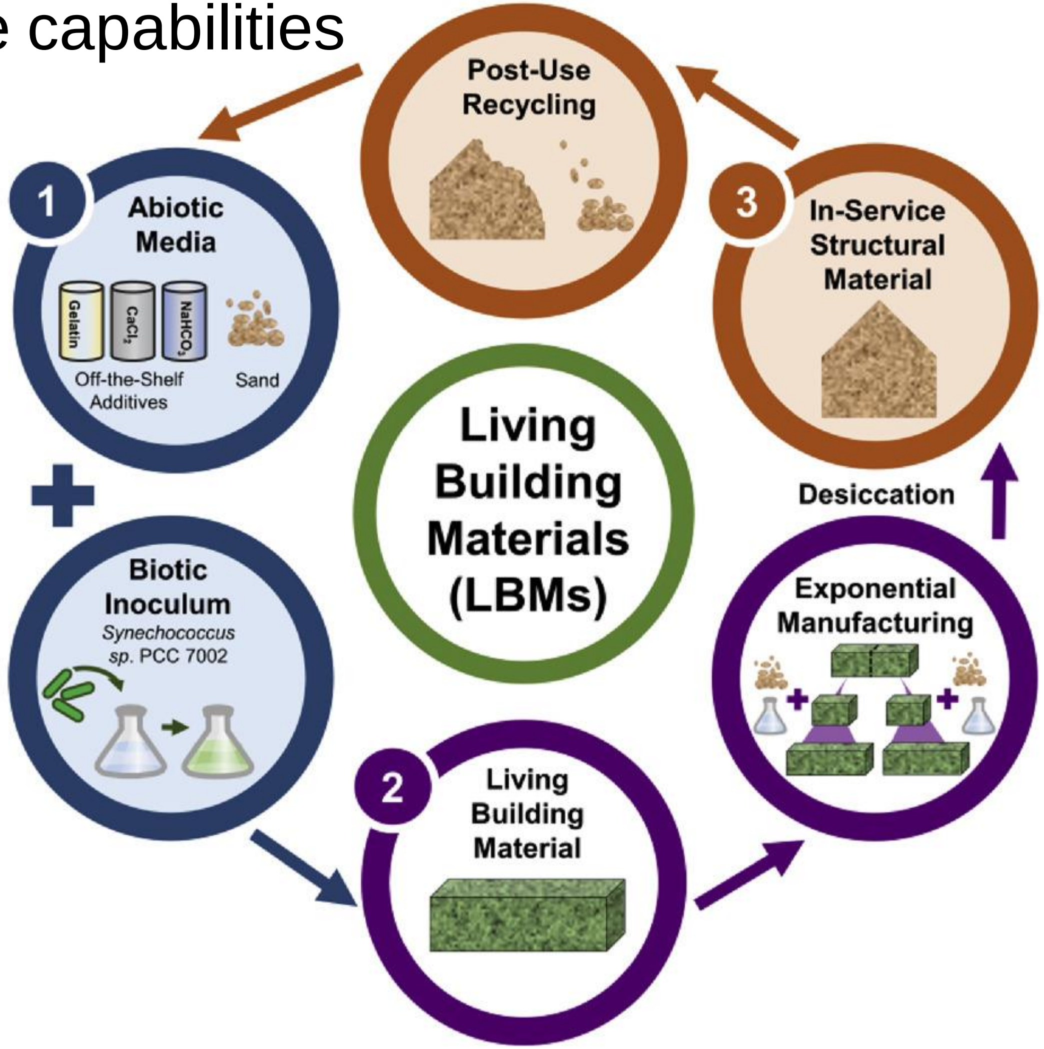
Switches:

- The viability and regenerative potential of Synechococcus were enabled by the use of temperature and humidity (i.e., rehydration) switches.
- The first high-temperature switch corresponded to the incubation and growth temperature (37°C), which was sufficient to dissolve the gelatin matrix and encourage bacterial metabolic activity and mineral precipitation.
- The low-temperature switch corresponded to the storage temperature (4°C). At this temperature, the gelatin matrix effectively encapsulated the cyanobacteria and medium to form a solid LBM.
- during LBM regeneration, the addition of new liquid abiotic media and higher temperatures were the high-humidity and high-temperature switches that rekindled metabolic activity.
- Material systems that protect ureolytic microorganisms with encapsulating gels or other solid media generally require physical damage to the encapsulant in order to trigger additional biomineralization

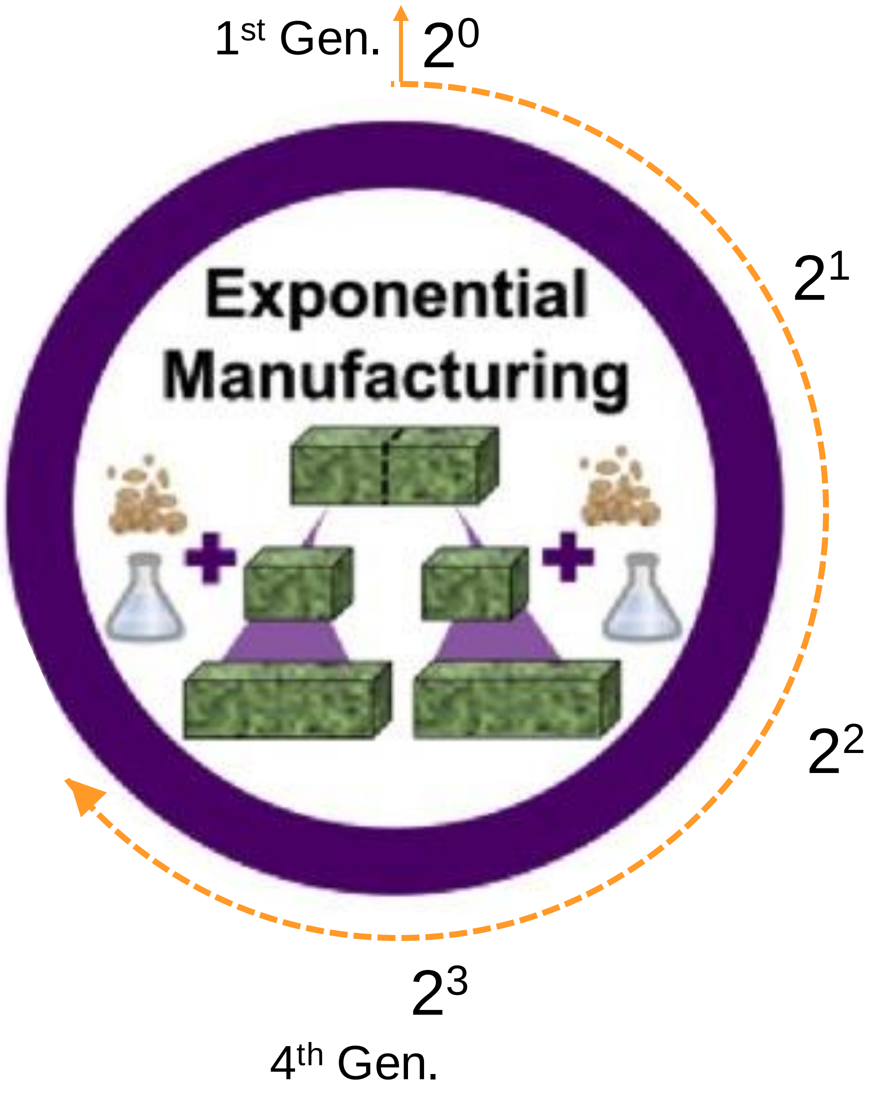
Engineered living materials enable next-generation **exponential manufacturing** due to their regenerative capabilities

- These materials are capable of exponential regeneration of the living component in response to **physical switches** (i.e. humidity, temperature)

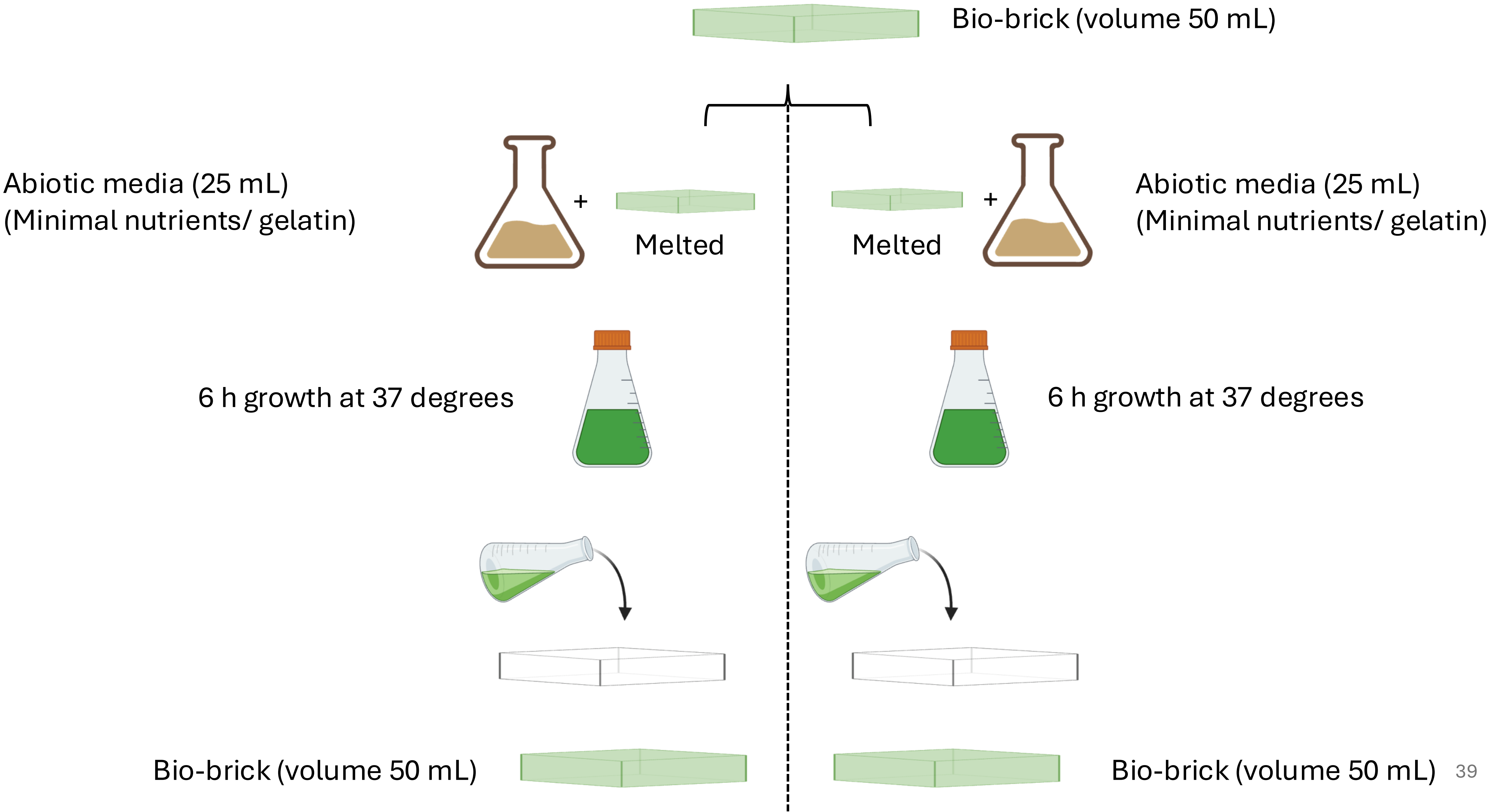
- Microorganism-precipitated calcium carbonate conferred **high fracture toughness** to the Living Building Materials (LBMs)



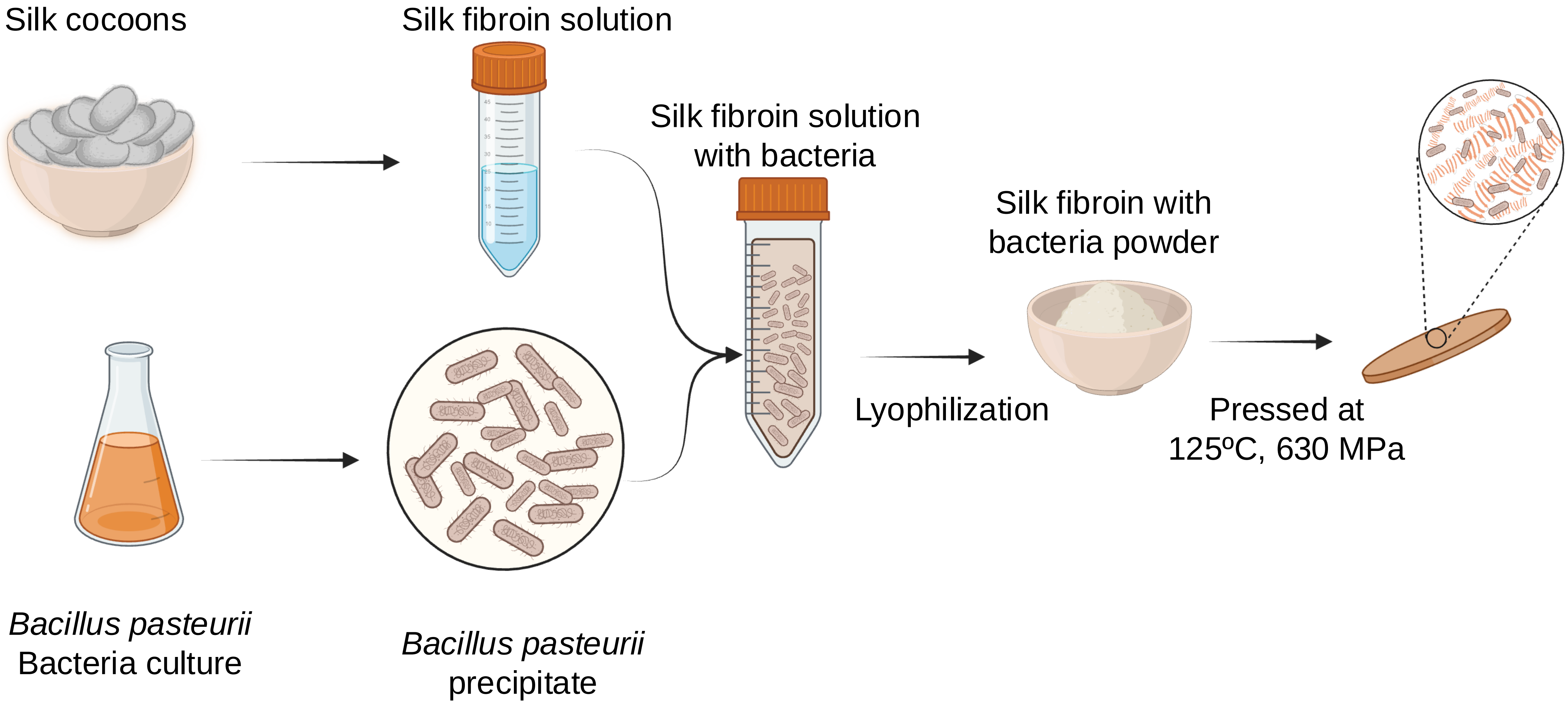
Successive regeneration is observed under physical switches



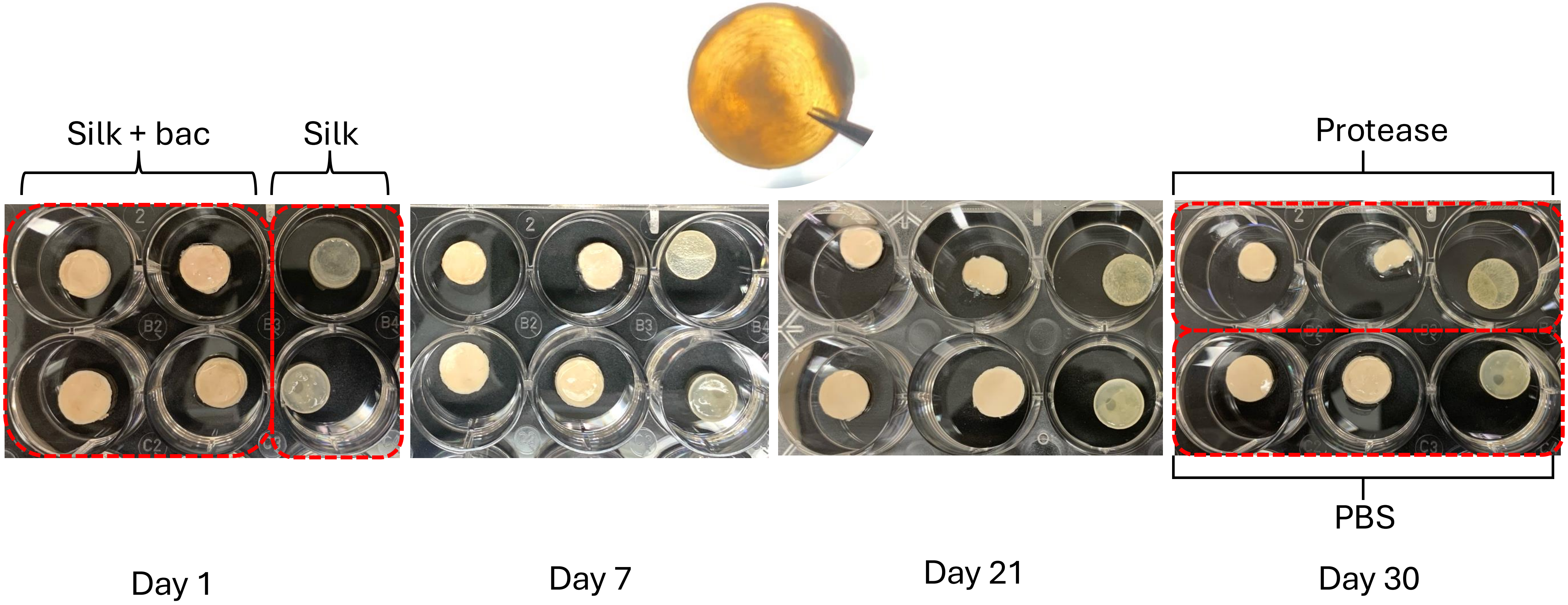
Regeneration of LBMs (exponential growth)

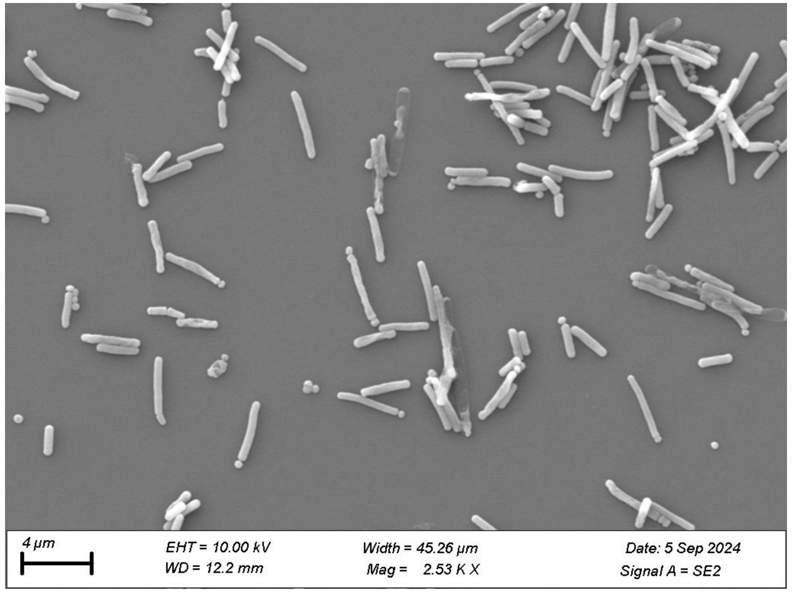


My research on mechanically superior silk plastics using calcifying bacteria



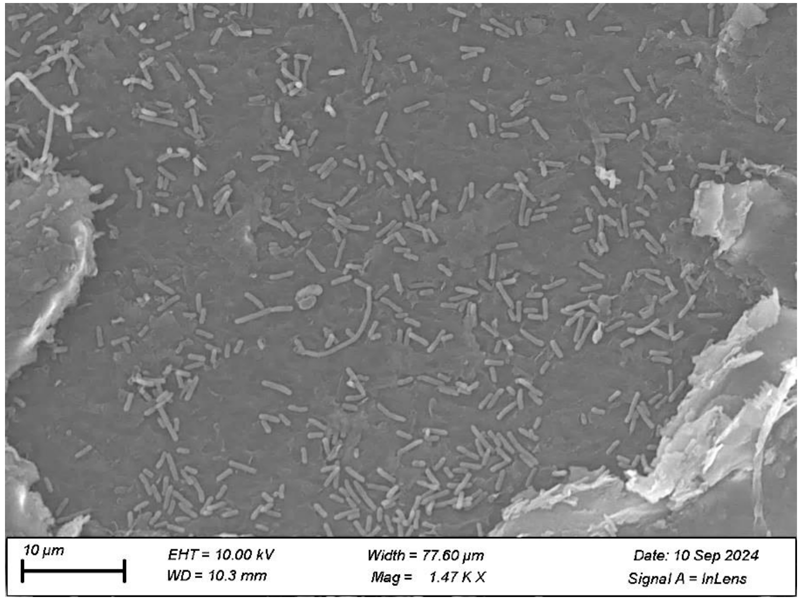
Protease based study to check bacteria release from silk and bacteria plastics

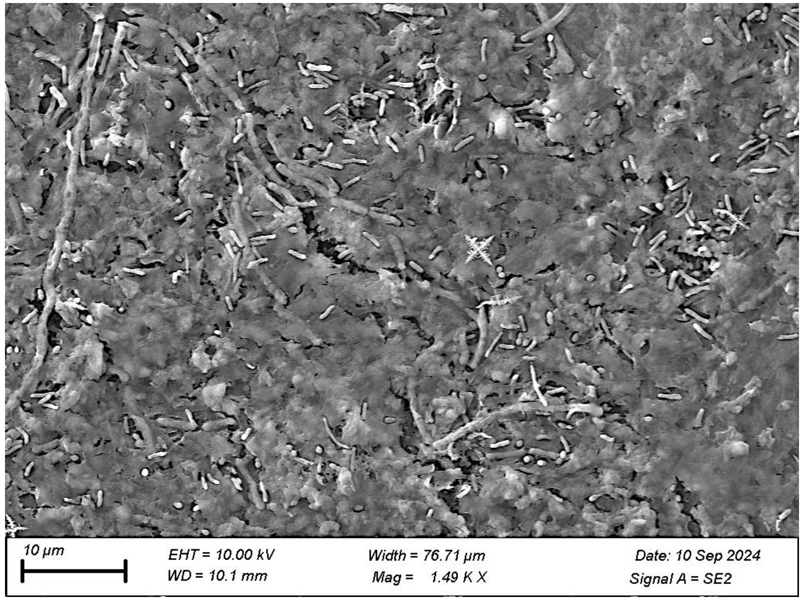


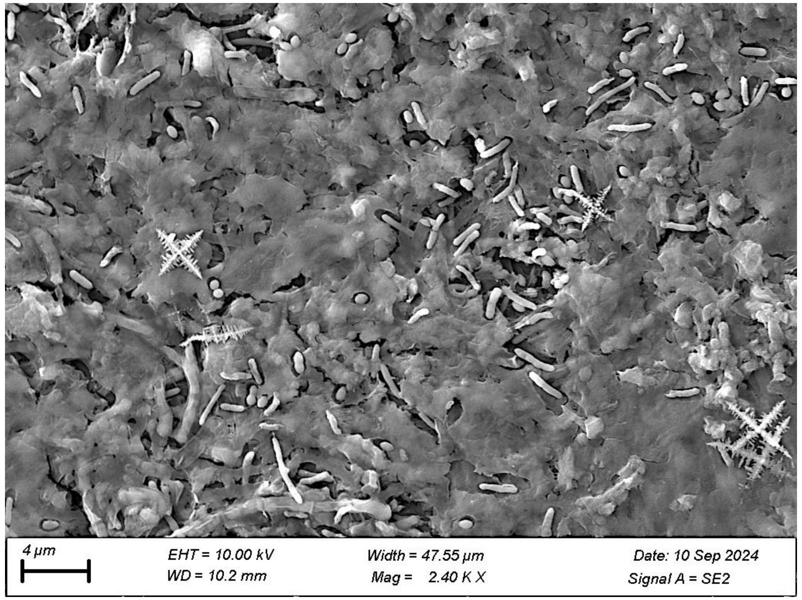


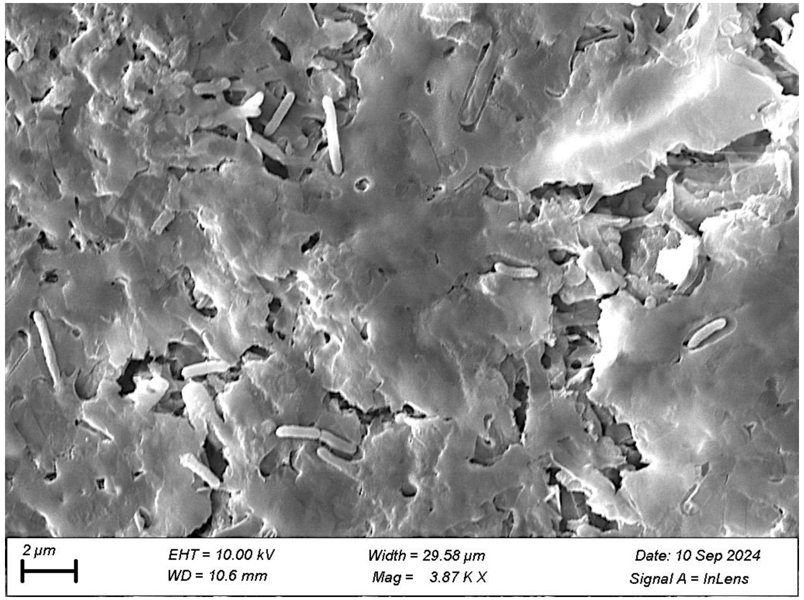
Silk/ *pasteurii* disc

Under SEM

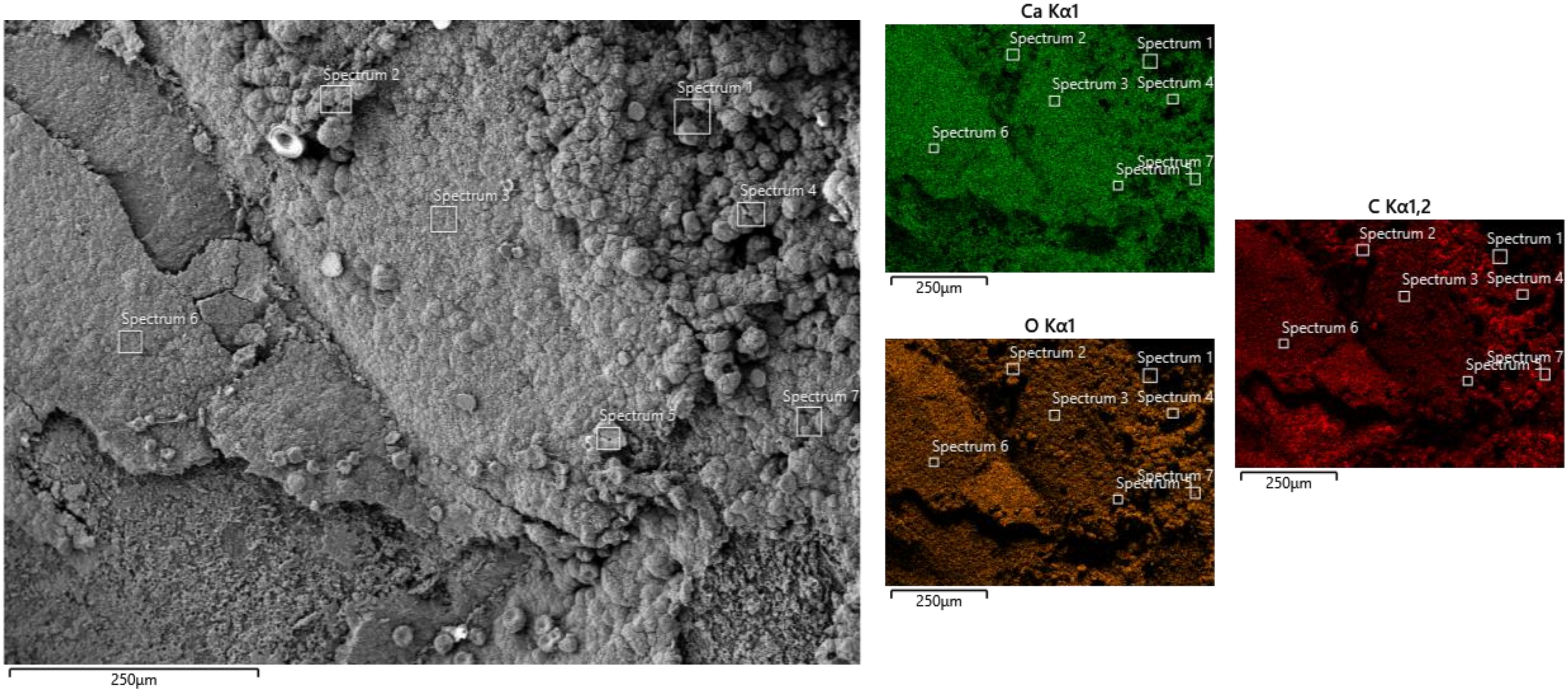








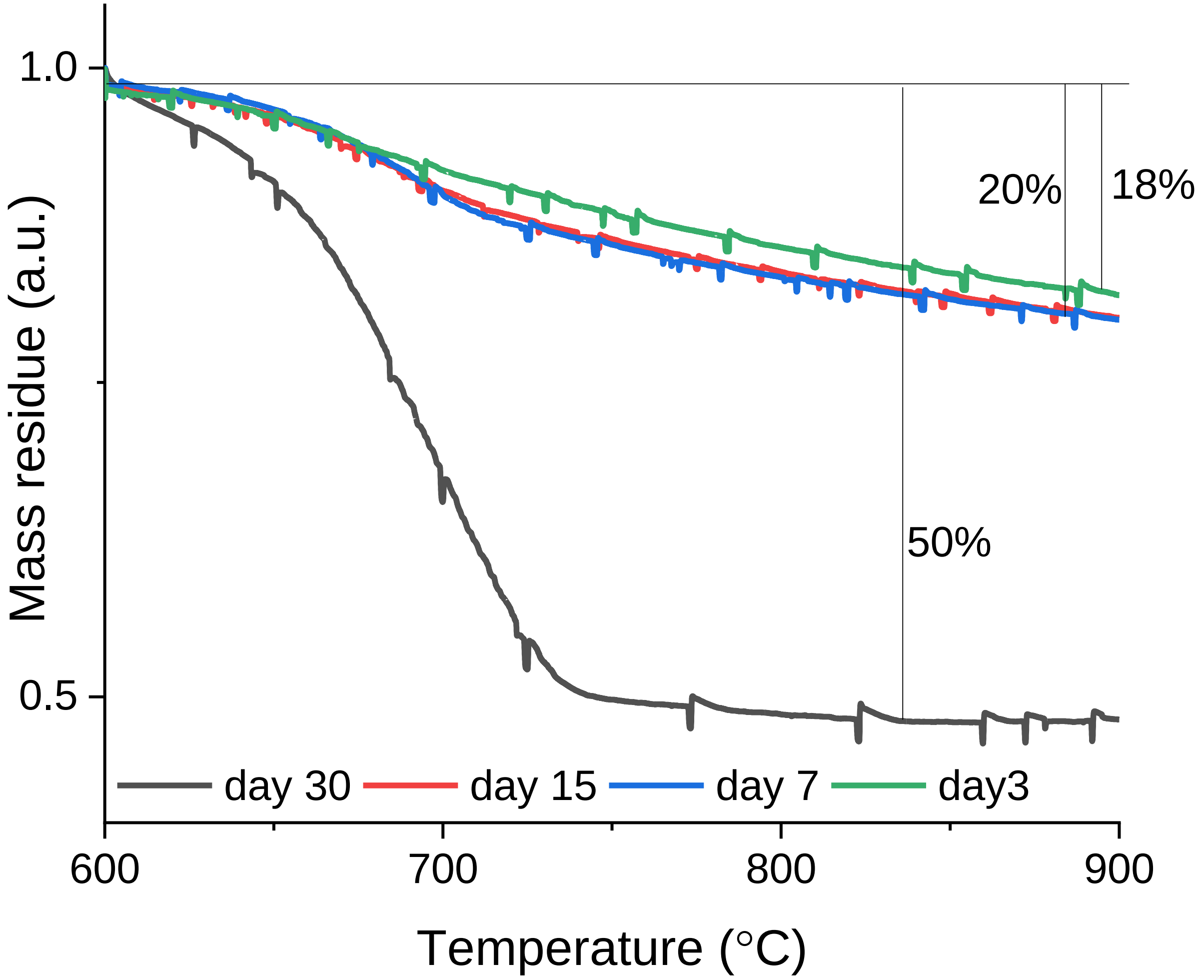
EDS- elemental mapping for cross-section of biocemented silk-bacteria plastic



$CaCO_3$ quantification using TGA

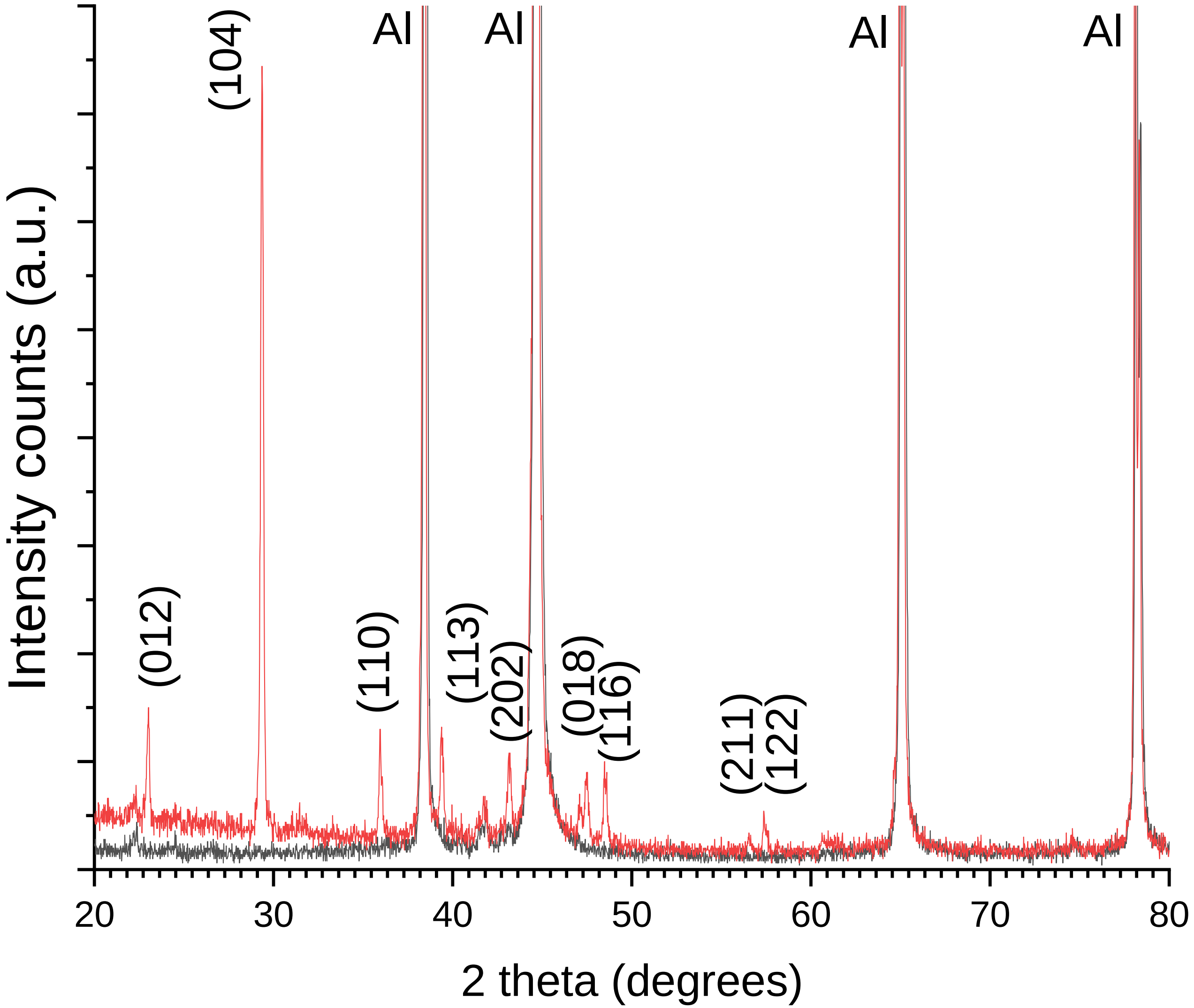
The plastics were incubated in the bio-cementation media for different time periods

$$CaCO_3 (s) \rightarrow CaO(s) + CO_2 (g)$$

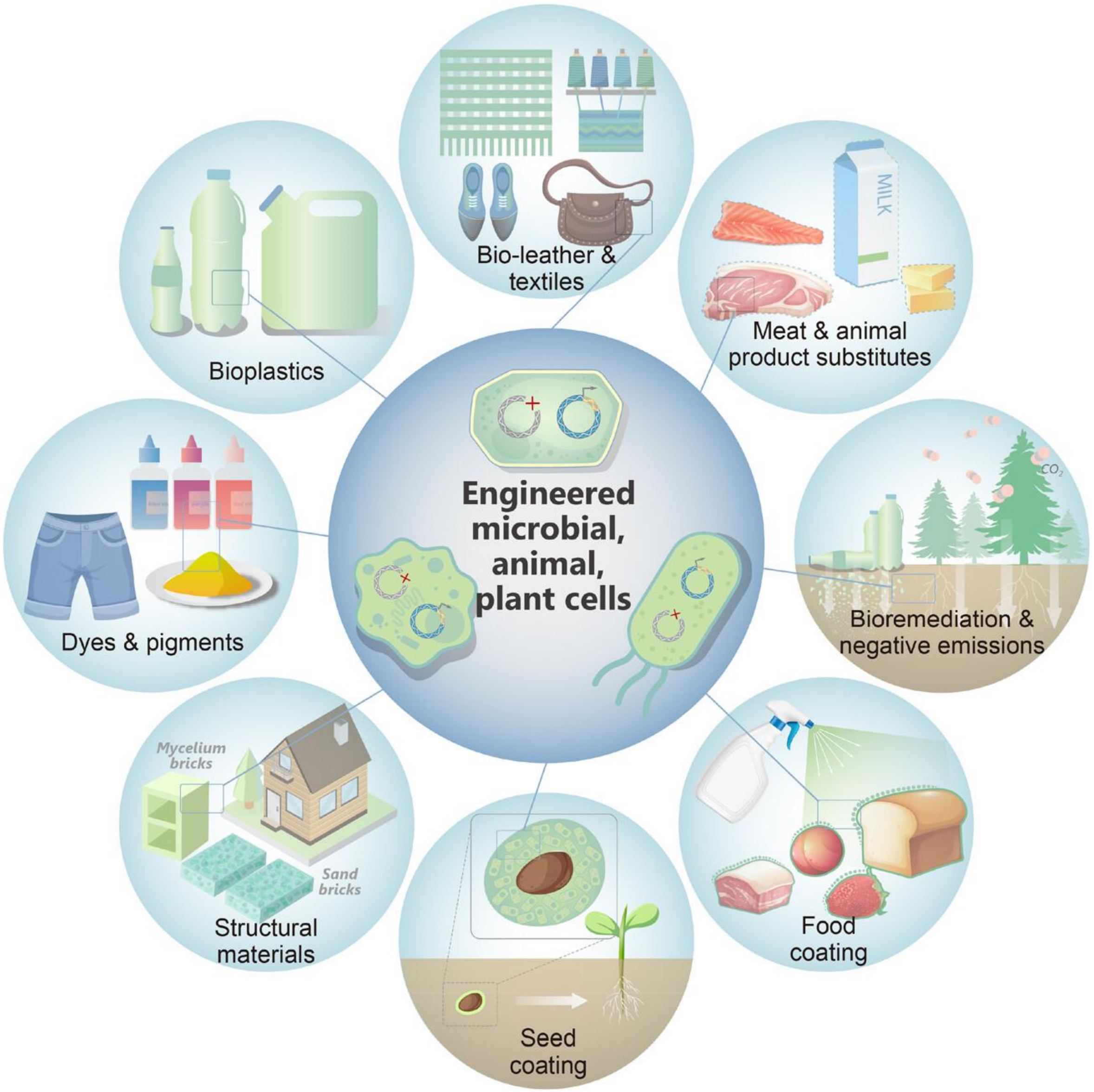


X ray diffraction to identify $CaCO_3$ phases

The obtained peaks are indexed to calcite phase of calcium carbonate (trigonal structure)



Living materials will touch many aspects of our daily life in the future



An, Wang, Huang, Wang, Liu, Xun, Zhong (2022). Engineered living materials for sustainability. *Chemical Reviews*

Limitations

Opportunity Areas

Limitations	Opportunity Areas
Low growth rates and yield	Use of Artificial Intelligence (AI) and Synthetic Biology Foundry (SBF) to find optimum conditions
Inferior material strength	Application of natural inorganics or artificially designed materials to render the living materials strong
Limited longevity	Multispecies consortia systems development to increase stress resistance

There are still many hurdles for widespread adoption of living materials

What we need to work on:

- Minimizing the Biohazards of Living Materials
- Manufacturing Living Materials from Inexpensive and Easily Accessible Resources
- Extending the Working Life of Living Materials
- Artificial Intelligence (AI)-Assisted Material Design
- Building Effective Biosynthetic Pathways in a Chassis for Large-Scale Production

AI-Art by DALL-E

Key Learnings

1. Living materials are inspired by nature's circularity

2. Living materials are comprised of living and non-living components

3. Living materials can offer the capacity for regeneration, self-repair, biosynthesis, and responsiveness