Application of bacteria as self-healing agent for the development of sustainable concrete

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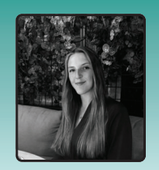

Aliana Desmeules

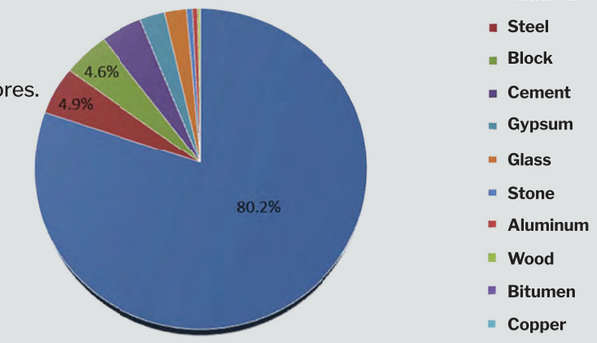

Lucie Caulier

Context

- This last century has been particularly prosperous for humanity, and one area that highlights this fact is construction :
  - Buildings have been constructed to house a growing population
  - Great stadiums have been made to host important sporting events
  - Skyscrapers have been built to demonstrate the power of commendatores.

Concrete : 80 % of the mass of the Building Materials used

- *Very low embodied energy* → *1.3 MJ/kg*
- *Cement Production* → *Chemical Reactions* → *More than 7 % of total anthropogenic CO_2 in Atmosphere*



Increase concrete's durability could mitigate the production of CO_2

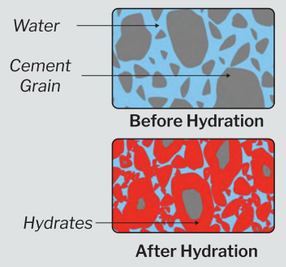
Concrete

Composition

- *Water*
- *Cement*
- *Aggregates* } *Inert Fillers*
- *Sand* }
- *Additives*

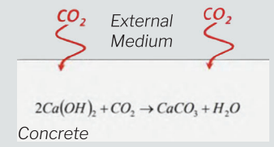
Hydration

- **Exothermic** reaction **between cement and water**
- **Formation of hydrates**
- From a slurry to a solid state



Carbonation

- Causes phase transformation that changes pH levels:
  - **Initial : highly basic** (pH ~13) (alkali concentration)
  - **Over Time** : Becomes more **neutral** (pH ~8)
- **Induces carbon capture**, contributing to the concrete's environmental impact.



Cracks in Concrete

Principle

Water Evaporation

Hydration Exothermicity → *Drying Shrinkage*

Consequences

- **Best Case : Increased porosity**
- **Worst Case : Surface cracking**
  - Reduced service life
  - Matrix degradation
  - Steel corrosion in reinforced concrete

Reduce Cracking

- **Design Stage Adjustments**
  - Modify water/cement ratio
  - Add chemical additives to the mix
- **Post-Production mesures**
  - Apply sealants to prevent water evaporation
  - Water the concrete to cool it down and minimize cracking risks

Bacteria to reduce cracks in concrete

Bacterial Self-Healing Study

- Bacteria from Bacillus genus incorporated into concrete for **calcium carbonate production**
  - Fills Cracks
  - **Enzymatic hydrolysis of urea**, which releases ammonia and carbon dioxide

Benefits and Limitations

- **Advantages**
  - Effective for crack repair
  - Regain in strenght
  - Decrease in permeability
- **Drawbacks**
  - Produces **ammonium ions** with each carbonate ion, leading to potential **nitrogen pollution**

Application Approaches :

- **Direct incorporation** of bacteria into concrete (preferred method) :
  - Reduces need for repeated surface applications
  - Cost Effective
- **External application** to cracks :
  - Repetitive effort for each new crack
  - Costly & Labor Intensive

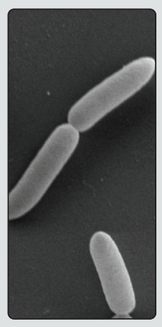
Which Bacteria ? Not that easy...

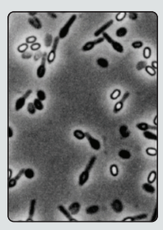
Bacterial Requirements for Concrete Application :

- Tolerant to high alkalinity (pH 11-13) (fresh concrete is highly alkaline due to portlandite (calcium hydroxide) formation)
- Withstand mechanical stress during mixing
- Oxygen tolerant (diffusion)
- Small enough spores to fit into concrete spores

Bacteria Tested in Study

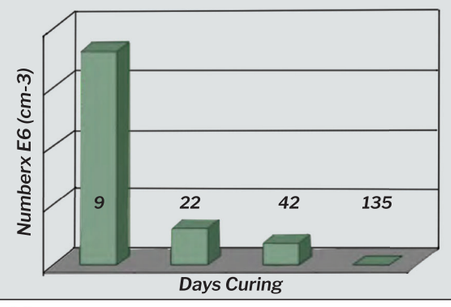
- **Alkalihalobacillus pseudofirmus DSM 8715 (formerly Bacillus pseudofirmus) :**
  - Gram-positive, alkaliphilic, alkalitolerant, aerobic, and facultative anaerobe
  - Discovered in hyperalkaline spring in Zambales, Philippines (2019)
  - **Can biodegrade LDPE plastic**

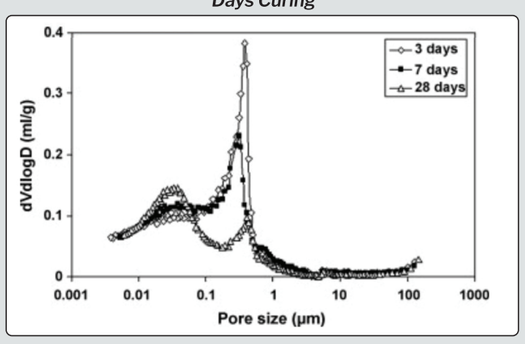




- **Bacillus cohnii DSM 6307 :**
  - Also from the Bacillus genus
  - High alkalinity tolerance

How bacteria do cope in concrete ?





Most-probable-number estimate of viable bacteria spores (B. cohnii) incorporated in aged cement

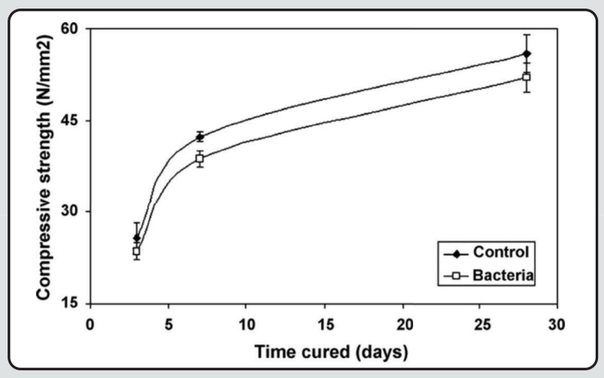
- **Dramatic decrease of bacteria**
- Simultaneous **decrease of pore size**

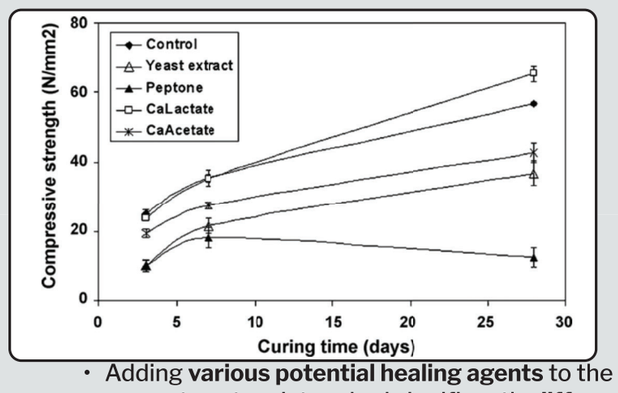
Young Concrete → Large spores accomodate spores

Older Concrete → Crushed spores, no mineral formation

Results

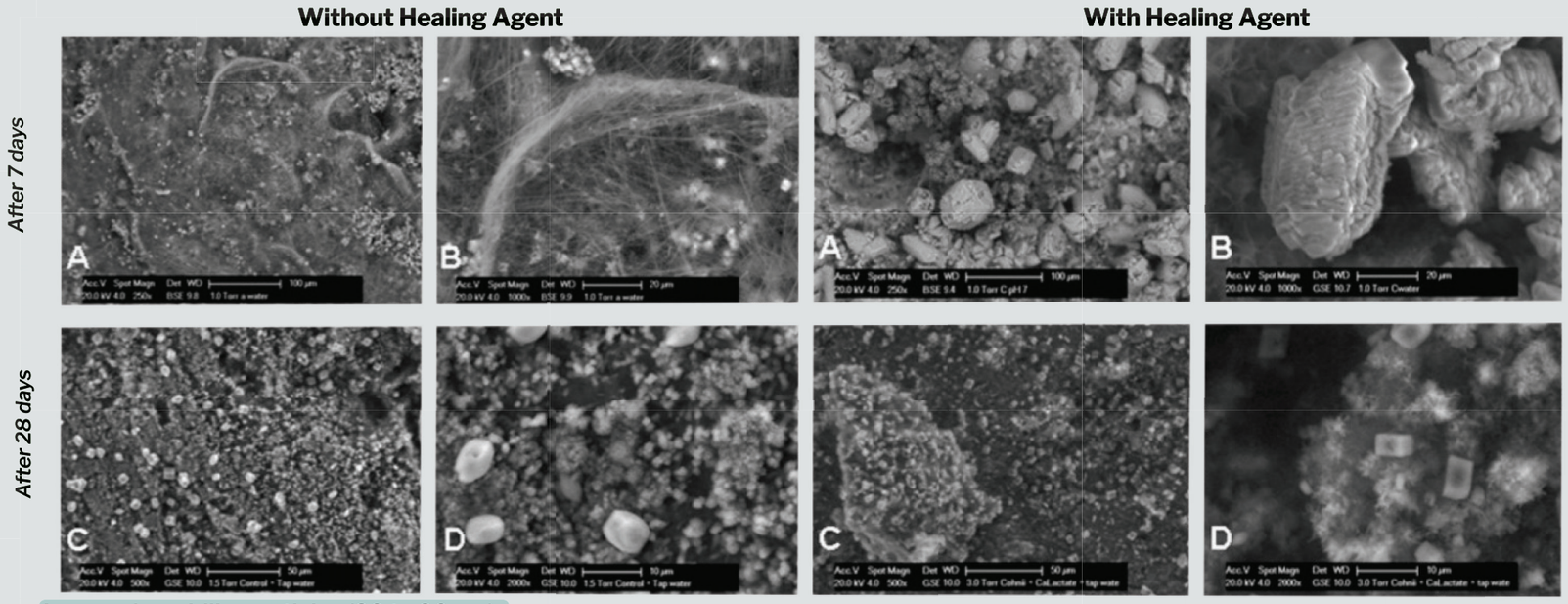
Compressive Strenght





- Adding **various potential healing agents** to the cement paste mixture had significantly **different impacts** on aging specimens
  - Incorporating a high concentration of bacterial spores (6×10^8 cm^{-3})
    - Less than a 10% reduction in strength for specimens cured for 3, 7, and 28 days

Microstructure



- **Large mineral-like particles (20 to 80 µm)**
  - Abundantly formed on the crack surfaces of specimens cured for 7 days
  - No formation on samples cured for 28 days
    - Crack surfaces of the older 28-day cured specimens, whether containing healing agents or not, showed no significant differences in appearance.
- **Bacteria combined with calcium lactate (two-component healing agent)**
  - Large mineral-like precipitates (20–80 µm), are abundantly found on the crack surfaces of young (7-days) cement specimens
    - **Reaction Mechanism : $CaC_6H_{10}O_6 + 6O_2 \rightarrow CaCO_3 + 5CO_2 + 5H_2O$**
  - Production of calcium carbonate-based minerals increases when the generated CO_2 reacts with portlandite
    - **Reaction Mechanism : $5CO_2 + Ca(OH)_2 \rightarrow 5CaCO_3 + 5H_2O$**

Conclusion

Strengths of the Study :

- **Innovative Approach :** The study investigates using specific bacteria, as self-healing agents in concrete to enhance its durability and minimize maintenance needs
- **Environmentally Conscious Method :** By using bacteria that induce calcium carbonate formation, the study aims to lower environmental impact, addressing concrete's high CO_2 emissions
- **Detailed Methodology :** Test of bacterial viability in high-alkaline concrete, addressing potential barriers like pore size and alkalinity

Limitations Noted :

- **Limited Bacterial Viability : Bacterial spores lose viability over time**, particularly as pore sizes shrink with concrete aging, **limiting the long-term effectiveness** of the self-healing mechanism

Suggestions for Improvement :

- **Explore Alternative Bacteria** or Metabolic Pathways : Investigating other bacterial species or metabolic processes
- **Encapsulation** Techniques : Encapsulating bacterial spores in protective materials could extend their viability and allow self-healing in older concrete
- **Scaling Up and Field Testing** : Testing in real-world conditions and at a larger scale would be crucial to understand the practical challenges of implementing this technology.

Personal Impressions

- **Good information display :** The introduction provides the reader with the necessary information, the structure used leaves a clear message
- **Promising yet experimental :** The study is promising for its potential impact on sustainable construction, though practical applications may still be experimental given the limitations
- **Room for Technological Advancements :** This research opens avenues for advancements in self-healing concrete, but technological improvements will be necessary for widespread application

References

"FIGURE 1: Material Percentage Contribution by Material Mass in Pre-Use..." ResearchGate, 2015, www.researchgate.net/figure/Material-percentage-contribution-by-material-mass-in-pre-use-stage-of-the-office-building_fig1_293804254. Accessed 12 Nov. 2024.

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Biosorption and biomagnetic recovery of La^{3+} by *Magnetospirillum magneticum* AMB-1 biomass

Based on the work of M. Mohammadi, B. Reinicke, K. Wawrousek, Department of Chemical Engineering, University of Wyoming [1]

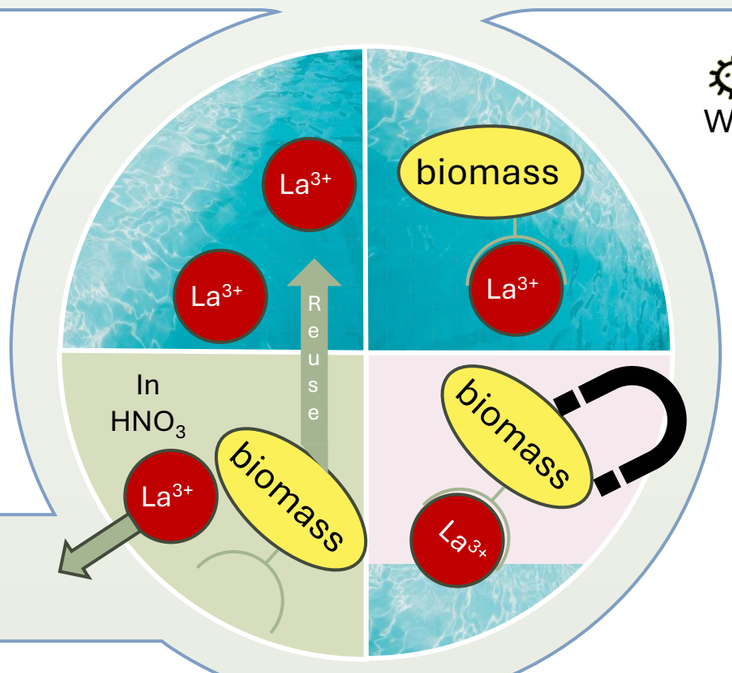
Authors: Aurélie Vuagniaux, Partick Klemme, Adrien Borgeat (Group 2)

Introduction

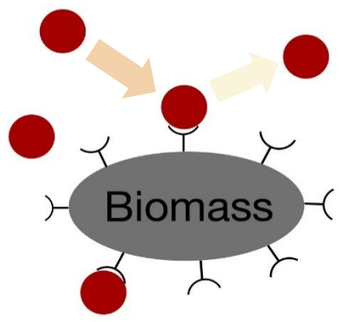
- Rare Earth elements (REEs) applications

Laptops Car batteries

- Lots of extractions techniques are known for REEs, but they require a lot of energy, and they release toxic compounds
- New promising sustainable extraction method for REEs present in solutions is using biomass, especially bacteria



Biosorption



Schematic of biosorption [3]

Functional group

Adsorption

Desorption mediated through a solution of HNO_3, vortex and incubation

Bacterium of interest

Magnetospirillum magneticum AMB-1

Why ?

- Magnetic bacteria (presence of Fe_2O_3 nanoparticles) [2]
- Easy to isolate the biomass from the solution thanks to its magnetic property (magnet)
- Use of biosorption to bind rapidly and reversibly metallic ions on the biomass surface in aqueous environment

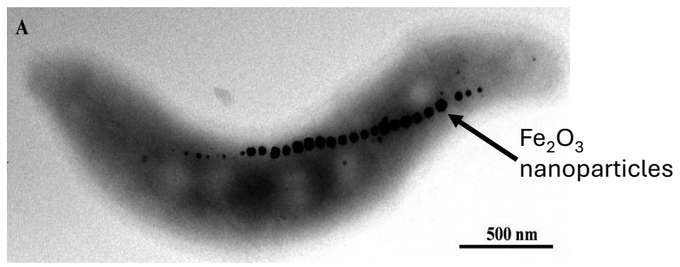


Figure 1, Image of AMB-1 biomass with visible magnetite nanoparticles [2]

Results I

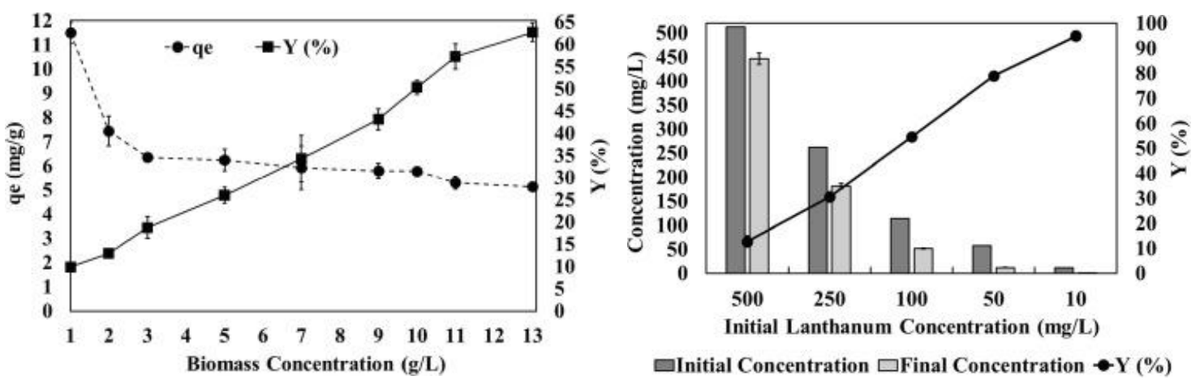


Figure 3, Variation of the binding capacity and biosorption efficiency as a function of biomass concentration (left), and biosorption efficiency as a function of Lanthanum concentration (right) [1].

- Increasing biomass concentration, leads to decrease in binding capacity (q_e) due to aggregation and increase in biosorption efficiency (*Y)* due to increase of additional binding sites.
- The Lanthanum ions spontaneously interact with the proteins of **AMB-1** and not the magnetite it synthesizes for temperatures ranging from 21 to 50 °C.

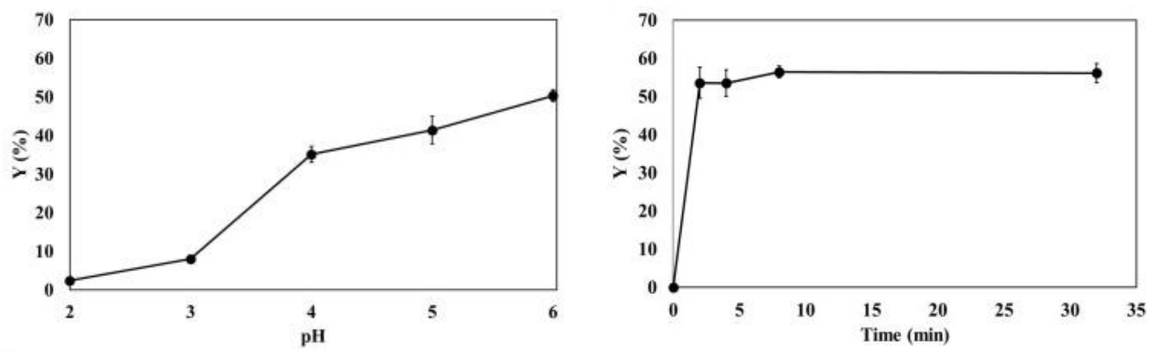


Figure 4, Effect of pH (left) and time (right) on biosorption efficiency [1]

Temperature does not affect efficiency in the 21 to 50°C range.

Efficiency decrease of 11% with salinity increasing from 0 to 1.5 M NaCl

57 La Lanthanum 138.91

Biosorption and desorption are not limited to La^{3+}, both processes have been tested with all REE apart from *Pm* with positive results.

Results II

Microbe species	Binding capacity (mg/g)	Time
AMB-1	37.2	8 min
Myxococcus xanthus	137.5	60 min
Pseudomonas sp.	950	24 h
Stichococcus bacillaris	51.2	6 h
Arthobacter nicotinae	4.2	180 min

Table 1, Comparison of binding capacities to La^{3+}(in terms of weight of **dry** biomass) between various bacteria at the same testing conditions.

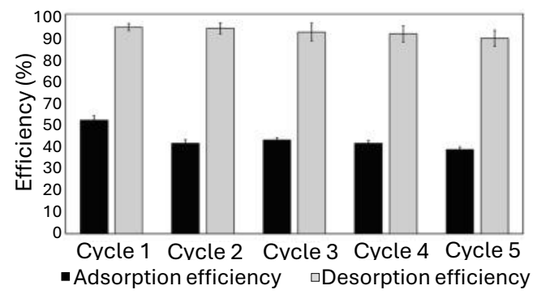


Figure 2, recyclability of AMB-1 [1]

AMB-1 has promising results for reusability as its efficiency is not greatly affected by recyclability.

Conclusion

Magnetospirillum magneticum **AMB-1** has proven to be efficient for the removal and recovery from aqueous solution of La^{3+}, but can also be applied for other REEs. Plus, it has been shown that:

- Adsorption capacity is 6.0±0.2 mg La^{3+}/g using 10g/L **AMB-1** biomass with a 100 ppm La^{3+} solution at pH 6.0
- The same biomass can be used for multiple cycles after desorption
- Biomass adapts to various external conditions (pH, T° and salinity)
- Binding capacity is lowered when the La^{3+} concentration is high

Article's strengths & weaknesses

• Well documented (85 references)	• Not in a famous journal
• Test for all REEs, not only La ➡ more general	• Tested only salinity, not the impact of other minerals found in rivers
• Well explained and concise	• Minimal temperature tested: 21°C

References:

[1] M. Mohammadi, B. Reinicke, and K. Wawrousek, ”Biosorption and biomagnetic recovery of La3+ by Magnetospirillum magneticum AMB-1 biomass,” Separation and Purification Technology, vol. 303, p. 122140, December 2022.

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Programmable microbial ink for 3D printing of living materials produced from genetically engineered protein nanofibers

Anna M. Duraj-Thatte, Avinash Manjula-Basavanna, Jarod Rutledge, Jing Xia, Shabir Hassan, Arjirios Sourlis, Andrés G. Rubio, Ami Lesha, Michael Zenkl, Anton Kan, David A. Weitz, Yu Shrike Zhang & Neel S. Joshi

Summarized by Mizuki Watanabe, Arthur Micaleff and Charles Malmasson (Group 3)

Introduction

The 3D printing of living material is a sector of research that has garnered significant attention in recent times. The creation of 3D structures using microbes is a question that has already been explored with many different approaches, which although successful, never leveraged the genetic programmability of the microorganisms they contained.

This work explores this aspect with 3 objectives:

- The design of an extrudable bio-ink featuring high print fidelity
- The production of said bio-ink entirely from engineered microbes via a bottom-up approach
- The creation of a programmable platform to enable advanced functions for macroscopic living structures

As such, this work presents the steps for the fabrication and characterization of engineered E. coli biofilms, their use as bio-ink and its functionalization using engineered bacteria.

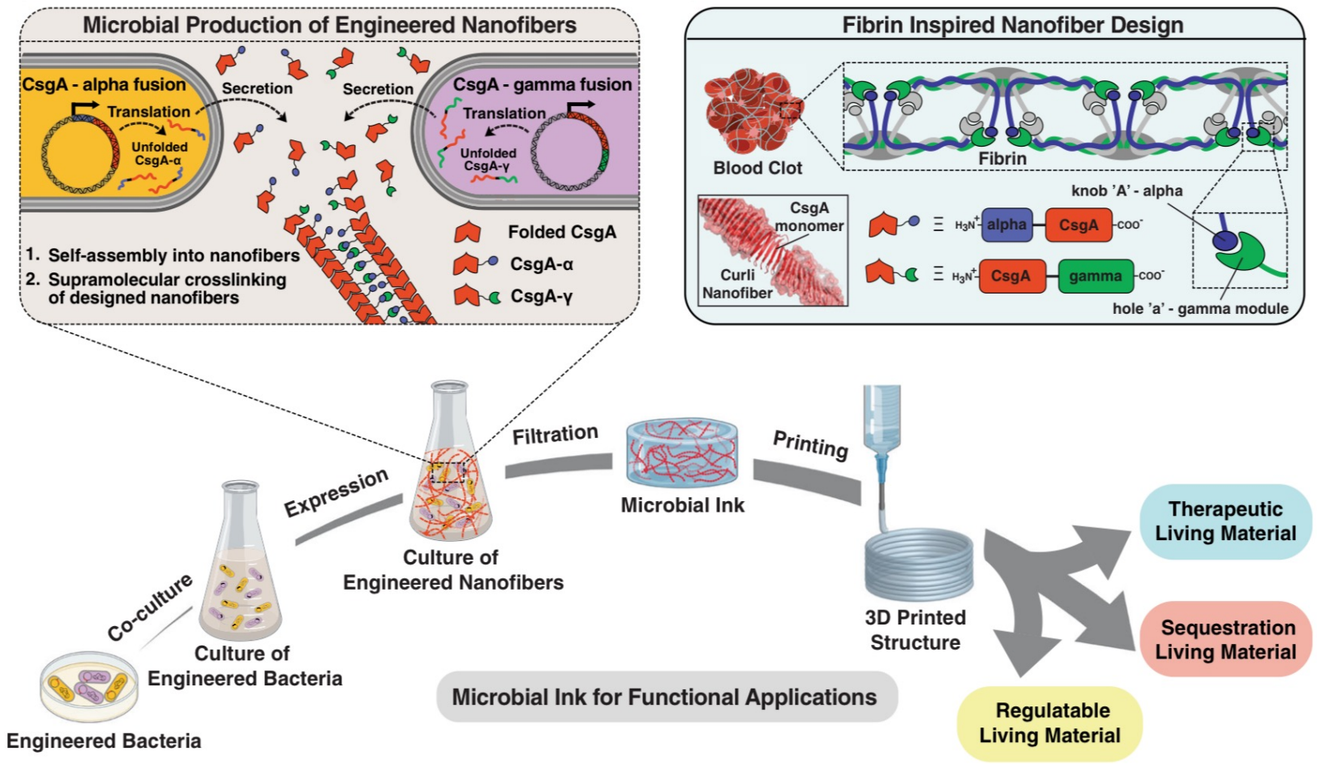


Fig. 1: Overview of the design strategy, production and functional application of microbial ink

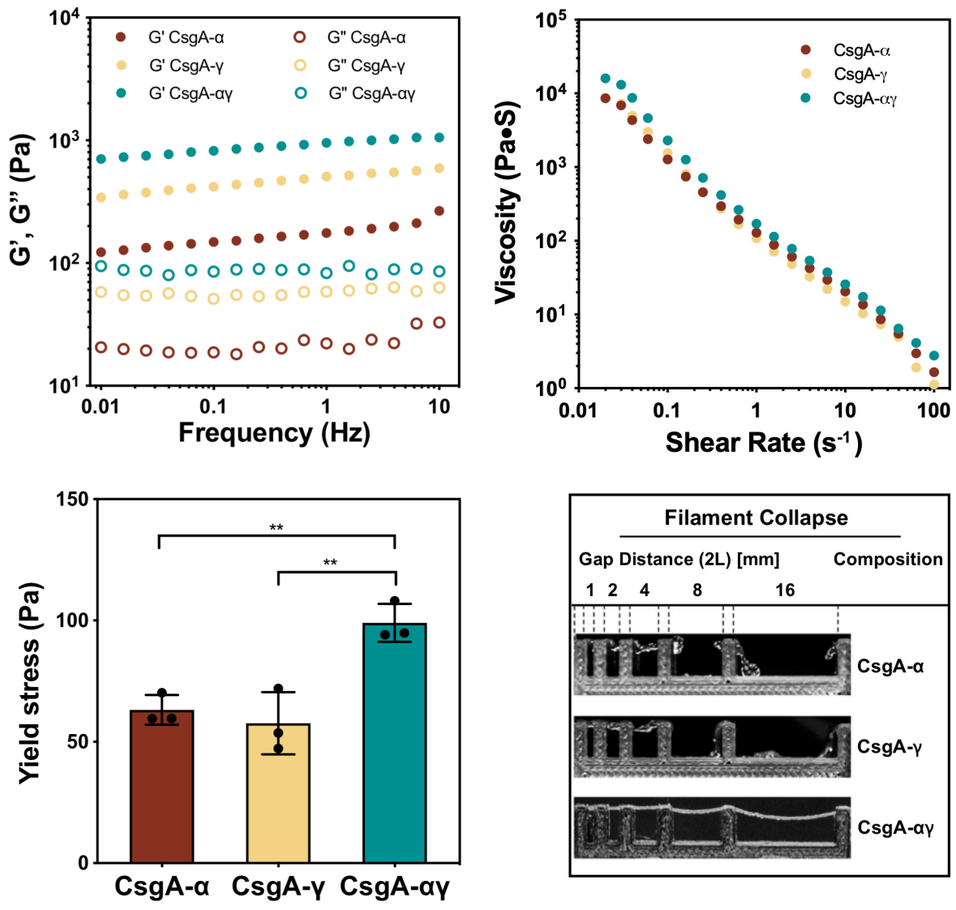


Fig. 2: Overview of the material behavior, with a comparison of the storage & loss moduli, shear thinning behavior, yield stress and filament collapse

Production of microbially produced bio-ink

Core aspects of microbial ink production

Good printability + Recapitulation of the extra-cellular matrix

Shear-thinning hydrogel + Use of the biofilm itself as matrix

→ Curli CsgA fibers with fibrin-inspired supramolecular cross-linking ←

Bio-ink production and initial structural characterization

- Fabrication of the nanofibers using 2 types of genetically engineered E. coli bacteria
- TEM analysis revealing larger fiber diameters in denser supra-cross-linked structures
- Filtration protocol (and optionally cell removal) to prepare for printing

Characterization of the rheological properties and printability of the bio-ink

- Rheological tests and actual printing experiments
- Desired shear thinning behavior and more solid-like characteristics thanks to supramolecular cross-linking, resulting in superior structural integrity and shape fidelity while printing

Functionalization

Therapeutic applications

- Azurin release via embedded modified E. coli, triggered by the chemical inducer IPTG

Sequestration applications

- Production of altered CsgA fibers featuring BPA binding peptides using modified E. coli bacteria

Regulation applications

- Production of protein synthesis inhibitors by the embedded E. coli, triggered by the chemical inducer IPTG

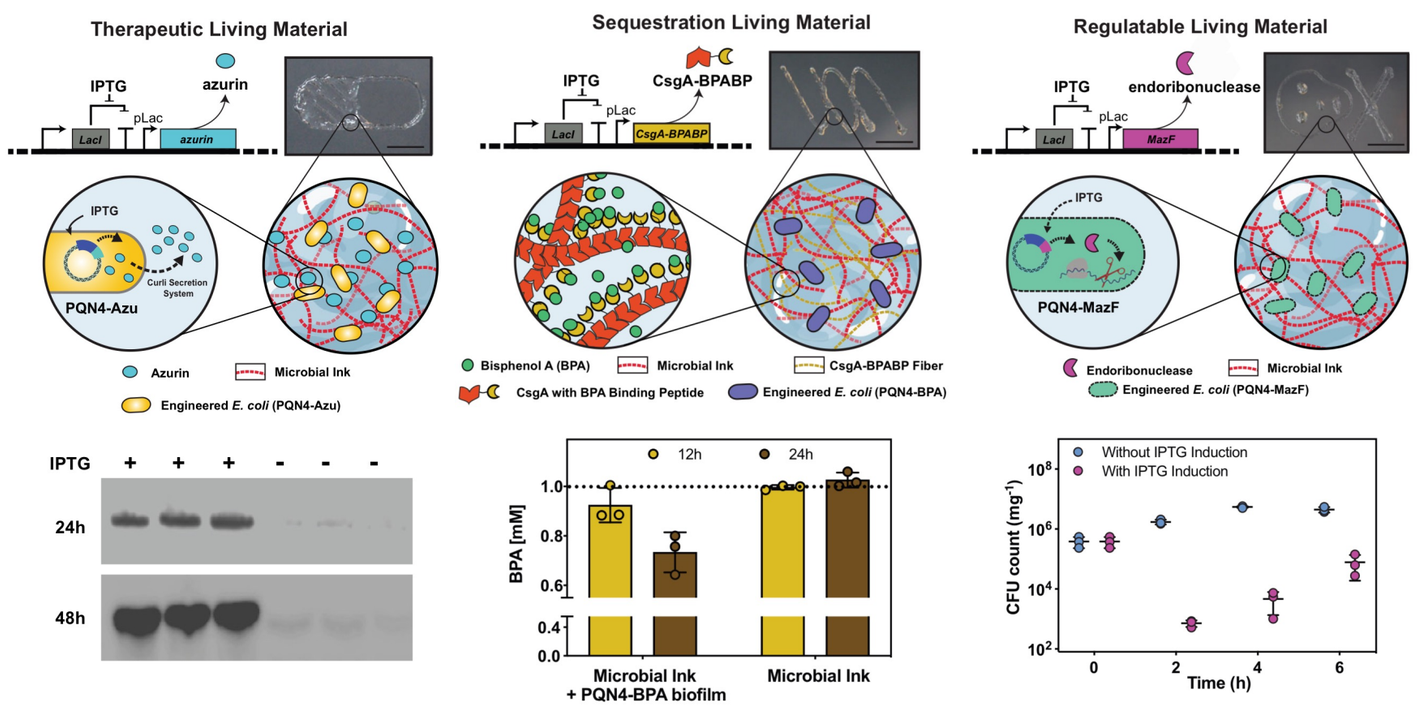


Fig. 3: Possibilities for functionalization

Take-away

It is possible to produce hydrogels from the biofilm produced by modified E. coli bacteria. The resulting gels show adequate printability and offer a versatile platform for multiple kinds of bacteria-based functionalization.

A.M. Duraj-Thatte, A. Manjula-Basavanna, J. Rutledge, J. Xia, S. Hassan, A. Sourlis, A.G. Rubio, A. Lesha, M. Zenkl, A. Kan, D.A. Weitz, Y.S. Zhang, N.S. Joshi, Programmable microbial ink for 3D printing of living materials produced from genetically engineered protein nanofibers, Nat Commun 12 (2021) 6600. https://doi.org/10.1038/s41467-021-26791-x.

Engineered Cyanobacteria-Based Living Materials for Bioremediation of Heavy Metals Both In Vitro and In Vivo

Tao Sun, Huaishu Huo, Yingying Zhang, Yaru Xie,Yize Li, Kungang Pan, Fenfang Zhang, Jing Liu, Yindong Tong, Weiwen Zhang, and Lei Chen

POSTER #4

Bioremediation of Heavy Metals (HM)

- Traditional remediation via chemical and physical methods.
- Bioremediation by using microorganisms.

It is less expensive, more eco-friendly and does not produce toxic sludge

- Typically high density
- Toxic at low concentrations
- Released in water and soil

Examples: cadmium (Cd), copper (Cu), lead (Pb), zinc (Zn), mercury (Hg), chromium (Cr)

Cyanobacteria

Responsible for ~25% of the global carbon fixation via photosynthesis. Remarkable potential for bioremediation (richness of binding sites for heavy metals).

However, they exhibit low tolerance to most heavy metals. 4.6 µM Cd^{2+} can inhibit the growth of *Synechocystis* sp. PCC 6803 cyanobacterium by roughly 50%

Evaluation of the bioremediation capability of PM/6803

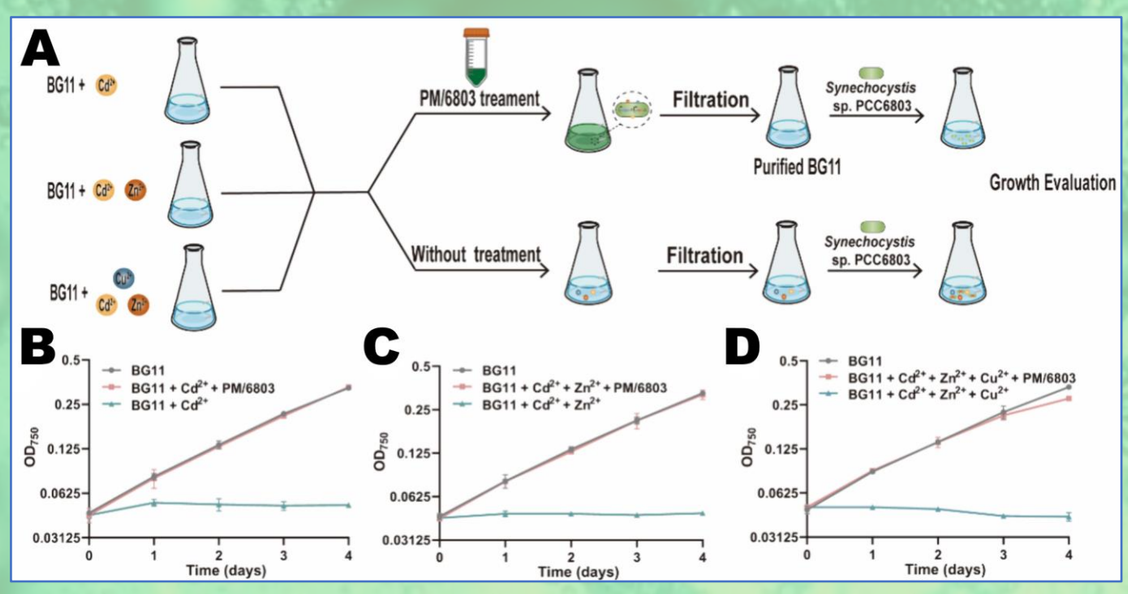


Fig.2: A) Characterization of the purification efficiency with PM/6803; Growth pattern of wild *Synechocystis* contaminated with B) Cd^{2+}, C) Cd^{2+} and Zn^{2+}, D) Cd^{2+}, Zn^{2+}, and Cu^{2+}, with and without purification

The medium (BG11) is contaminated with heavy metals. Part of it is purified with PM/6803. A wild type of *Synechocystis* sp.PCC6803 is added in pure BG11, and contaminated and purified medium (Figure A).

Results show that Cd and Zn are effectively remediated as purified and pure media witness the same thrive of *Synechocystis* (Figures B-D).

Solution for the tolerance to HM

To enhance metal tolerance in *Synechocystis* sp. PCC 6803, two genes encoding for specific proteins that can chelate heavy metals were introduced: phytochelatins (PCS) and metallothioneins (MT).

To regulate the gene activation, a riboswitch was used to produce three strains (Figure A): PCS/6803, MT/6803 and combined both to get PM/6803 (expressing both proteins).

With PM/6803, it has been observed that modified bacteria can grow in a medium containing a higher amount of Cd^{2+} (Figure B).

Light absorbance analysis where bacteria containing PM/6803 can keep a photosynthetic yield of 51% under Cd^{2+} exposure (Figure C)

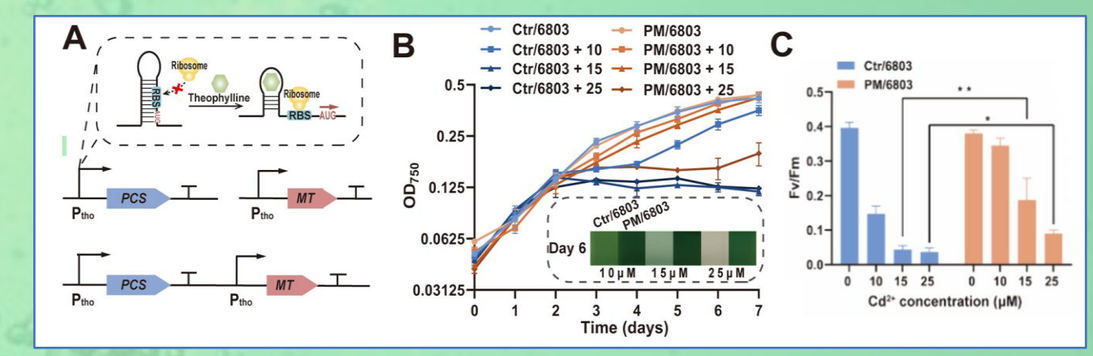
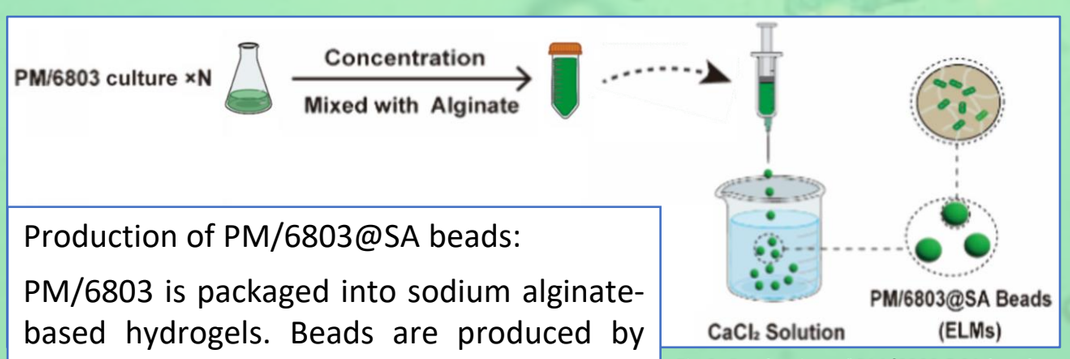


Fig.1: A) Principle of a riboswitch B) Growth patterns of Ctr/6803 and PM/6803 depending on Cd^{2+} concentration (0 to 20 µM) C) Photosynthesis yield depending on Cd^{2+} concentration (0 to 20 µM)

Making a Living Material



Production of PM/6803@SA beads:

PM/6803 is packaged into sodium alginate-based hydrogels. Beads are produced by releasing the mixture dropwise into a calcium chloride solution.

Fig.2: Production of PM/6803@SA beads

Characterisation of the Living Material

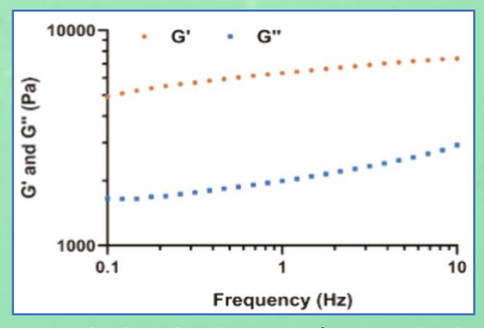


Fig.3: Rheological analysis of PM/6803@SA beads

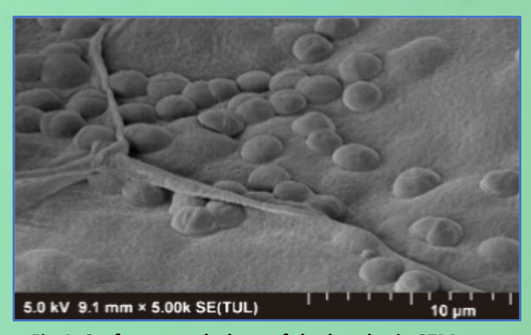


Fig.4: Surface morphology of the beads via SEM

Rheological analysis showed a stable structure, and the elastic behaviour expected from gels

A homogeneous distribution of bacteria was observed via SEM within the hydrogel

In vitro application

Figure A lists the different groups and their composition.

Cd^{2+} concentration proved fatal to all of group 2 after 12h, and to group 3 after 24h. It is possible that the zebrafish ingested heavy metals chelated on PM/6803 thus leading to acute poisoning despite Cd^{2+} concentration decrease in vitro in group 3 (Figure B). PM/6803@SA was best able to remediate the environment.

Pure alginate beads can chelate almost as much HM as lone PM/6803. Inside the zebrafish's main body, Cd^{2+} was found only in groups 2 and 3, which showcases the synergistic bioremediation capacities of PM/6803 and alginate (Figure C).

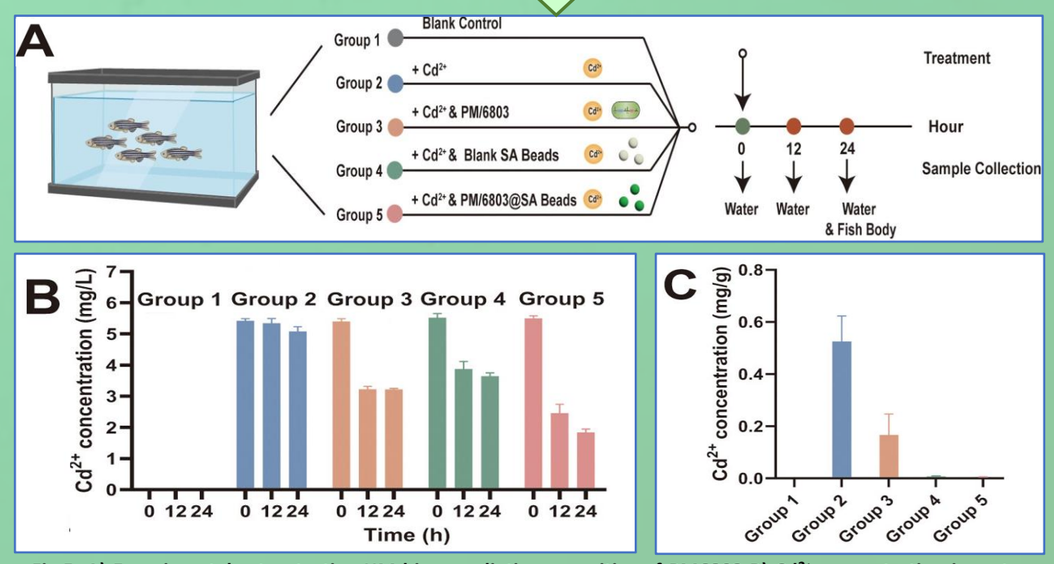


Fig.5: A) Experimental setup testing HM bioremediation capacities of PM6803 B) Cd^{2+} concentration in water after 0, 12 and 24h C) Cd^{2+} concentration in fish main body after 24h

Conclusion

This study has proven that gene-modified cyanobacteria encapsulated in an alginate matrix can perform HM bioremediation in vitro. The same has been observed in vivo in another part of the study.

Discussion

Cyanobacteria display a vulnerability towards Ag, which limits its survivability. Furthermore, leakage of engineered strains into the environment is a concern when using current hydrogel encapsulation.

Reference: Sun, T. et al. Engineered Cyanobacteria-Based Living Materials for Bioremediation of Heavy Metals Both In Vitro and In Vivo. ACS Nano 18, 17694–17706 (2024), DOI:10.1021/acsnano.4c02493

RESEARCH POSTER 5

EDIBLE MYCELIUM BIOENGINEERED FOR ENHANCED NUTRITIONAL VALUE AND SENSORY APPEAL USING A MODULAR SYNTHETIC BIOLOGY TOOLKIT

AUTHORS

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INTRODUCTION

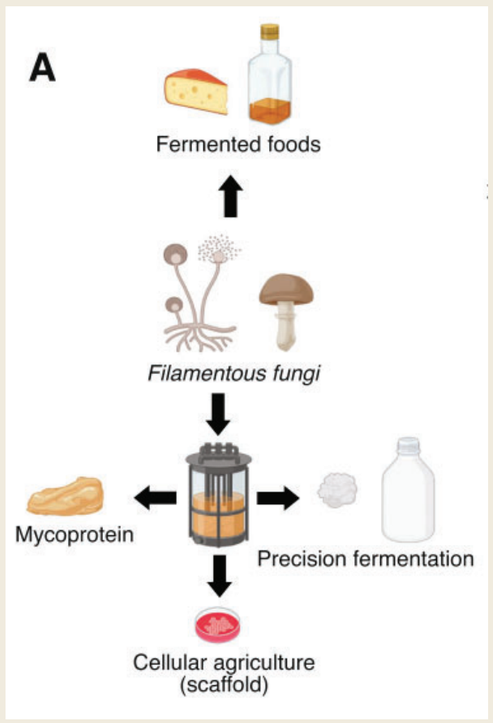


Fig 1: Fungal applications in sustainable food production

"A recyclable CRISPR-Cas9 method for efficient gene integration and expression"

They selected the filamentous fungi **A.oryzae** as it is already widely used in human fermented foods, has umami flavour and is commercially available.

CRISPR-Cas9 was used to selectively modify the DNA to insert a gene at a specific locus of interest.

RNP-based genome editing because it can be formed in vitro from commercially available Cas9 protein and sgRNAs, and minimizes off-target effects.

Locus-specific **marker recycling** for multiple rounds of genetic engineering by removal with 5-FOA

Methodology

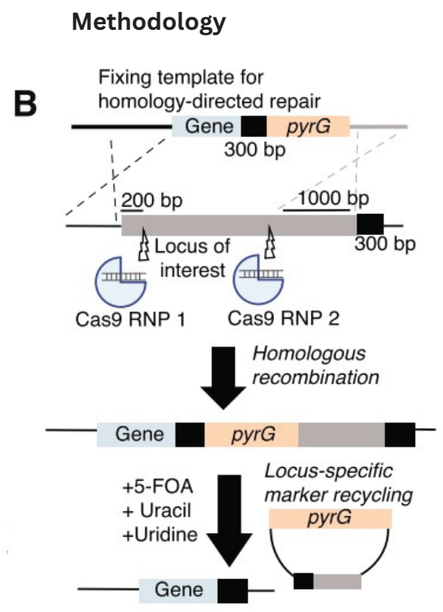
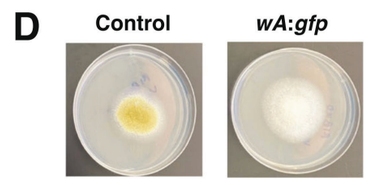


Fig 2: Strategy for RNP-based CRISPR-Cas9 editing. Counter-selection using 5-FOA allows locus-specific marker excision

Results

- **90% integration efficiency** of the GFP gene at the correct wA locus
- **Sequential engineering** possible with pyrG marker excision



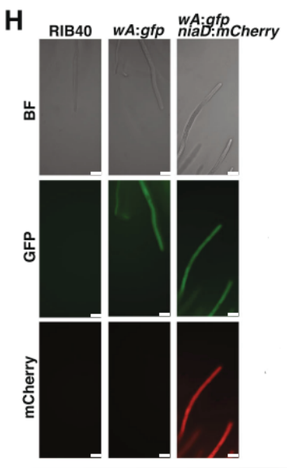


Fig 3: **D** Pigmentation change with wA:gfp **H** ΔpyrG recycled to integrate mCherry at the niaD locus confirmed with protein expression

"Expansion of the promoter toolkit using a synthetic expression system and bidirectional promoters"

A promoter is a region of DNA that controls the start of gene transcription, by allowing the transcription machinery to bind to it.

Methodology

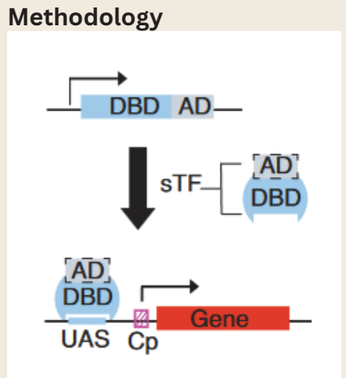


Fig 7: Design of synthetic expression system (SES)

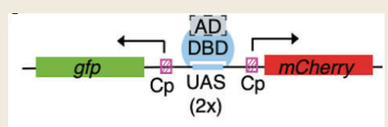


Fig 8: Design of bidirectional core promoter

"Identification and evaluation of neutral loci for gene expression"

A neutral loci is a precise position on a chromosome where variations do not affect the fitness or phenotype of the organism. This makes it an ideal site for gene integration and controlled protein expression levels.

Methodology

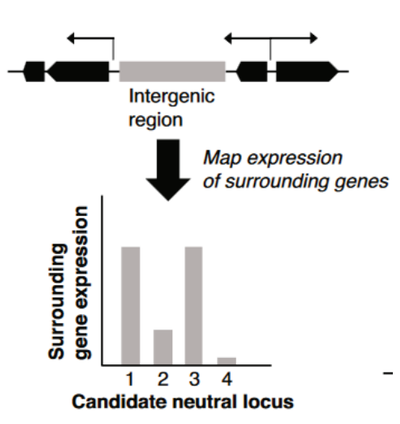


Fig 4: Analysis of RNA sequencing data

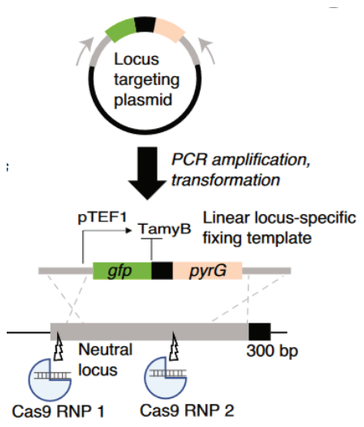


Fig 5: Integration of a GFP expression cassette

Results

- Of the 10 loci tested, 9 showed efficient integration and 8 expressed GFP in a stable manner.

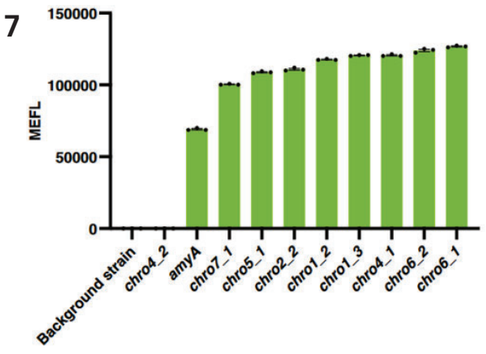


Fig 6: Expression of GFP (expressed as mean fluorescein equivalents, or MEFL) across neutral loci

Results

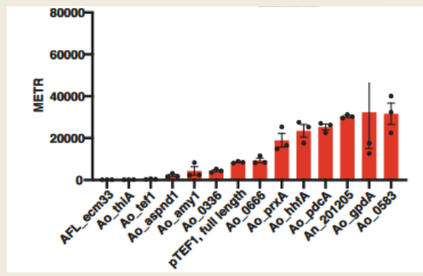


Fig 9: Expression of mCherry (expressed as mean fluorescein equivalents, or MEFL) for selected core promoters

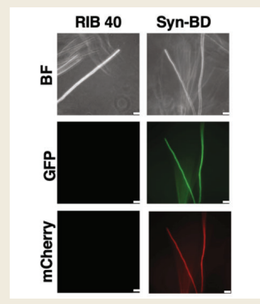


Fig 10: Impact of Syn-BD on the expression of bidirectional promoter

"Edible mycelium bioengineered for enhanced nutritional value and sensory appeal"

The team engineered A. oryzae to produce significantly higher levels of **ergothioneine**, an amino acid with antioxidant properties that supports cardiovascular and neurological health A. oryzae homologs of Egt1 and Egt2 were identified bioinformatically and expressed from neutral loci using a bidirectional promoter (strain VMR-Eg1-2) or as two separate genes at two different genomic locations, with each gene under the control of its own promoter (strain VMR-Eg1_2). They found that engineered strains could increase ergothioneine levels by up to **21-fold**, reaching or surpassing the levels in ergothioneine-rich foods like mushrooms. .

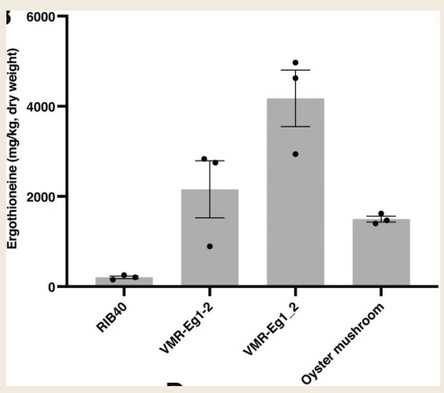


Fig 11: Ergothioneine level comparison in oyster mushroom, the engineered strains (VMR-Eg1-2 and VMR-Eg1_2) and the background strain (RIB40).

The researchers also sought to enhance sensory qualities by engineering **heme biosynthesis**, critical for color and flavor in meat analogs. The team first identified A. oryzae homologs for heme biosynthetic enzymes based on sequences found in S. cerevisiae and optimized expression of rate-limiting enzymes. With these modifications, they achieved a **4-fold increase** in heme levels, bringing them to nearly 40% of those in commercially produced meat alternatives like IMPOSSIBLE™ meat.

The bioengineered mycelium also maintained a fibrous texture and contained a full profile of essential amino acids, presenting it as a promising candidate for the meat alternative market with minimal post-processing required.

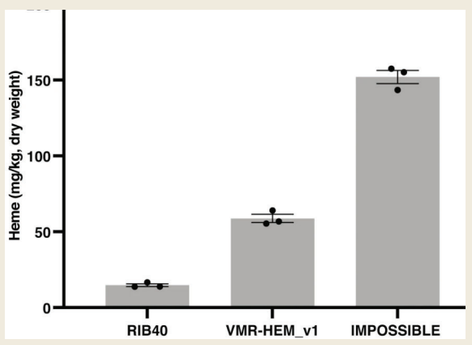


Fig 12: The intracellular heme levels in the engineered strain (VMR-HRM_v1) were 4-fold higher than in the background strain, RIB40, and 40% of those found in IMPOSSIBLE™ burger made from plants.

Discussion and conclusion

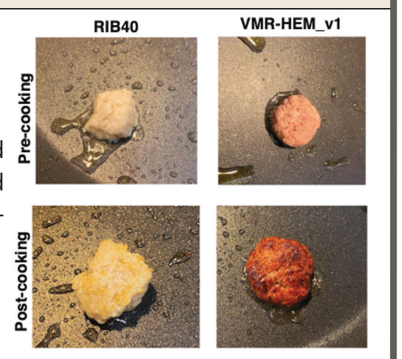


Fig 12: Color of harvested background (RIB40) and engineered heme strain (VMR-HEM_v1) after culturing.

This toolkit enables modifications, such as increasing ergothioneine levels and boosting heme content to simulate the flavor and appearance of red meat in **meat alternatives**. Unlike the traditional approach of producing animal proteins through fungi, this work highlights the efficiency and environmental advantages of **directly engineering** fungal biomass.

Although these initial prototypes demonstrate promising applications, further testing is essential to evaluate **consumer acceptability**, **safety**, and the **regulatory considerations** for genetically modified organisms (GMOs) in food. Researchers anticipate that genetically modified edible fungi could similarly address **sustainability**, ethical considerations, and public health concerns associated with industrial animal farming.

This poster is a paper presentation of this paper: Maini Rekdal, V., van der Luijt, C.R.B., Chen, Y. et al. Edible mycelium bioengineered for enhanced nutritional value and sensory appeal using a modular synthetic biology toolkit. Nat Commun 15, 2099 (2024). https://doi.org/10.1038/s41467-024-46314-8

Engineered living materials - MSE 493

Students : M. Debouvry, B.Lunven, Y. Zhou
Supervisor : T.Abitbol Group 6

Biocomposite thermoplastic polyurethanes containing evolved bacterial spores as living fillers to facilitate polymer disintegration

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INTRODUCTION

Thermoplastic Polyurethane (TPU) is the **6th most produced plastic in the world**. Knowing plastic pollution challenges, our group proposes a new end-of-life for TPU by introducing spores from polymer-degrading bacteria into TPU to :

1. Improve **mechanical properties**
2. Increase polymer **bio-degradability**

Applying an innovative technique : the adaptive laboratory evolution (ALE) to increase heat resistance of spores for hot melt extrusion of TPU.

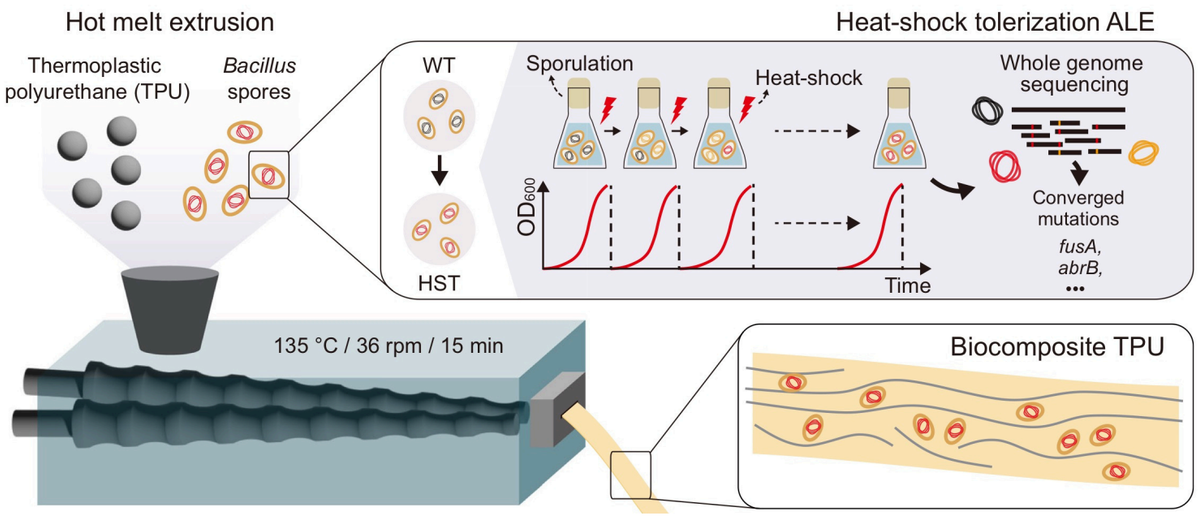
MATERIALS

- ***Bacillus subtilis* spores** ATCC 6633, d=500nm, l= 10µm
- Difco sporulation medium
- TPU pellets
- Mini-screw extruder (slit size = 5mmx0.7mm)
- Autoclaved and microbial compost

METHOD

1- Adaptive laboratory evolution (ALE)

- 40 cycles of heating with increasing time in boiling water
- Selective mutations on fusA and abrB genes of *B. subtilis*
- Increase the **heat shock tolerance** of spores

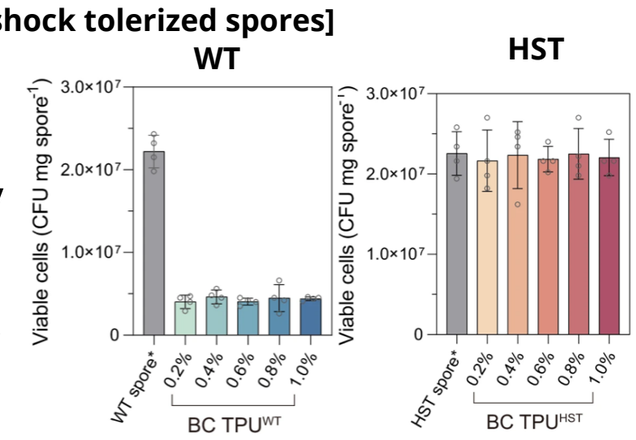


General method scheme :

2- Hot Melt extrusion (HME) of TPU

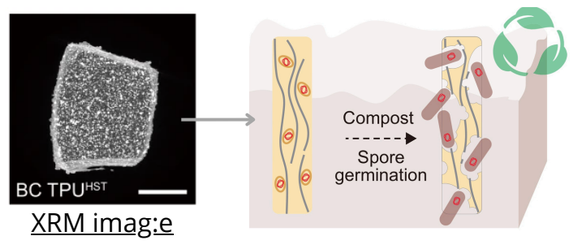
- 2 types of biocomposites (BC-TPU) formed : *WT* and *HST*
  [WT : Wild type spore; HST : heat-shock tolerized spores]
- Controlled dispersion of spores
- In **BC-HST ~100% viability** of the spores

Viability of spores after HME:



3- Disintegration of BC-TPU

- In Microbial active and autoclaved composts, 37°C, 45-55% RH
- Test with BC-TPU 0.8 w/w% spore concentration



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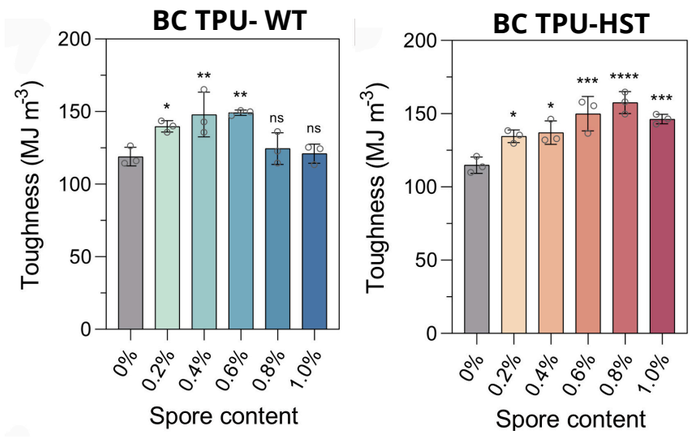
RESULTS

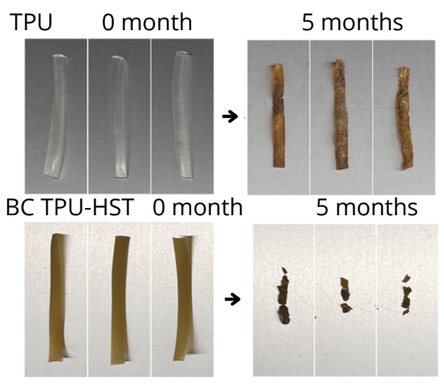
1- Tensile properties : Spore as reinforcing fillers



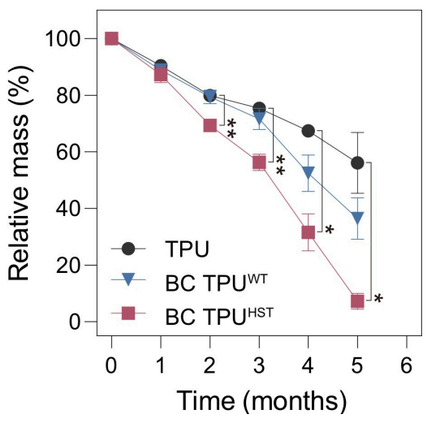
Reinforcement effect of spores on the tensile properties of TPU:

- **Increased toughness** with BC-WT and BC-HST compared to pure TPU
- Critical concentration of 0.8% of spores (HST) to achieve maximum toughness
- Suggests spore content has an effect on dispersion of spores in the BC

2- Biocomposite TPU disintegration :



Images of biodesintegration test:



Mass-loss during degradation in autoclaved compost:

- Dormant **spores are activated** by germination due to nutrients in compost.
- BC-HST: **92.7% mass loss** after 5 months.
- **Biodegradation** in low-microbial environments (autoclaved) still possible.

CONCLUSION

- Spores are reinforcing fillers and biocatalyst for TPU disintegration.
- ALE results in an **increased viability** of dormant spores to hot melt extrusion
- **Mechanical improvements** with HST spores of BC-TPU up to a critical concentration.
- Disintegration of BC-HST even in low degrader strain concentration media, and generally **faster disintegration** than BC-WT and TPU
- **Further research** : Scalability, price and accessibility to spore bacteria

Reveals efficient new end-of-life path for TPU plastics with improved mechanical properties.

Kim, H.S., Noh, M.H., White, E.M. et al. Biocomposite thermoplastic polyurethanes containing evolved bacterial spores as living fillers to facilitate polymer disintegration.Nat Commun 15, 3338 (2024). https://doi.org/10.1038/s41467-024-47132-8

An Ingestible Bacterial-electronic System to Monitor Gastrointestinal Health

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Poster by : Antoine Fotius,[4] Pierre-Arnaud Vals,[4] Mohammadreza Hassanzadeh[4] – Group No. 7

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Context & Project

Microorganisms in the human body can adapt in response to environmental changes by adjusting their gene expressions. New advances in synthetic biology enable the engineering of cells with genetic circuits that sense biological inputs.[1] Whole-cell biosensors can detect health-related analytes[2] but haven't yet achieved real-time, noninvasive diagnostics. Additionally, the advancements in microelectronics have led to ultra-low-power, miniaturized sensors with wireless connectivity. These technologies could enable live patient monitoring.[3]

In this study, an ingestible micro-biosensor capsule was created by coupling both microelectronics and synthetic biology for noninvasive detection of biomarkers related to gastrointestinal tract (Fig. 1). Engineered bacteria inside the capsule can emit light when they sense target biomarkers. This light is detected by embedded photodetectors and wirelessly transmitted to an external device. As a proof of concept, the ingestible micro-bio-electronic device **(IMBED)** (Fig. 3) used *E. coli* bacteria to detect stomach bleeding but demonstrated potential for detecting other gut diseases.

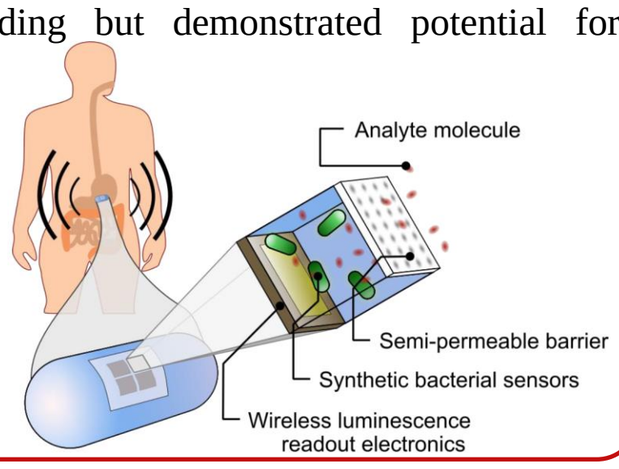

Figure 1. Capsule for sensing biomarkers in vivo with whole-cell bacterial sensors and wireless electronic readout.

Methods

Biosensor engineering

The biosensors have been devised by combining the desired DNA fragments into one using the Gibson assembly method.

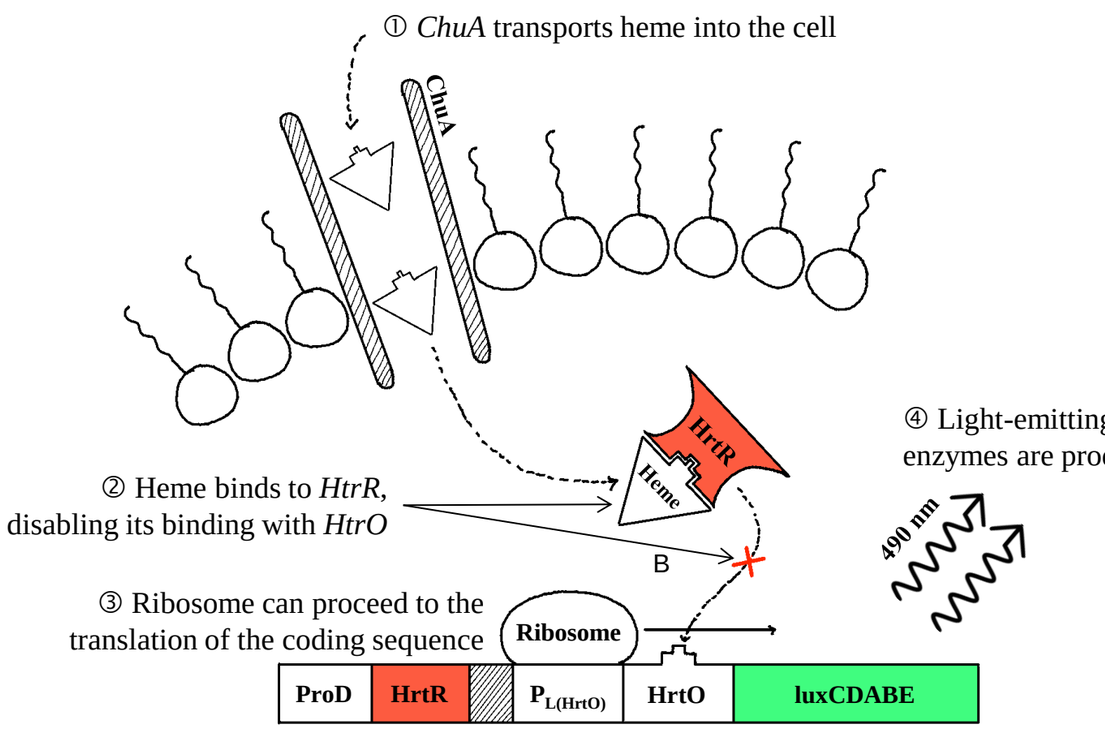

Figure 2. Simplified architecture and functionning of the biosensor. Fotius, Antoine.

IMBED Capsule

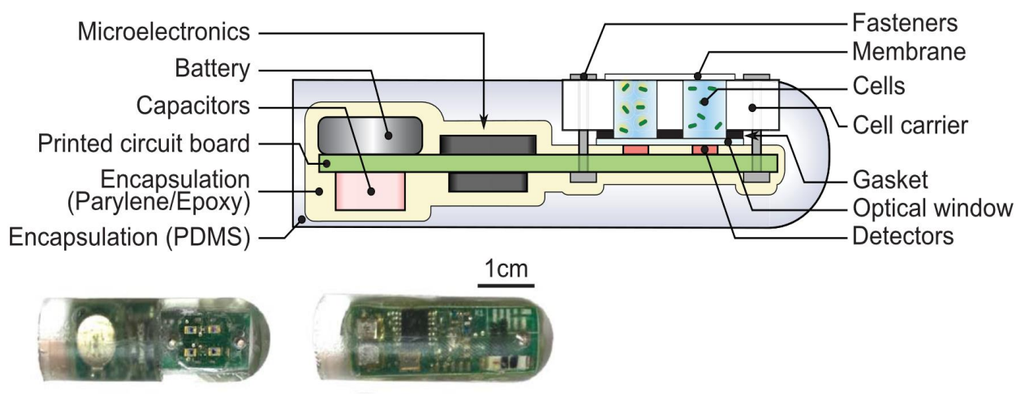

Figure 3. Cross section, front- and back-side photos of the device.

Experimental Results

In-vitro testing

To demonstrate the functioning of the biosensor, it was first tested in laboratory conditions. The IMBEDs have been exposed to different blood concentrations, between 0 and 500 ppm for two hours, as seen in Fig. 4B. The evolution of the photocurrent measured during the experiment is specified for 500 ppm in Fig. 4A.

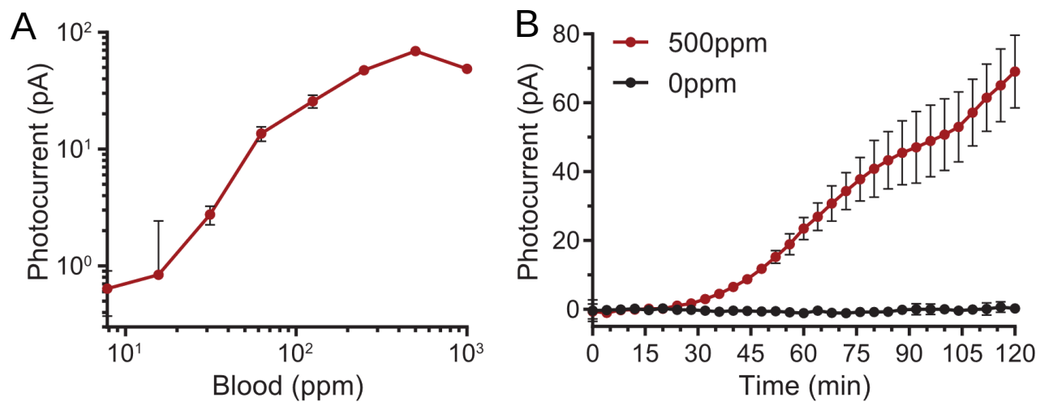

Figure 4. A) Kinetic response of blood between 0 and 500 ppm blood. B) Dose-response of blood sensor containing different blood concentrations 2 hours after exposure.

Based on Fig. 4A, the limit of detection of IMBEDs is 32.5 ppm of blood, and we can differentiate between 500 ppm and 0 ppm of blood after 30 min.

In-situ testing

Pigs have been chosen for this study because of the similarity of their intestinal system with humans. A solution of a neutralization solution of bicarbonate and glucose, with or without 0.25 mL of blood, is administered to pigs. The IMBED is then inoculated to both groups.

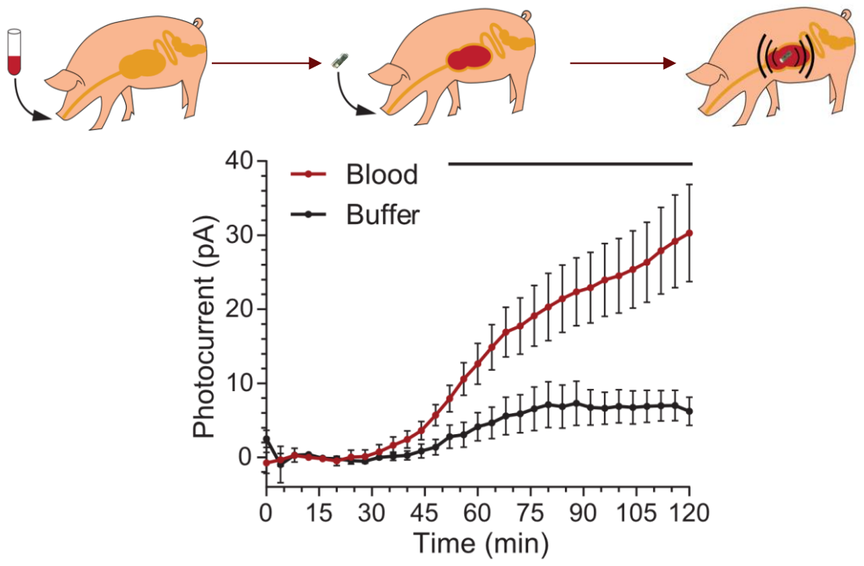

Figure 5. Data collected during the in-situ experiment

The IMBED has successfully started to detect blood after 30 min (Fig. 5), showing that the device can be used in real conditions to detect internal bleeding.

Applications & Perspectives

- According to the authors, the device can be used for the detection of various gut diseases by changing the analytes, the results have not been presented.
- IMBEDs could be used more broadly to detect specific bacteria in fluidic environments in a cost effective and fast way.
- Although it is functional, the device is quite bulky and will need miniaturization to be viable under clinical conditions.

References

1- Brophy JA, Voigt CA. (2014). *Principles of genetic circuit design*. Nature Methods 11, 508–520,doi:10.1038/nmeth.2926.

2- Slomovic S, Pardee K, Collins JJ. (2015). *Synthetic biology devices for in vitro and in vivo diagnostics.* Proceedings of the National Academy of Sciences of the U.S.A. 112, 14429-14435, doi:10.1073/pnas.1508521112.

3- H. Wang. (2013). *Magnetic Sensors for Diagnostic Medicine: CMOS-Based Magnetic Particle Detectors for Medical Diagnosis Applications*, IEEE Microwave Magazine 14, 110-130, doi: 10.1109/mmm.2013.2259402.

4D PRINTING OF ENGINEERED LIVING MATERIALS

Laura K. Rivera-Tarazona, Tarjani Shukla, Kanwar Abhay Singh, Akhilesh K. Gaharwar, Zachary T. Campbell, and Taylor H. Ware

Materials able to change shape under a specific stimuli have been found very useful in the context of a controlled drug delivery system. Here is reported a method which uses direct-ink-write printing to fabricate engineered living materials (ELMs) that fulfill such functions. It does so by printing 4D ELMs by integrating genetically engineered yeast which will lead to a shape change of the printed material in response to specific biomolecules.

OBJECTIVE

- Develop engineered living materials (ELMs) that change shape in response to biochemical stimuli.
- Create 4D materials using genetically engineered yeast that proliferates when exposed to specific biomolecules.
- Achieve controlled shape changes in the material.

➡ Demonstrate feasibility of ELMs for controlled drug delivery.

METHODOLOGY

- **Bioink Preparation:** Bioinks contain yeast, cellulose nanocrystals (CNCs) for rheology, acrylamide, and crosslinkers. [1]
- **Printing Technique:** Direct-ink-write 3D printing with photopolymerization to control spatial distribution of yeast.
- **Genetic Engineering:** Yeast strains engineered to only proliferate in the presence of specific nutrients (e.g., L-leucine or uracil), inducing localized shape changes [2]

VISUALS AREA

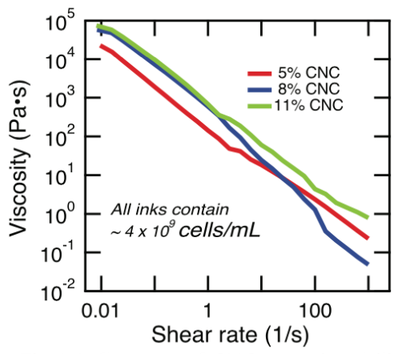

Fig 1. Influence of CNC on printability of ELMs and cell-free inks.

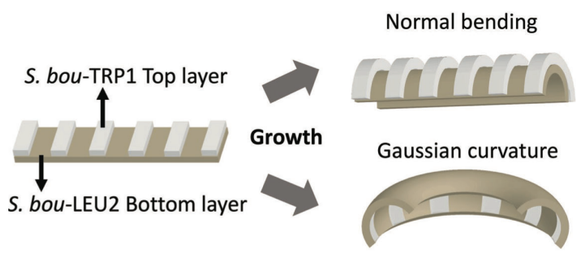

Fig 2. Schematic of a printed bilayer composed of two engineered S. boulardii mutants. Bilayer is capable of sequential shape change.

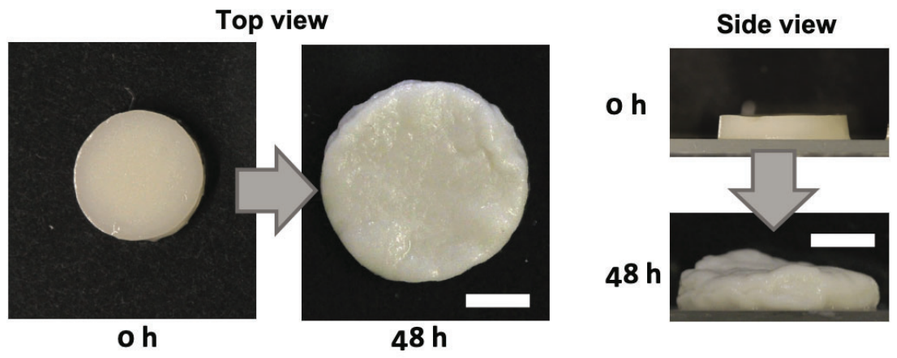

Fig 3. ELM disk before and after growth in synthetic complete medium. Top view shown on the left and side view shown on the right (Scale bars: 5 mm)

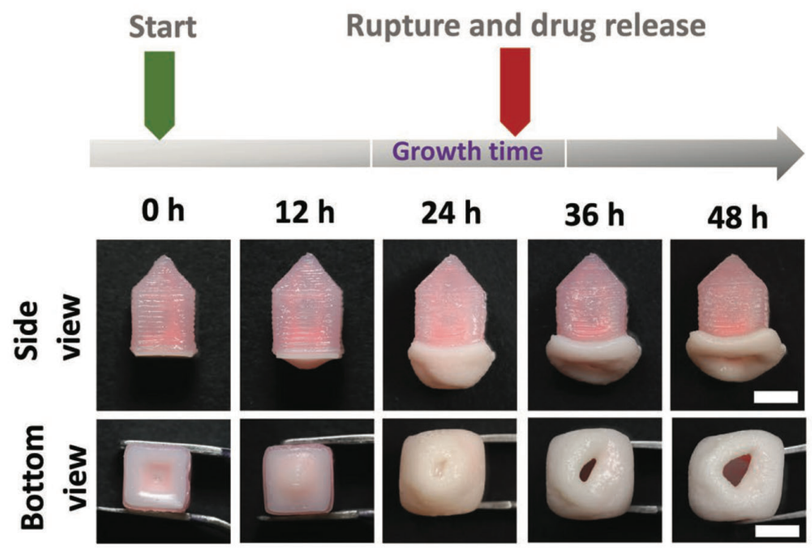

Fig 4. Influence of CNC at varying concentrations on mechanical properties and shape change

RESULTS

- **Ink Characterization:** Bioinks show strong shear-thinning behavior, essential for precise printing.
- **Volume & Shape Change:** ELMs expand up to 370% in volume when exposed to growth-promoting conditions, controlled by CNC concentration.
- **Drug Delivery Application:** Printed ELM capsules release a model drug upon structural change, triggered by the presence of specific amino acids

ANALYSIS

- Use of **CNC gives a higher shear storage modulus**, which allows the 4D printed material to be resistant enough to form a solid like material, as shown in figure 1.
- **Shape can easily be given to the material** due to the deformation the printed material undergoes after being placed in a specific environnement as shown in figure 2. [3]
- The **swelling also allows the film to break** at some point as shown in figure 4. It can be very useful to **deliver drugs** in the body at a given time.

SUGGESTIONS FOR FUTURE RESEARCH :

- Is the **long term biocompatibility** of the material good enough not to arm the human body?
- It could be more relevant to apply it **directly to decease-specific biomarkers.**
- A study should be made on the safety for **in vivo use of genetically modified yeast.**

CONCLUSION

This study shows that the use of yeast inside of a polymer matrix is **a promising method for drug delivery** in the human body.
First, By proving that adjusting bioink viscosity with **CNC make the matrix solid** like. Then we tested the **response of encapsulated yeast to stimuli** and the transformation that follows which could released the drug. This show great possibility for drug delivery even if there are still **untested parameters that could endanger the human body** such as long term biocompatibility.

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Living materials with programmable functionalities grown from engineered microbial co-cultures

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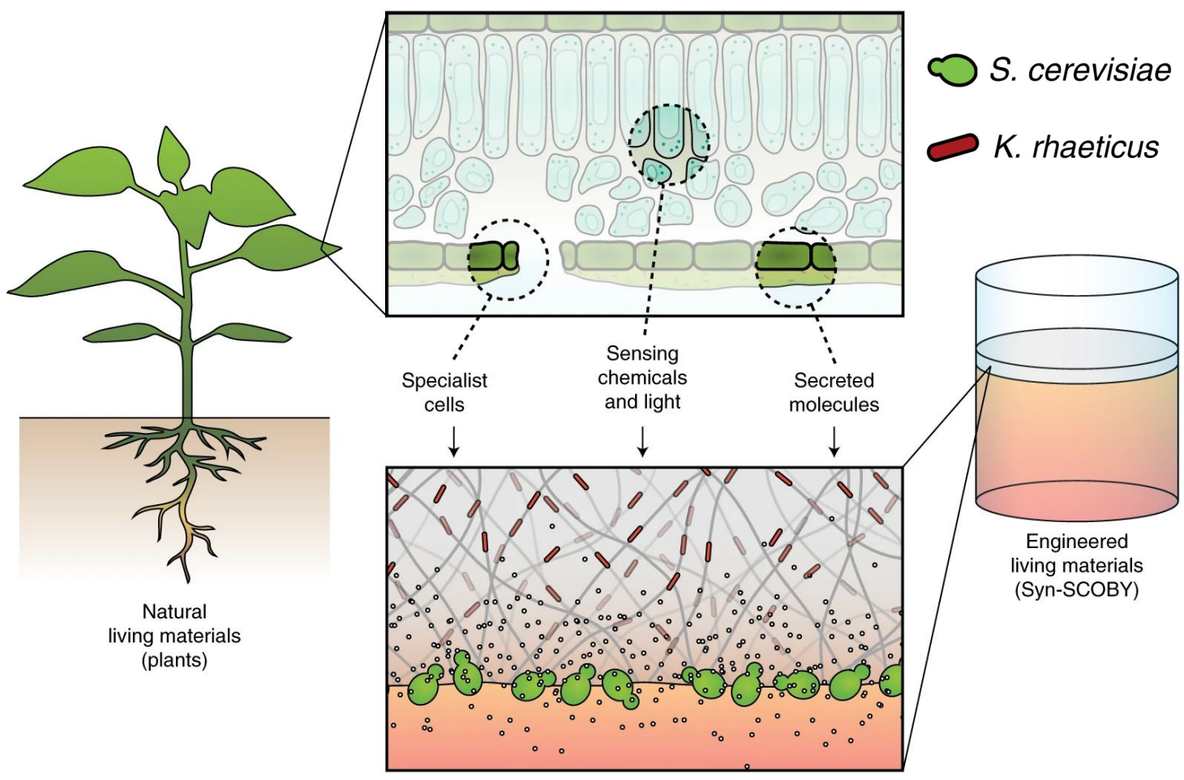
Charlie Gilbert, Tzu-Chieh Tang, Wolfgang Ott, Brandon A. Dorr, William M. Shaw, George L. Sun, Timothy K. Lu & Tom Ellis
DOI: 10.1038/s41563-020-00857-5

Summary

We present a method for creating functional living materials based on bacterial cellulose (BC), achieved through a stable co-culture of the yeast *Saccharomyces cerevisiae* and the bacterial cellulose-producing bacterium *Komagataeibacter rhaeticus*. This symbiotic yeast-bacteria culture offers a flexible platform for producing bacterial cellulose-based living materials with applications in biosensing and biocatalysis.

Introduction

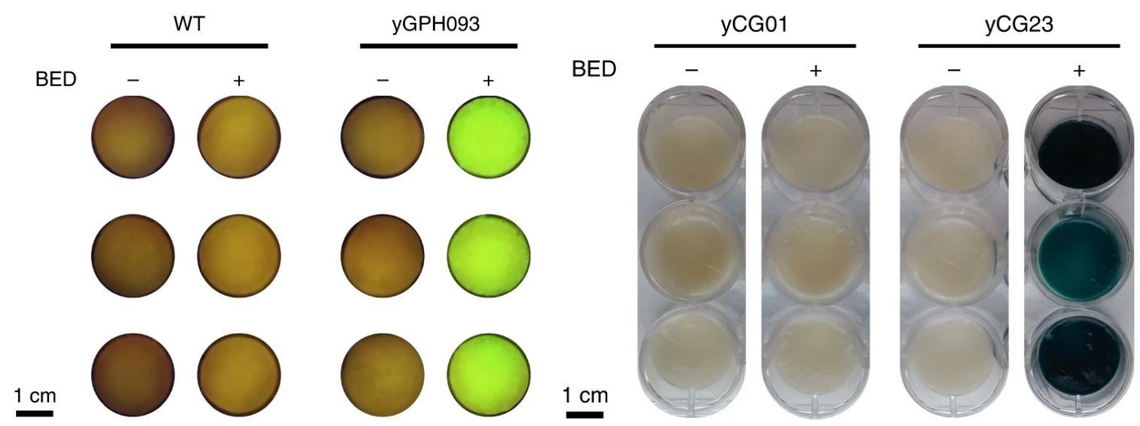
Co-culturing enables a nature-inspired division of tasks among cell types, integrating both scaffold production and functionalization within a single platform—a capability not commonly seen in other ELM research[1].



Utilizing a synthetic symbiotic culture of bacteria and yeast (Syn-SCOBY), BC serves as a durable, high-yield scaffold, while *S. cerevisiae* provides functionality.

Biosensing and biocatalysis

S. cerevisiae sensing strain (yGPH093) produces a strong signal upon exposure to β-oestradiol (BED). Biocatalysis strain (yCG23) secretes laccase enzymes, which degrades BED.

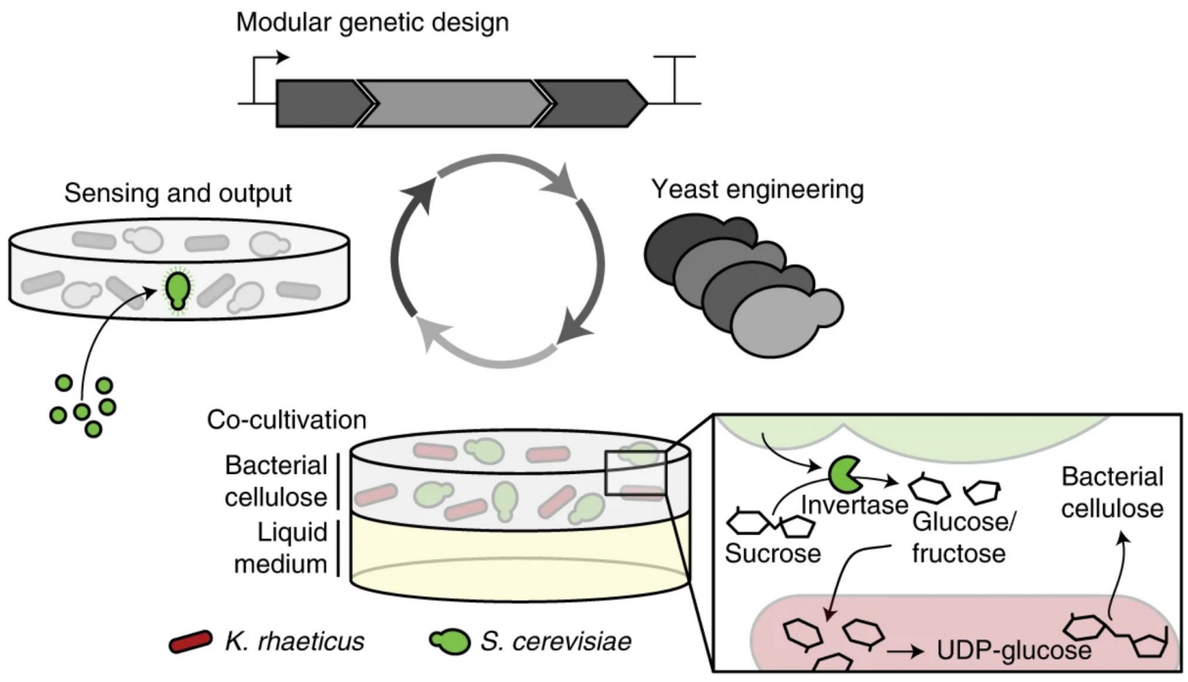


Left image: Pellicles grown with either wild-type or BED-responsive (yGPH093) yeast embedded in the BC matrix. BED detection is visualized through GFP fluorescence. Right image: BED-responsive pellicles containing the yCG23 strain were assayed for laccase activity using colorimetric ABTS[1].

Discussion and outlook

We demonstrated that a Syn-SCOBY approach could be used as a novel way to grow ELMs with self-assembling, responsive, and adaptable properties. The ELMs could be used as biosensors able to detect pathogens[1] and environmental pollutants[2]. However, further testing is needed to study the long-term stability and reusability of these ELMs. Additionally, real-world applications may require more functionalities embedded in the Syn-SCOBY materials.

Conditions for Syn–SCOBY growth



Invertase is secreated by yeast, favoring BC production. Yeast can be further engineered to functionalize the BC matrix.

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Group 10 : Théo Altherr, Éric Greulich, Osama Ounallah Khamis

COMPLEX LIVING MATERIALS MADE BY LIGHT-BASED PRINTING OF GENETICALLY PROGRAMMED BACTERIA

MARCO R. BINELLI, ANTON KAN, LUIS E. A. ROZAS, GIOVANNI PISATURO, NAMITA PRAKASH, ANDRÉ R. STUDART

1. INTRODUCTION

As it is well known, engineered living materials present unique opportunities for creating structures with self-healing, regenerating and adaptive properties. By using microorganisms and embedding bacteria and other microbes within synthetic matrices, such as hydrogels, these materials can dynamically interact with their environment, enabling applications across various fields, from robotics to bio-medicine.

In this research, biocompatible hydrogels are designed and optimized for light-based 3D printing to create complex, bacteria-laden structures with functional properties. Specifically, "living inks" are developed by incorporating bioluminescent bacteria for light emission and melanin-producing bacteria to provide a visual response upon chemical stimulation. These inks are combined within a single hydrogel matrix to produce a self-powered sensing device that displays clear visual cues in reaction to environmental changes. This method establishes a sustainable, reproducible pathway for creating programmable engineered living materials that offer potential for further genetic modifications that could expand their functionality across various different fields.

2. RESULTS AND DISCUSSION

Two types of photopolymerization-based 3D printing were used: digital light processing (DLP), a layer-by-layer process that enables to print complex shapes from a single ink with high resolution, and volumetric printing (VP), a process which allows to cure an entire 3D printed object in one go.

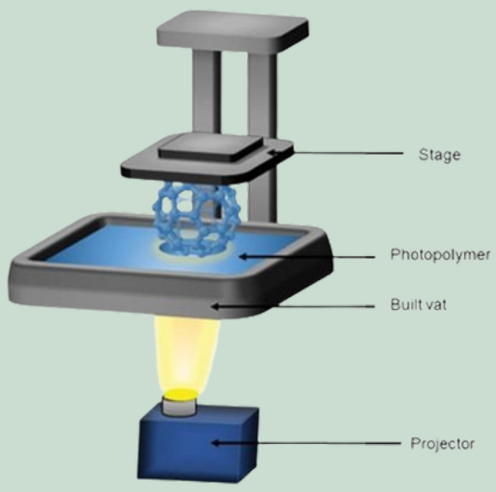

Figure 1: Schematics of DLP setup used for printing of bacteria containing structures

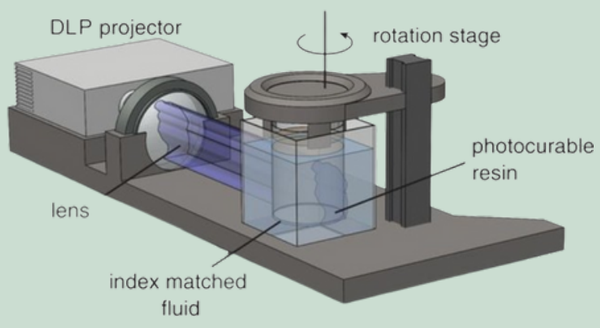

Figure 2: Schematics of Volumetric printing process

The ink used was a mixture of methacrylated hyaluronic acid (HAMA), ensuring cell viability and a methacrylated PEO-PPO-PEO copolymer (PluDMA) ensuring sufficient mechanical strength after polymerization through dense crosslinking. Pre-cultured P. kishitanii and genetically modified E.coli were then added to obtain two bacteria-laden inks. One with bioluminescent properties and the latter with chemical sensing (IPTG) properties through melanin formation.

After assessing that both these inks result in active bacteria containing structures (luminescence and production of melanin when exposed to IPTG), the high resolution of DLP and the geometry influence on the bioactivity was clearly shown with the bioluminescent ink.

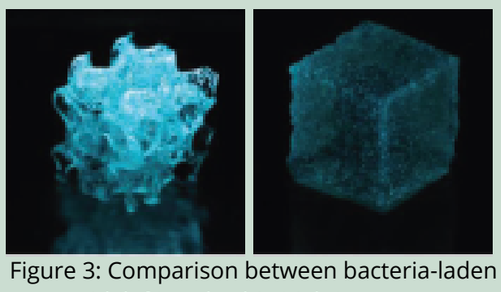

Figure 3: Comparison between bacteria-laden gyroid (left) and cubic (right) structures

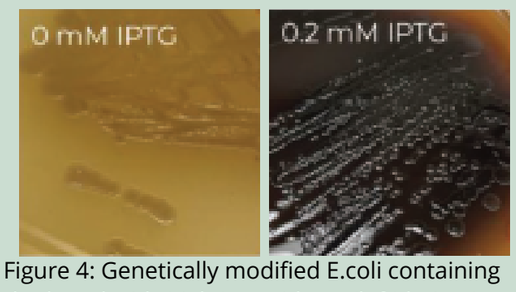

Figure 4: Genetically modified E.coli containing hydrogel with (right) or without (left) being in contact with IPTG

Finally, using VP, a structure containing both materials, was printed. An outer shell containing E.coli was firstly formed, later on filled by a P. kishitanii loaded hydrogel. Since the ink base is the same, this structure maintained its structural integrity between both phases, while still showing the living microorganisms activity.

Figure 5: Printed structure showing the evolution of darkening of the outer shell and bioluminescence of the filling when in contact with IPTG

3. CONCLUSION

This research has proven that light-based 3D printing of hydrogel loaded with microorganisms was possible. In this study, two different bacteria with two antagonist properties were embedded in a hydrogel in order to create a "metabolically powered chemical sensor". This article is really interesting because it proves that we can create materials with precise and complex shapes and structures that exhibit natural properties and behaviours.

Regarding the applications, the number of possibilities is very large, as many different bacteria could be used in this technology, as well as different genes they possess, and they can all be genetically modified to serve a certain purpose.

We think it could be interesting to use this technology in the biomedical field. Indeed, the hydrogel matrix is a biocompatible system, and the different bacteria embedded in it could be chosen or genetically modified to act together as a visual detector for specific diseases or toxic substances.

Again, because this technology seems to be very versatile and modulable, many different options are possible. Adjusting the hydrogel matrix or changing the bacteria could result in a living system with completely different properties and uses. This paper shows that understanding living materials and finding ways to tweak them to use them to our advantage is becoming a crucial part of engineering in many domains.

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Development of a photosynthetic hydrogel as potential wound dressing for the local delivery of oxygen and bioactive molecules

Rocío Corrales-Orovio, Felipe Carvajal, Christopher Holmes et al.

Introduction

The ideal wound dressing…[1] → **HYDROGELS**

…is biocompatible
…maintains local moisture
…avoids microbial contamination
…has sufficient mechanical strength
…can be easily & painlessly removed

HBOT, TOT[2]	No consistent/significant results
H_2O_2, CaO_2, PFC	Toxic, unstable, short term 0_2 delivery
C. reinhardtii	Decrease tissue hypoxia, biocompatible

Oxygen in wound healing process…[3]

…facilitates oxidative killing of bacteria
…supports aerobic cell metabolism
…enhances collagen maturation
…promotes angiogenesis

Methods

A. Cultivation

C. reinhardtii was grown photomixotrophically in TAP, at 20°C and constant illumination.

B. Polymerization

GDL is added to initiate polymerization (H+ release)

Internal gelation happens through alginate Ca^{2+}-induced crosslinking

External gelation for enhancement of hydrogel mechanical properties

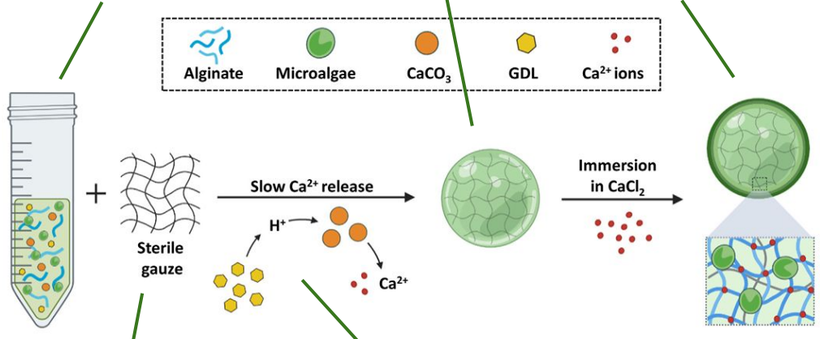

Fig. 1 Photosynthetic hydrogel fabrication.

Gauze acts as a secondary network for enhanced strength

Decrease in pH causes Ca^{2+} release

C. Characterisation

Swelling - measured mass of drying hydrogels characterised through swelling ratio = $m_{swollen}/m_{initial}$

Mechanical properties - evaluated through compression testing & confirmed with SEM imaging

***In vitro/in vivo* testing** - conducted respectively on human fibroblasts/zebrafish larvae"

Skin irritation - tested by application on healthy skin

Oxygen production - evaluated by oxygraphy in darkness and light

Bioactive molecules release - HG's ability to release cefazolin & GM algae production of VEGF were monitored

Results & Interpretation

Swelling behavior

- 65% shrinkage after 72h usage
- Presence of *C. r.* does not affect the swelling behavior
- HG is not expected to shrink in clinical essay due to the moist environment[4]. It could even be dried by half to improve exudate absorption in wounds

In vitro & *In vivo* testing

- HG + *C. r.* showed great biocompatibility
- Photosynthetic HG has the capacity to sustain the metabolic oxygen requirements of zebrafish larvae and skin explants

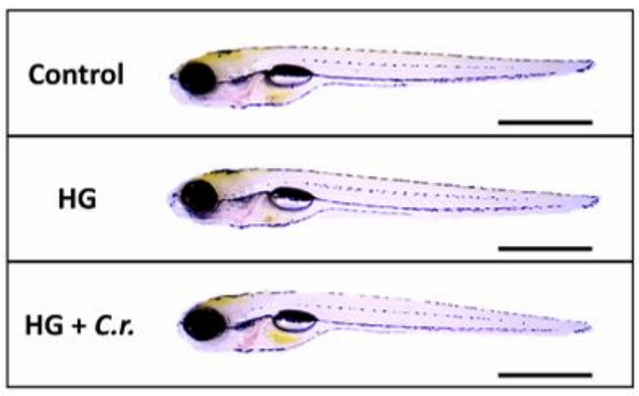

Fig. 2 HG-exposed zebra larvae survival and good health was verified.

Mechanical properties & Microstructure

- SEM images show the gauze layer was properly integrated in the polymer structure, not affecting the crosslinking pattern. In return, no interference of the algae on the gauze
- A rougher surface in HG + *C. r.* shows the successful encapsulation of the algae in the alginate

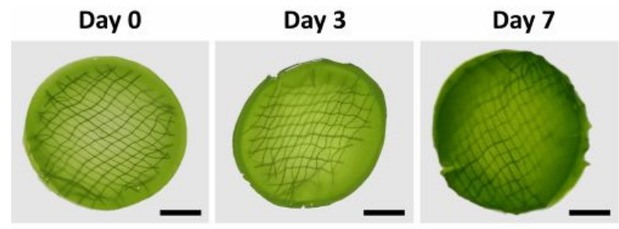

Fig. 3 HG + C. r. patch appearance after different use periods.

- Pore diameter and distribution is unaffected by the presence of *C. r.*
- Uniaxial compression test shows no change of $E_{Compression}$ upon *C. r.* addition
- Stability of the HG + *C. r.* is ensured for up to 7 days

Skin irritation

- Tested on 20 human volunteers, HG + *C. r.* showed no particular sign of irritation
- Calculated irritation index of HG + *C. r.* is lower than commercially available dressings

Cefazolin release & antibiotic effect

- Progressive drug release; 68% after 2 hours & 80% after 72 hours
- No impact of *C. r.* on drug release

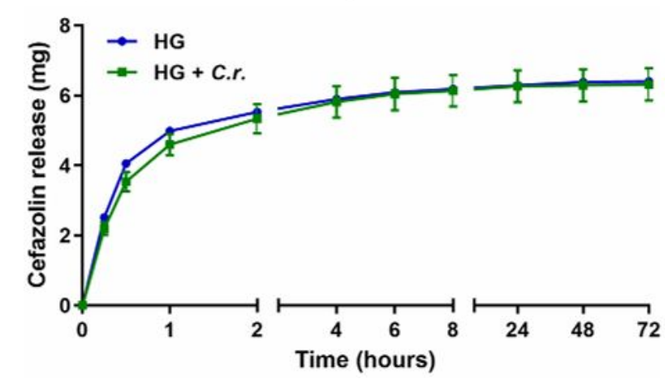

Fig. 4 Cefazolin release rate in HG and HG + C. r.

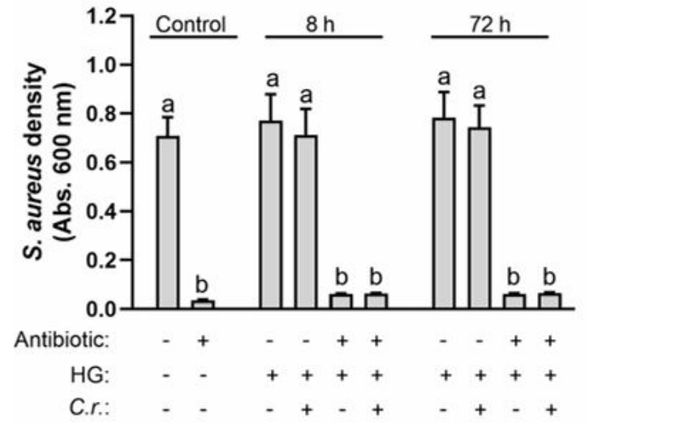

Fig. 5 Functionality of the released cefazolin.

- Cefazolin released after 8 & 72 hours is as effective as freshly produced one

VEGF release

- Constant release (& production) of VEGF for 7 days

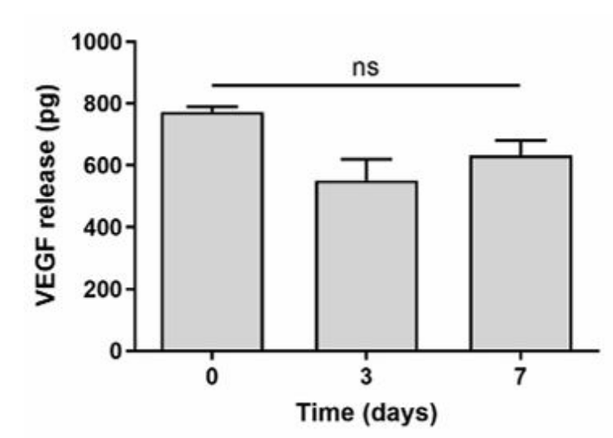

Fig. 6 VEGF release rate over time.

Oxygen production

- Optimal O_2 production $5 \cdot 10^7$ algae/mL

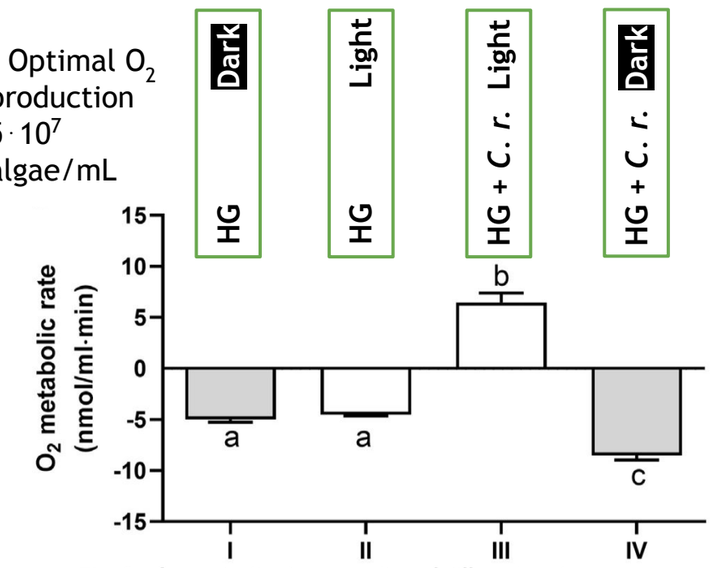

Fig. 6 O_2 production/consumption of different systems.

Conclusions

Takeaways

Biocompatibility: HG + *C. r.* is biocompatible, showing no skin irritation on human volunteers and allowing survival of zebrafish larvae.

Oxygenation: Light-exposed HG + *C. r.* showed oxygen release capability.

Bioactive molecules release: Embedded cefazolin and GM *C. r.*-produced VEGF are successfully released without affecting HG properties.

Structural stability: Crosslinking technique & addition of gauze resulted in preservation of mechanical properties & great stability.

Limitations & further research

Relevance of oxygen production: Evaluate whether the threshold algae density to sustain the oxygen requirements of a wound is practically reachable.

Extended testing: Further research using different bioactive molecules & thorough *in vivo*/*in vitro* testing on other species.

Scalability and storage: Address production and storage challenges to enable clinical use.

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Group 11

Virus Disinfection from Environmental Water Sources Using Living Engineered Biofilm Materials [1]

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École Polytechnique Fédérale de Lausanne (EPFL)
MSE-493 Engineered Living Materials, Poster Assignment

1.Introduction

Virus outbreaks are reported every year and are of primary concerns for public health In 2003, the World Health Organization estimated that worldwide **3.4 million deaths each year** can be attributed to water-related transmission of pathogens [2]. In that sense, water-treatment technologies play a crucial role and need constant improvements.

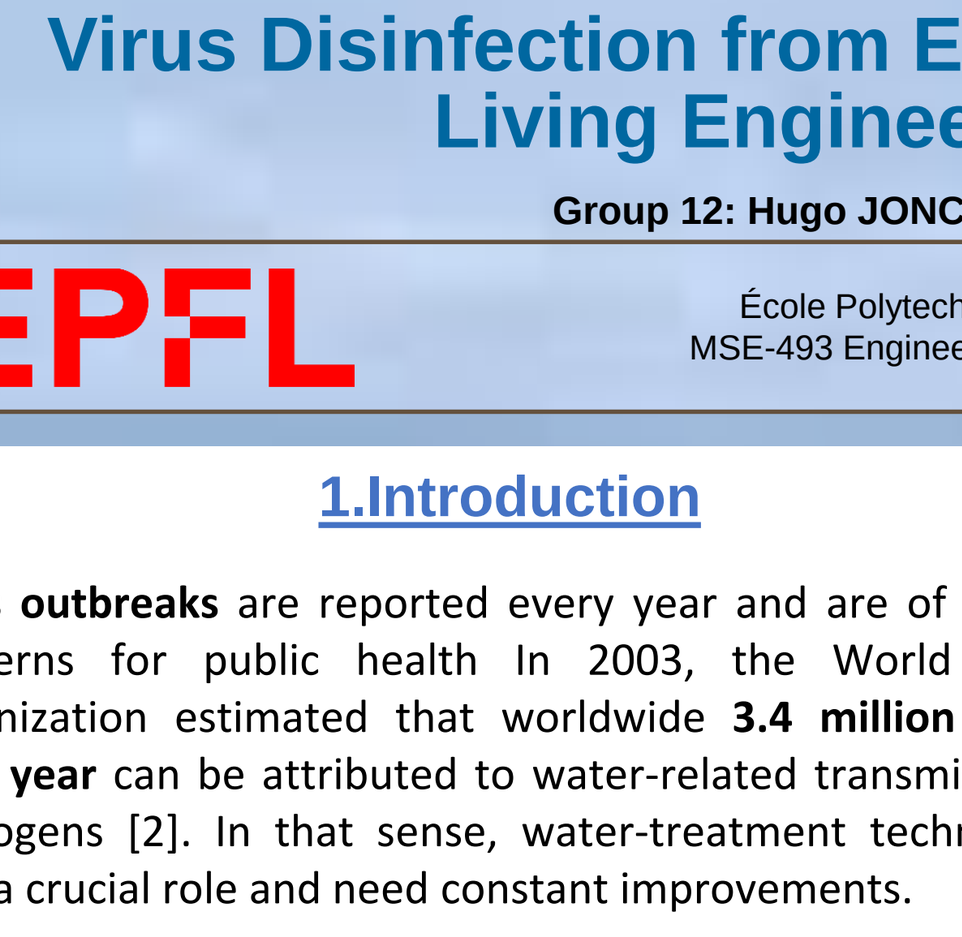


Figure 1: Schematic of *E.Coli* biofilm binding waterborne viruses.[1]

2.Biofilm Formation

1. Genetic engineering of ***E.coli***, introduction of gene coding for **C5 peptide** capable of binding to Hemagglutinin (HA) of H1N1 virus.
2. *E.Coli* synthesizes **CsgA-C5** protein monomers.
3. Self-assembly into amyloids.
4. Forms a biofilm which binds to HA.

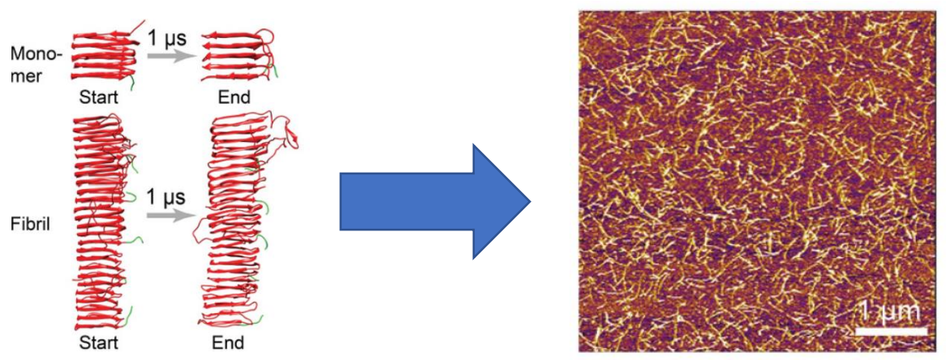


Figure 2: Simulated structures of CsgA-C5 monomer and fibril (left) and AFM image showing the morphology of self-assembled CsgA-C5 fibers (right).[1]

Hemagglutinin (HA): glycoprotein found on the surface of influenza viruses.
Amyloids : rigid protein aggregates with the cross-β structure, resistant to most solvents and proteases.

3.Virus Capture

HA-C5 Binding

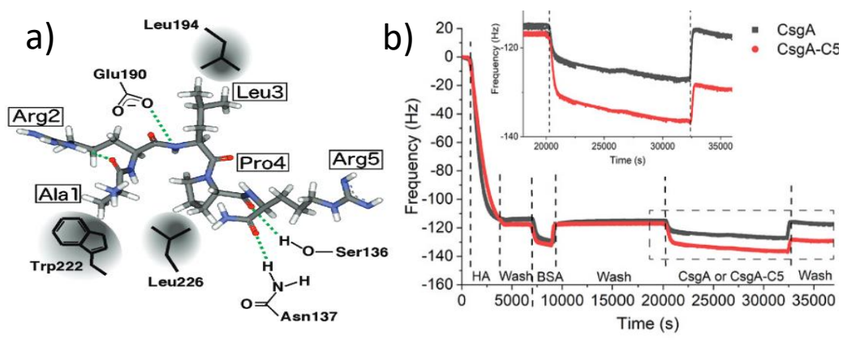


Figure 3: a) Proposed C5-HA binding mechanisms (molecular dynamics).[3] b) QCM analysis of the affinity strength between CsgA-C5 monomers and hemagglutinin.[1]

Virus binding in Aqueous Solutions

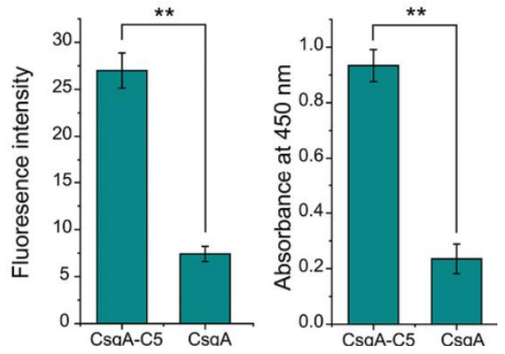


Figure 4: Immunofluorescence intensity (left) and ELISA (right) analysis to assess the binding of CsgA-C5 (and CsgA) nanofibers with whole virus particles.[1]

River water disinfection

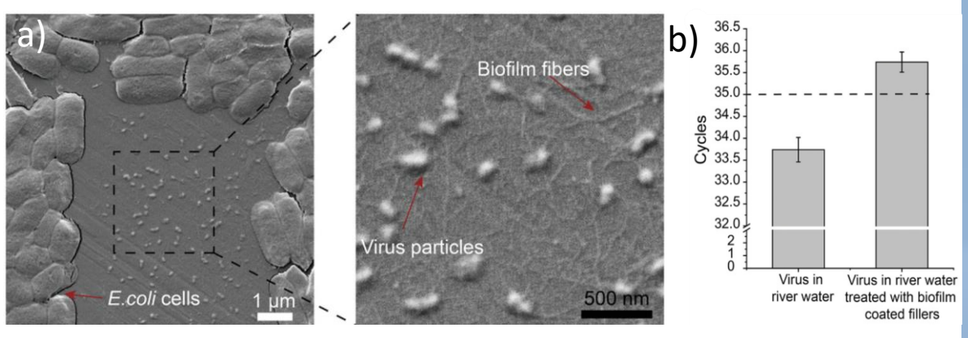
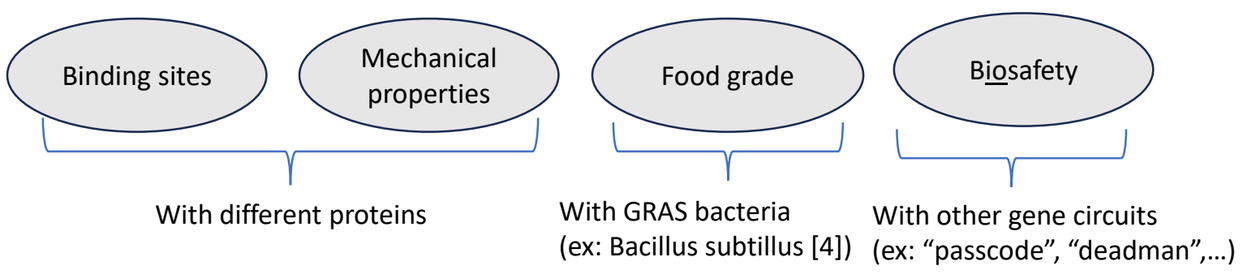


Figure 5: a) SEM images of virus bound to CsgA-C5 biofilm b) qPCR analysis of infected river water without and with CsgA-C5.[1]

4.Conclusion and Future Perspectives

- CsgA-C5 biofilm displays great **virus-binding** properties.
- ELM alternatives are promising for virus **disinfection** application.

Tunable parameters



5.Critical Analysis

- **Clear** and well explained
- No comparison of **efficiency** regarding other methods
- Retrieval of **fillers** and disposal of captured viruses not mentioned

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