

The background of the slide is a high-magnification scanning electron micrograph (SEM) showing a dense, granular surface texture with many small, rounded features and some elongated structures.

# **Tibbitt paper MSE 493**

**Prof. Tiffany Abitbol**  
**2024**

# Title (break down)

## Dual carbon sequestration with photosynthetic living materials



### What is a "good" title?

The title will be read by many people. Only a few will read the entire paper, therefore all words in the title should be chosen with care. Too short a title is not helpful to the potential reader. However too long a title can sometimes be even less meaningful. Remember a title is not an abstract. Also a title is not a sentence.

Goals:

- Fewest possible words that describe the contents of the paper.
- Avoid waste words like "Studies on", or "Investigations on"
- Use specific terms rather than general
- Watch your word order and syntax
- Avoid abbreviations and jargon

# What's actually supposed to be in an abstract?

## What is an abstract?

There are as many kinds of abstracts as there are types of research papers. The classic abstract is usually a "Informative" abstract. This kind of abstract communicates compressed information and includes the purpose, methods, and scope of the article. They are usually short (250 words or less) and allow the reader to decide whether they want to read the article.

The goal is to communicate:

- What was done?
- Why was it done?
- How was it done?
- What was found?
- What is the significance of the findings?

242 words

# Abstract (break down)

What is not here?

4

Experimental details! (and in most cases, they shouldn't be)

**What was done?:** dual CO<sub>2</sub> sequestration

**Why was it done?**

- As a way to permanently remove CO<sub>2</sub> from the atmosphere

**How was it done?** Immobilizing photosynthetic microorganisms within a printable polymeric network

**What was found?**

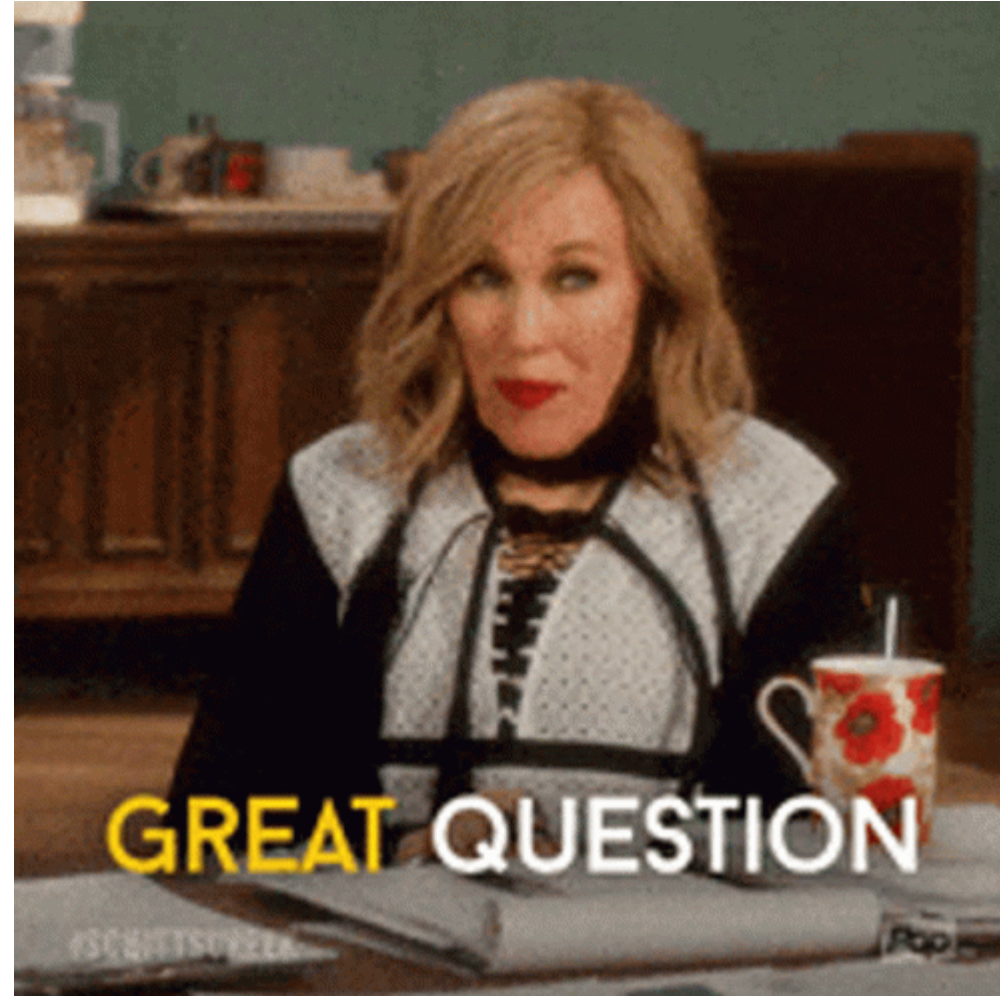
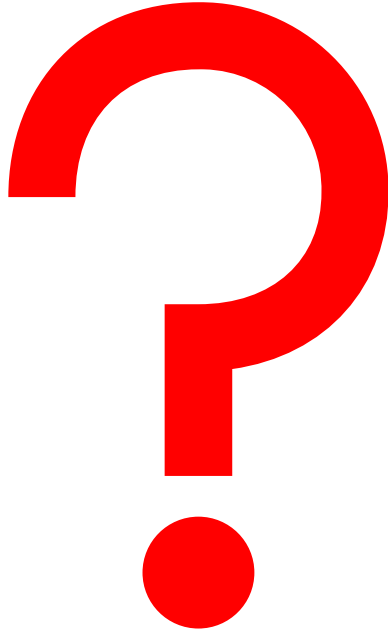
- Carbon sequestration resulted in biomass production
- Metabolic production of OH<sup>-</sup> ions promoted insoluble carbonate formation via MICP
- Digital design and fabrication ensured sufficient access to light and nutrient transport
- Sequestered approximately 2.5 mg of CO<sub>2</sub> per gram of hydrogel material over 30 days, with  $2.2 \pm 0.9$  mg stored as insoluble carbonates
- Over 400 days, the living materials sequestered  $26 \pm 7$  mg of CO<sub>2</sub> per gram of hydrogel material in the form of stable minerals

**What is the significance of the findings?**

- Potential for scalable carbon sequestration, carbon-neutral infrastructure, and green building materials
- Photosynthetic living materials as a complementary strategy to mitigate CO<sub>2</sub> emissions



# Why “Dual carbon sequestration?”



# Why “Dual carbon sequestration?”

- Biomass growth (reversible)
- Carbonate formation (irreversible)

Duh

Biological ecosystems, such as forests, aquatic systems, and wetlands, offer efficient pathways for carbon sequestration (storing carbon in a carbon pool) and conversion into carbon-based materials<sup>1</sup>. Natural systems operate under ambient conditions with sunlight and commonly available small molecules as their sole inputs. Further, living systems can sense self-repair, and respond to their surroundings, making them resilient to environmental changes<sup>1,2</sup>. Biological carbon sequestration, for example via afforestation or the growth of marine phytoplankton and algae, is also cost-efficient and environmentally-friendly<sup>3</sup>. In this context, natural carbon sequestration can serve as a passive, low-impact complement to industrial carbon sequestration, which normally requires specific, extreme, and energy-intensive conditions<sup>1,4</sup> and proximity to large emission sources<sup>5</sup>. However, natural carbon sequestration is typically slower than industrial carbon sequestration, and the control of living systems outside of their native environments is often challenging<sup>5,6</sup>.

Strategies to engineer living systems for active CO<sub>2</sub> sequestration would provide an additional approach to mitigate the accumulation of human-generated CO<sub>2</sub> in the atmosphere. The CO<sub>2</sub> concentrating mechanism of many photosynthetic microorganisms accumulates CO<sub>2</sub> within the cell body up to 1000-fold above ambient levels<sup>6,7</sup>. Subsequently, concentrated carbon can be fixed in the form of biomass generated during growth<sup>8,9</sup>. In addition to biomass production, microbially-induced calcium carbonate precipitation (MICP) in certain species can sequester CO<sub>2</sub> irreversibly in the form of inorganic carbonate precipitates. MICP proceeds via multiple metabolic pathways, including ureolysis, sulfate reduction, and denitrification<sup>10,11</sup>. In some organisms, MICP can occur as a direct by-product of photosynthesis, whereby the inorganic precipitates effectively act as an additional carbon sink<sup>12</sup>, enabling dual carbon sequestration. In this context, immobilizing photosynthetic microorganisms, such as algae and cyanobacteria, within a support matrix may provide an approach to drive biological CO<sub>2</sub> sequestration in the form of engineered photosynthetic living materials via dual carbon sequestration.

To date, engineered living materials have primarily been used for applications in biomedicine, sustainable materials production, and as living building materials<sup>13–17</sup>. For example, MICP has been exploited, primarily via ureolysis, to mechanically reinforce living materials based on the in situ formation of a stiff mineral phase<sup>18</sup>. Robust composites were produced via biomineralization using ureolytic MICP within a cellulose matrix<sup>19,20</sup>. Similarly, precipitate deposited in porous materials filled cracks and improved mechanical properties of composite building structures as well as consolidated soils<sup>21</sup>. Ureolytic MICP is attractive due to its short incubation period (typically 1–4 days), resistance to contamination, and rapid biomineralization; however, it poses substantial environmental concerns due to the associated production of large amounts (1–2 equimolar) of ammonia<sup>22</sup>. Further, ureolytic MICP requires a constant supply of urea and only proceeds in a narrow range of environmental conditions<sup>23–25</sup>. These challenges restrict the use of ureolytic MICP for long-term CO<sub>2</sub> sequestration<sup>26</sup>. Many of these limitations can be addressed with photosynthetic MICP, which requires no additional feedstocks and produces no toxic by-products<sup>18,25</sup>. Recently, photosynthetic MICP was used to design living building materials that mineralized over time<sup>17</sup>. While photosynthetic living

# Introduction

(Let's keep breaking things down)

# Elements of an introduction?

Mostly – I sort of missed the nature and scope?  
Where are they suggesting implementing the technology?



## What is a "good" introduction?

This is where you describe briefly and clearly why you are writing the paper. The introduction supplies sufficient background information for the reader to understand and evaluate the experiment you did. It also supplies a rationale for the study.

Goals:

- Present the problem and the proposed solution
- Presents nature and scope of the problem investigated
- Reviews the pertinent literature to orient the reader
- States the method of the experiment
- State the principle results of the experiment

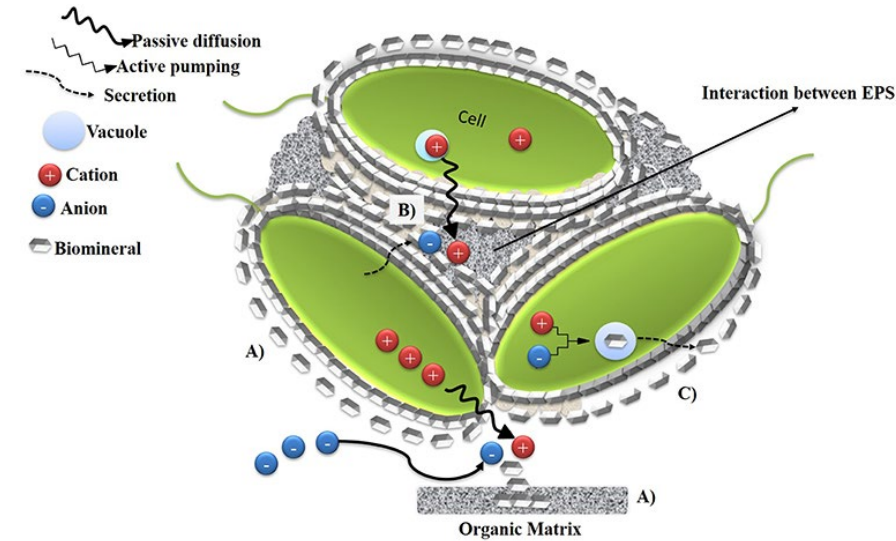


- Need to “mitigate the accumulation of human-generated CO<sub>2</sub> in the atmosphere”
- “Photosynthetic living materials have not been explored for CO<sub>2</sub> sequestration via biomass accumulation *and* irreversible MICP using atmospheric CO<sub>2</sub> as the main carbon source and light as the sole source of energy”

- Engineered living systems for active CO<sub>2</sub> sequestration as complement to other technologies
- “In this context, immobilizing photosynthetic microorganisms, such as algae and cyanobacteria, within a support matrix may provide an approach to drive biological CO<sub>2</sub> sequestration in the form of engineered photosynthetic living materials via dual carbon sequestration.”

# Nature and scope of the problem (sorta)

- ELMs mostly used in biomedicine, sustainable materials productions, and as living building materials (*is this fully true?*)
- Ureolytic MICP: reinforce living materials via mineral phase formation or fill cracks in composites and soils, disadvantage is build up of ammonia, need to constantly supply urea, narrow range of environmental conditions
- Photosynthetic MICP to the rescue! No additional feedstocks, no toxic byproducts
- ELM can be used to irreversibly fix  $\text{CO}_2$  into carbonates (CCS)



MICP

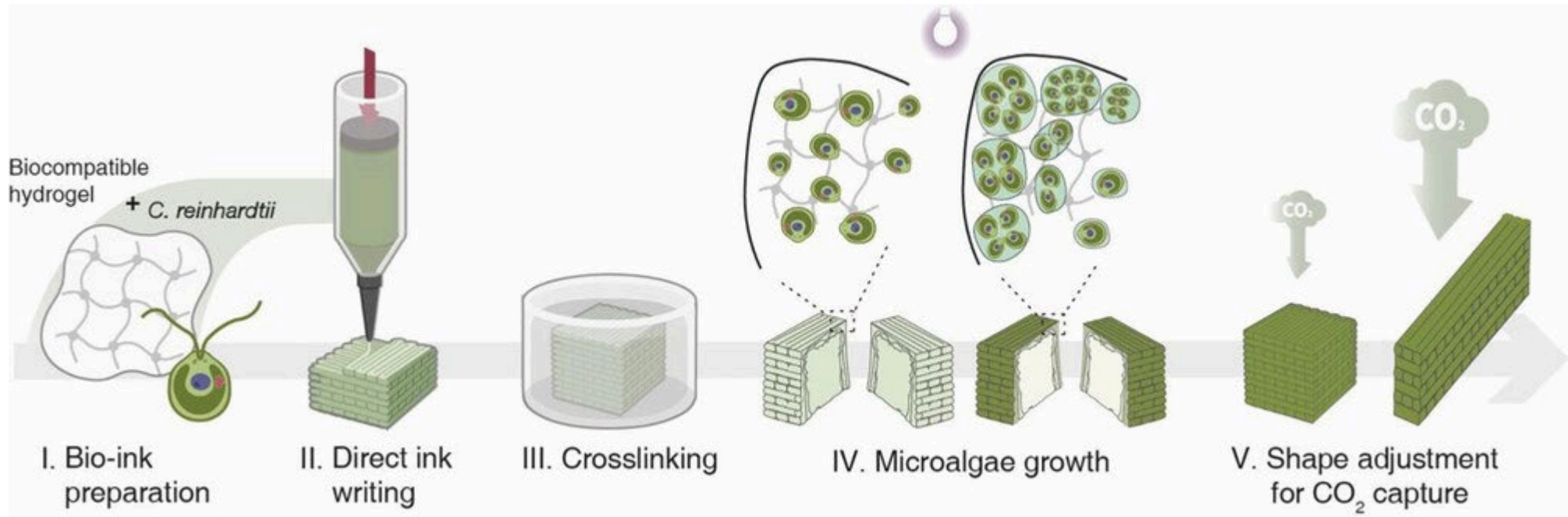
- C sequestration via photosynthesis
- Microalgae
- No MICP
- “reversible” upon biomass degradation

## RESEARCH ARTICLE

ADVANCED  
MATERIALS  
[www.advmat.de](http://www.advmat.de)

## Growth, Distribution, and Photosynthesis of *Chlamydomonas Reinhardtii* in 3D Hydrogels

Jeong-Joo Oh, Satya Ammu, Vivian Dorine Vriend, Roland Kieffer, Friedrich Hans Kleiner, Srikanth Balasubramanian, Elvin Karana, Kunal Masania,\* and Marie-Eve Aubin-Tam\*



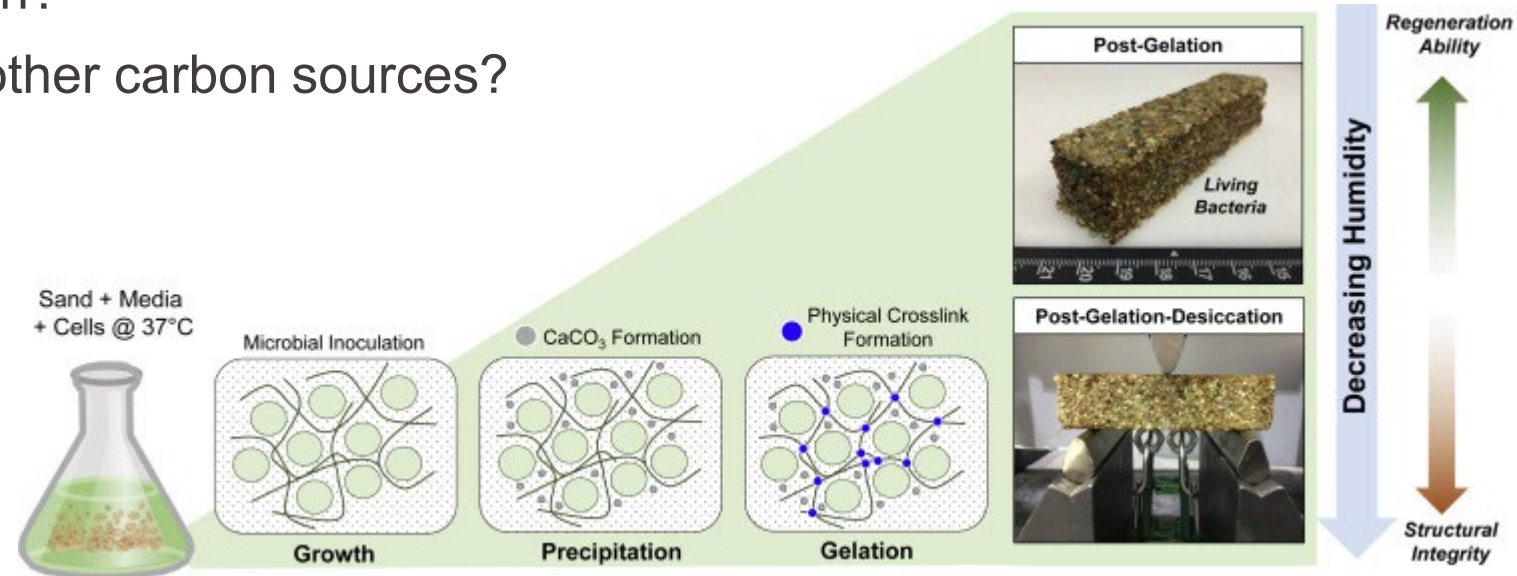
## Matter

### Article

# Biomining and Successive Regeneration of Engineered Living Building Materials

Chelsea M. Heveran,<sup>1,2</sup> Sarah L. Williams,<sup>6</sup> Jishen Qiu,<sup>1</sup> Juliana Artier,<sup>3</sup> Mija H. Hubler,<sup>1</sup> Sherri M. Cook,<sup>1</sup> Jeffrey C. Cameron,<sup>3,4,5</sup> and Wil V. Srubar III<sup>1,6,7,\*</sup>

- Carbon sequestration via photosynthesis
- MICP
- Cyanobacteria
- Isn't this also dual carbon sequestration?
- Media with other carbon sources?





Cyanobacterium *Synechococcus* sp. strain PCC 7002. **Why?**

- Can synthesize complex carbohydrates using light, inorganic nutrients found in seawater, and atmospheric CO<sub>2</sub> as the main carbon source
- Capable of photosynthetic MICP, exhibits a fast doubling time (~2.6 h under optimal conditions)
- Tolerates variations in light intensity and osmotic pressure

Pluronic F-127 (F127)-based hydrogel. **Why?**

- Bio-inert
- Transparent
- 3D printable – enables design to enhance access to light and nutrient exchange (open lattices, branched forms, and discrete pillars)

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- “Dual carbon sequestration via biomass generation and insoluble carbonate formation proceeded over the lifecycle (beyond one year) of the bio-printed structures.”
- “The mineral phase mechanically reinforced the living materials and stored sequestered carbon in a more stable form.”

A lot of supporting results in an SI section. No need to have everything in the main manuscript, only most important parts. A lot of journals limit the amount of figures.

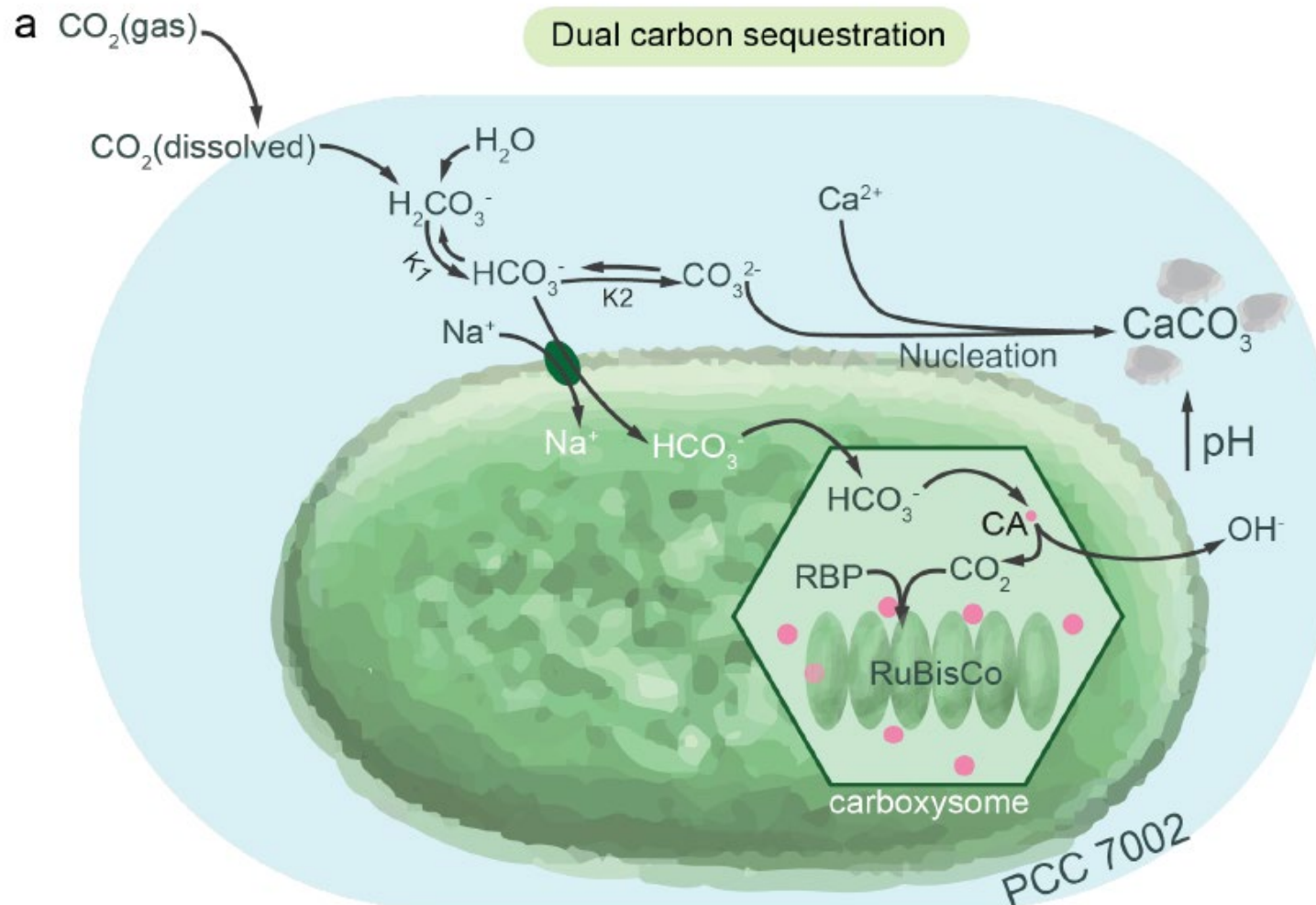
## Writing a "good" results section

This is the core of the paper. Don't start the results sections with methods you left out of the Materials and Methods section. You need to give an overall description of the experiments and present the data you found.

Goals:

- Factual statements supported by evidence. Short and sweet without excess words
- Present representative data rather than endlessly repetitive data
- Discuss variables only if they had an effect (positive or negative)
- Use meaningful statistics
- Avoid redundancy. If it is in the tables or captions you may not need to repeat it

# Now onto the results (not really a result, more like how cyanobacteria work)

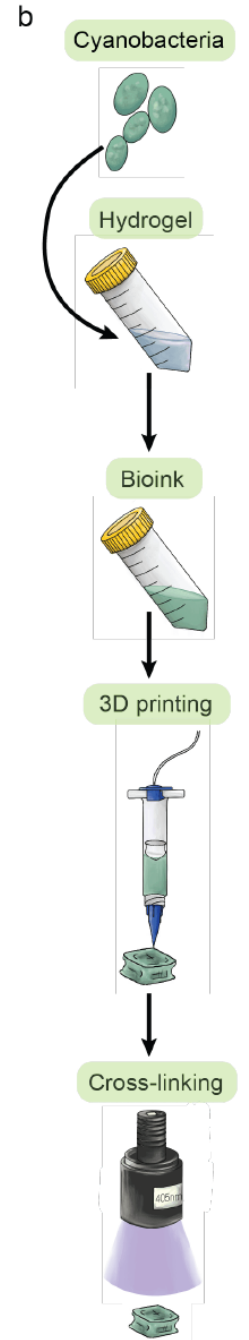


- $\text{CO}_2$  dissolves to give  $\text{HCO}_3^-$
- $\text{HCO}_3^-$  transported to “carboxysome”
- $\text{HCO}_3^-$  converted to  $\text{OH}^-$  and  $\text{CO}_2$  by carbonic anhydrase (CA)
- $\text{OH}^-$  secreted, local pH increases
- RuBisCo fixes  $\text{CO}_2$  into 2 molecules of phosphoglycerate, **which is enzymatically converted to sugars for biomass development**
- Local pH and anion EPS on cell membrane create a favorable environment for carbonate nucleation and growth
- In presence of  $\text{Mg}^{2+}$  or  $\text{Ca}^{2+}$ ,  $\text{CO}_3^{2-}$  is consumed and fixed as an insoluble carbonate

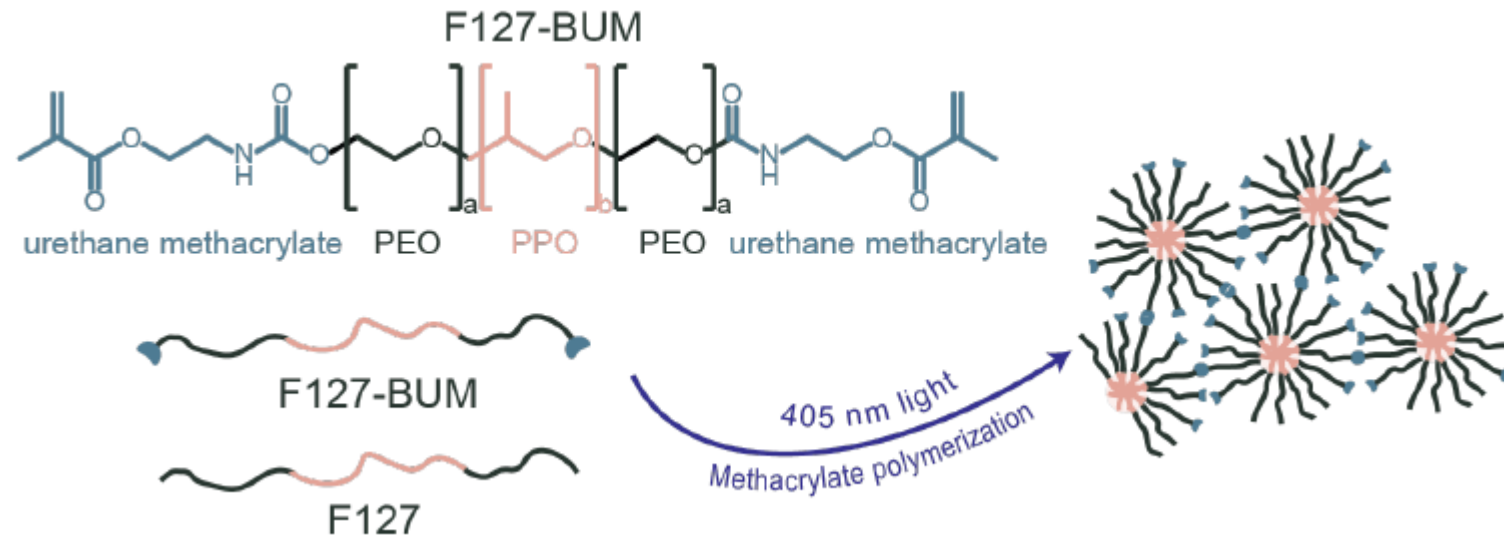


# Now onto the results

- A cute vertical schematic of concept of the work
- P7002 encapsulated into hydrogel to give bioink
- Give us more info on F127 – chosen because bioinert, processing versatility, easy diffusion of small molecules
- Bioink is 3D printed
- 3D printed object is photo crosslinked (?)

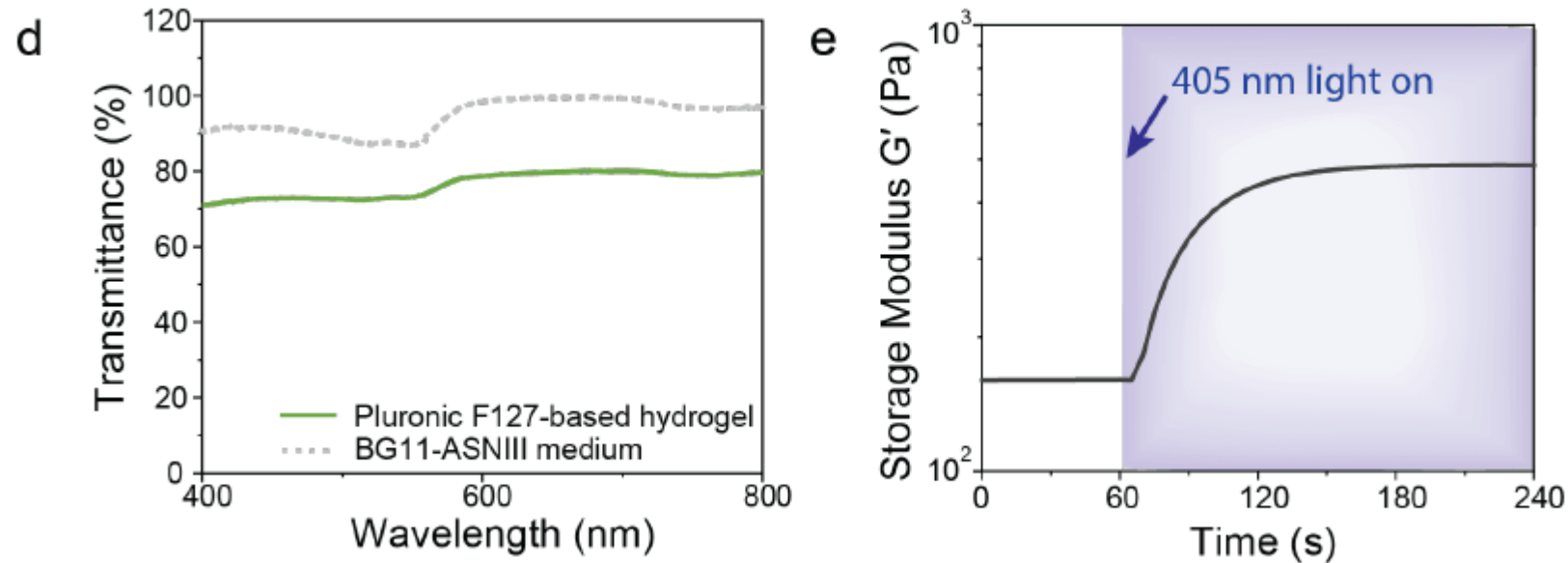


# Now onto the results (is this a result?)



- Bioink is actually made of 2 polymers
- F127 (13.2%) and F127-BUM (7.3%)
- High viability of 7002
- Good printability (DIW and light-based additive manufacturing)
- F127-BUM and LAP (?) synthesis described in ESI
- Cells suspended into ink by centrifugation

# Now onto the results (this looks like results)

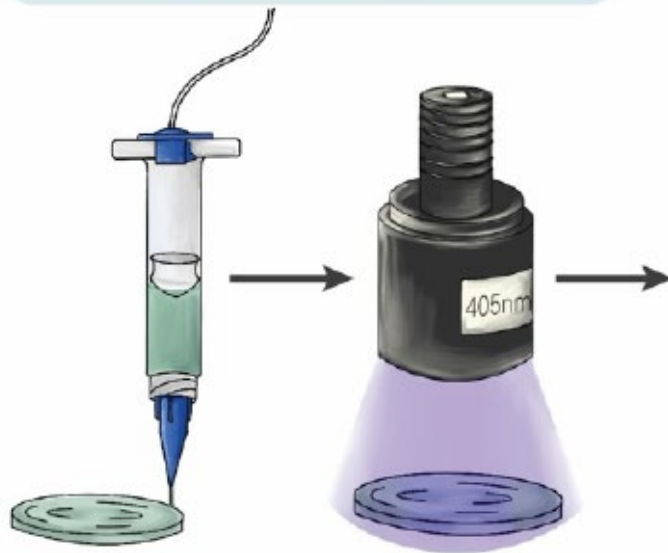


- Transparency is needed for photosynthesis
- Storage modulus increase with illumination at 405 nm due to photo crosslinking
- Any cells in this figure? I don't think so, just properties of the hydrogel
- They also say: "The ink exhibited shear-thinning and elastic recovery (~90%) after high shear, demonstrating its feasibility for extrusion-based printing" (SI section)...photo crosslinking for long term stabilization

# Now onto the results (dual sequestration schematic)

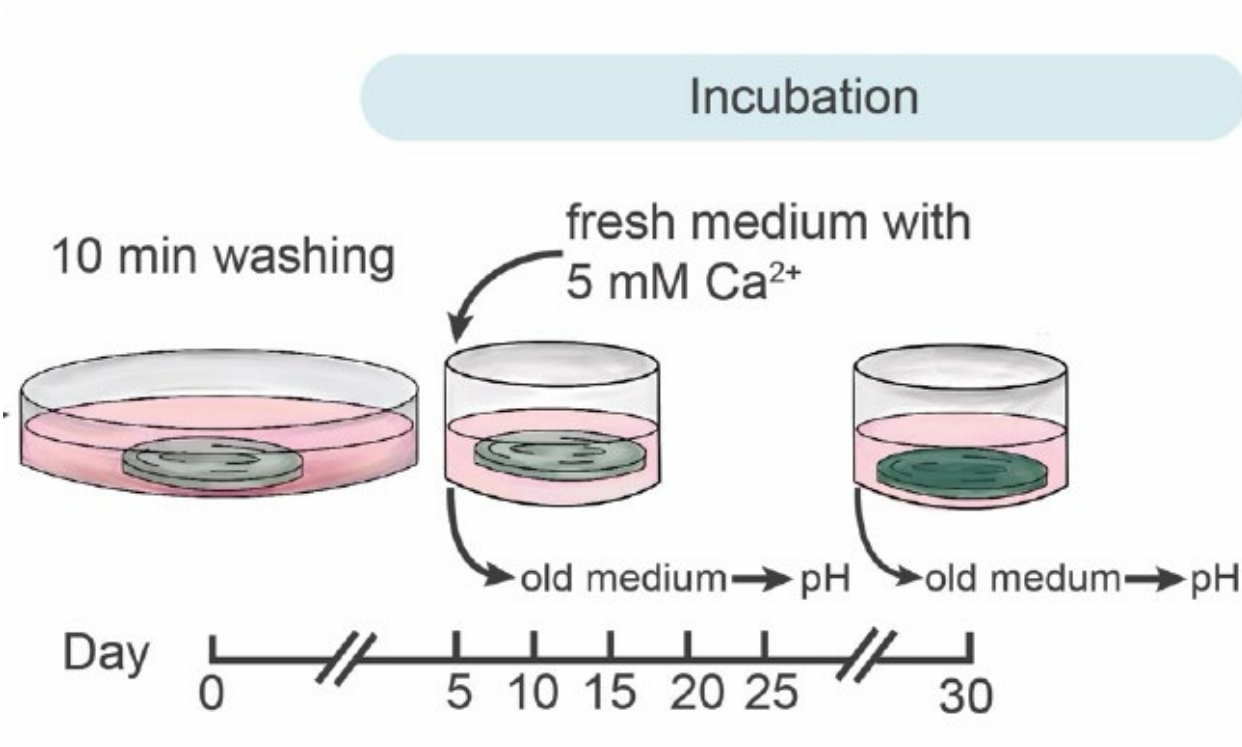
a

Printing and cross-linking



- 10 mm diameter discs; 40  $\mu$ L volume
- DIW and photocrosslinking

# Now onto the results (another schematic)



- Disks incubated for 30 days
- Media changed every 5 days
- From day 5, Ca<sup>2+</sup> in medium was set to 8.65 mM via CaCl<sub>2</sub> to mimic seawater
- All HCO<sub>3</sub><sup>-</sup> needed was provided by atmospheric CO<sub>2</sub>

# Now onto the results (yes!)

b

## Biomass generation

Day 0



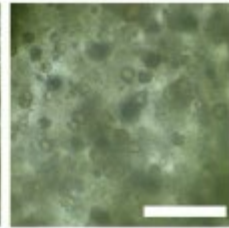
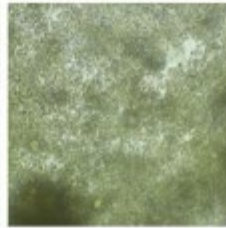
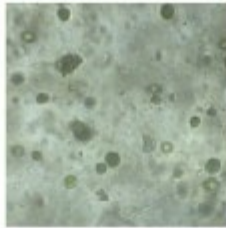
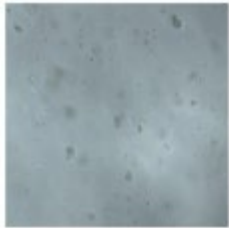
Day 10



Day 20



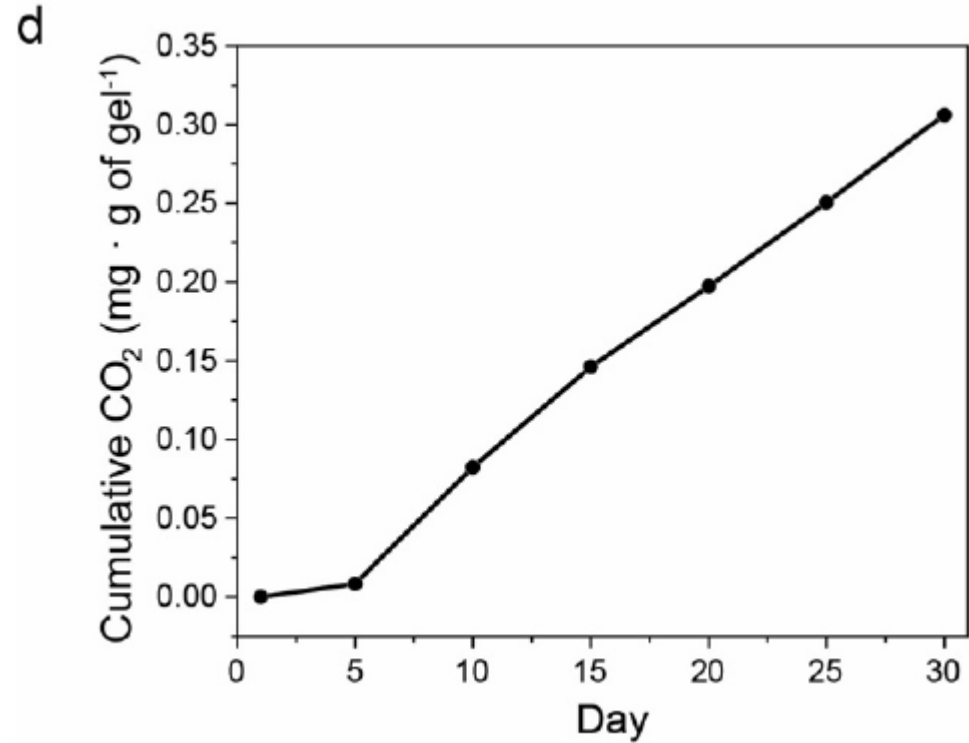
Day 30



- Biomass growth confirmed by microscopy and pH
- No pH change in abiotic
- *How does growth affect transparency/photosynthetic efficiency?*

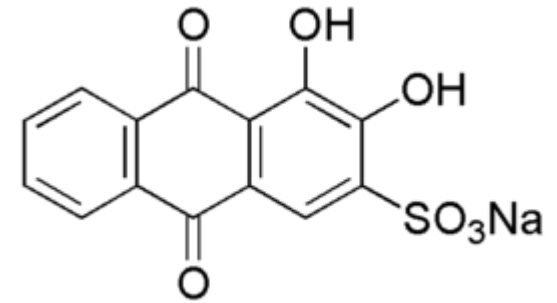


# Now onto the results (yes!)



- Cumulative CO<sub>2</sub> sequestered was calculated from the pH change (shown in SI)
- 0.31 mg/g living material of sequestered CO<sub>2</sub> after 30 days
- This is only due to biomass growth

- Water soluble, sodium salt
- Used to stain calcium deposits in tissues and to stain and differentiate carbonate minerals
- Goes from orange to red with binding
- Can stain other divalent ions
- Considered semi-quantitative

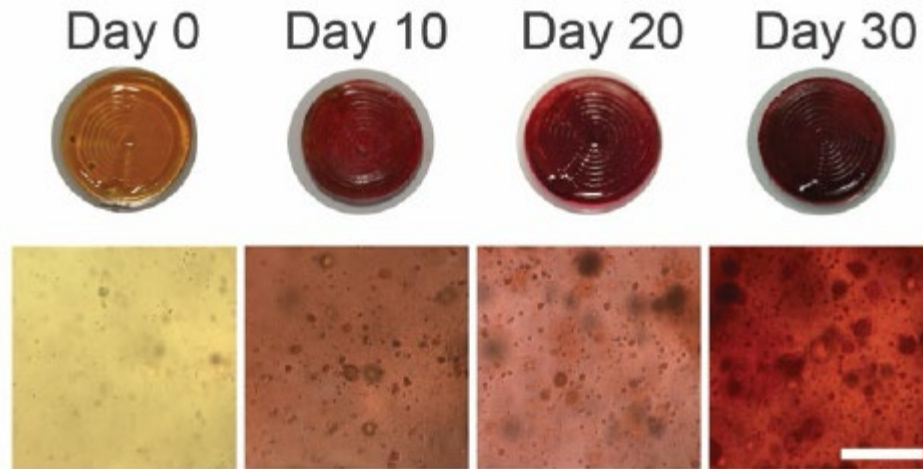


Alizarin Red

# Now onto the results (yes!)

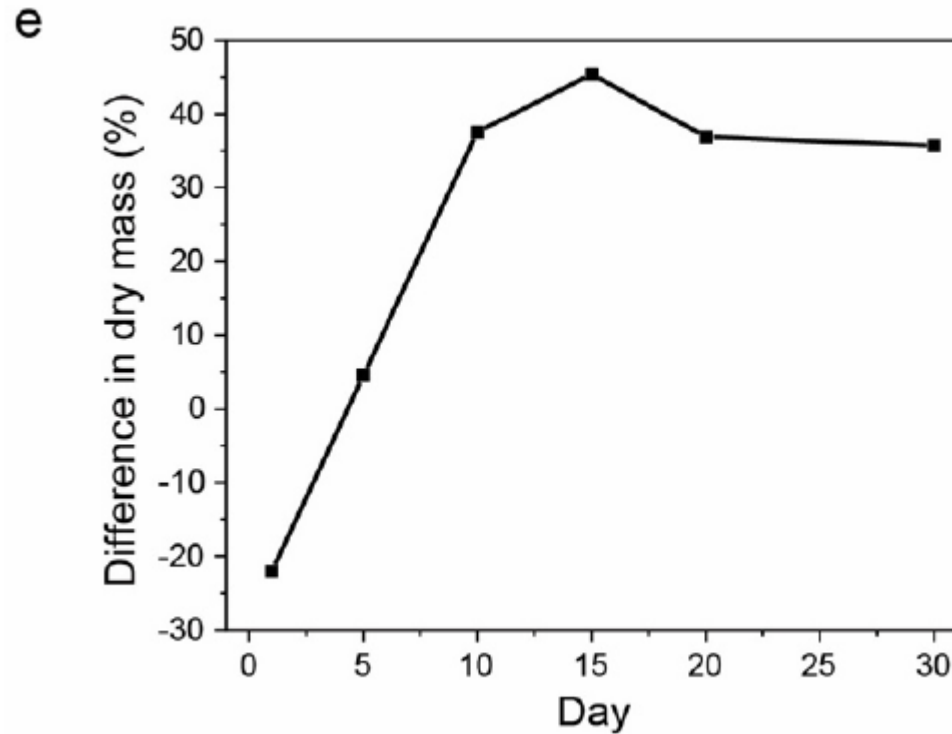
c

## Carbonate precipitation



- To confirm MICP, calcium staining with Alizarin red
- Abiotic samples (and day 0 samples) remain orange
- Biotic samples turn red at day 10 indicating  $\text{Ca}^{2+}$  accumulation

# Now onto the results (yes!)

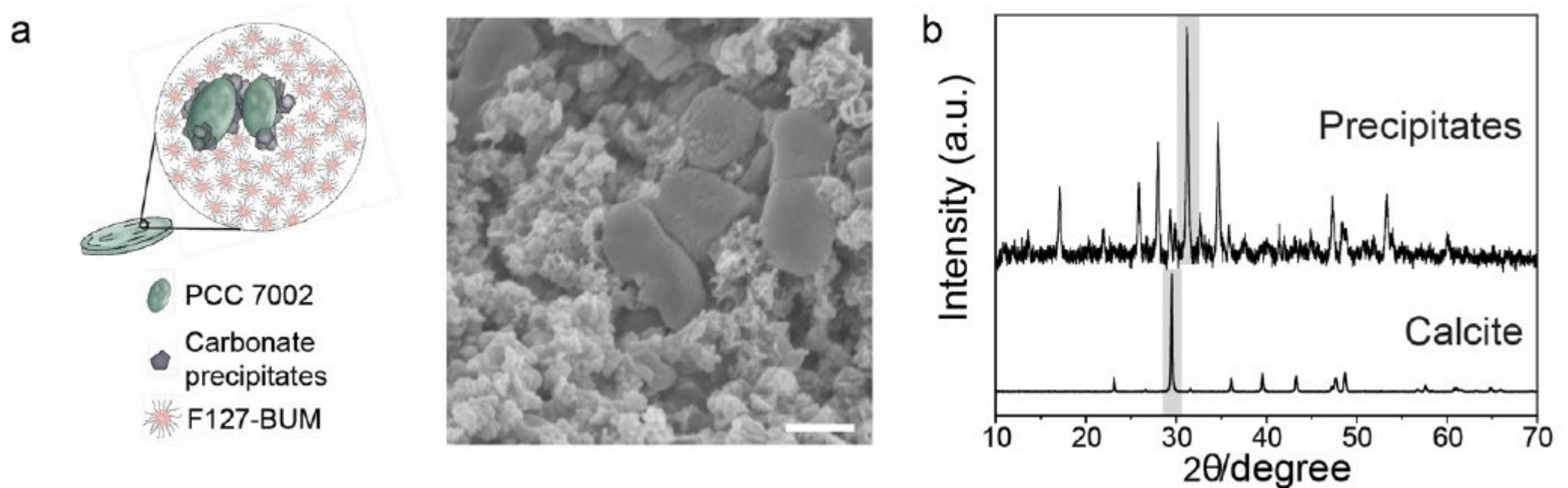


- To assess amount of biomass and precipitate compared biotic and abiotic samples
- Over 30 days, approx. 36% more mass in biotic sample
- Biomass and carbonate precipitates account for 45% of final sample mass

# Now onto the results (yes!)

- How much is carbonate precipitates vs, biomass?
- Determined by thermal decomposition
- At 600 °C, whatever is left is the carbonate
- 50  $\mu\text{mol}$  ( $2.2 \pm 0.9$  mg) of  $\text{CO}_2$  sequestered via MICP per gram of hydrogel – 1 order of magnitude higher than what was achieved by biomass growth!

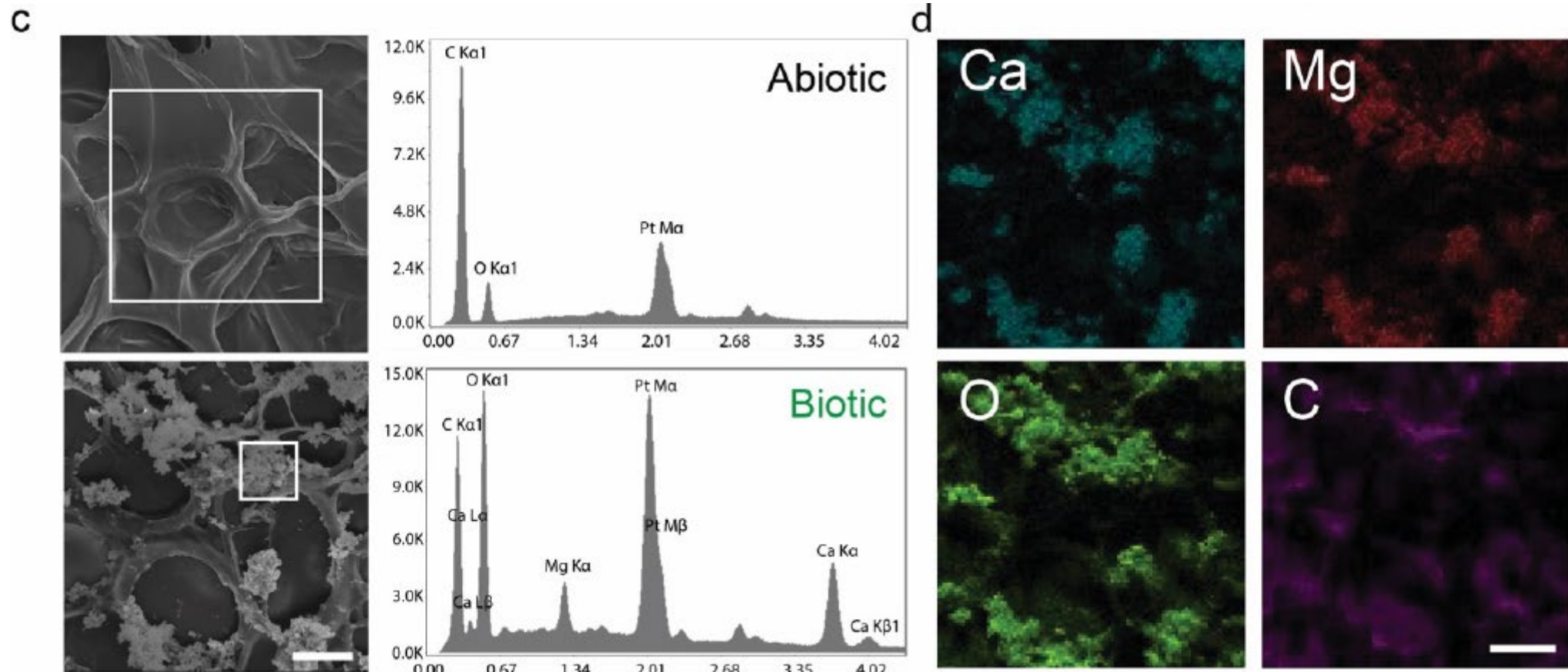
# Now onto the results (yes!)



- XRD confirms a crystalline calcite phase (done after thermal degradation)
- Shift in main diffraction peak at (104) plane (why? due to ANSII medium with magnesium and calcium ions)

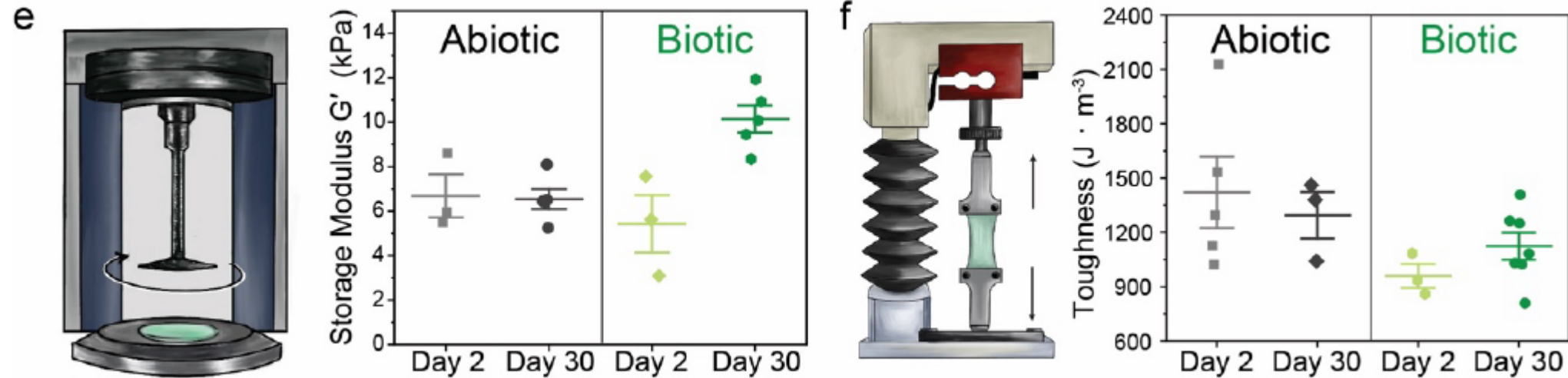


# Now onto the results (yes!)



- 20  $\mu$ m scale bars
- Abiotic EDS (energy dispersive spectroscopy) confirms “pericellular” carbonate formation

# Now onto the results (yes!)

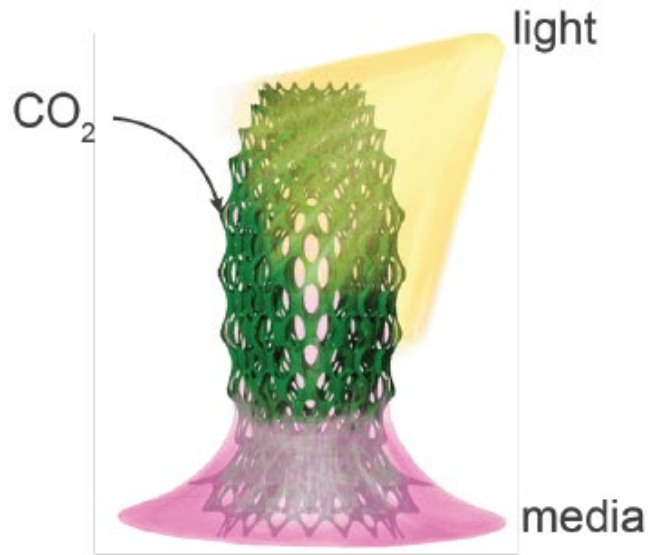


- $G'$  slightly lower on day 2 for biotic
- At day 30, see increase of  $G'$  for biotic, no change for abiotic
- Similar for toughness, day 30 biotic is tougher than day 2
- Attributed to reinforcing precipitates

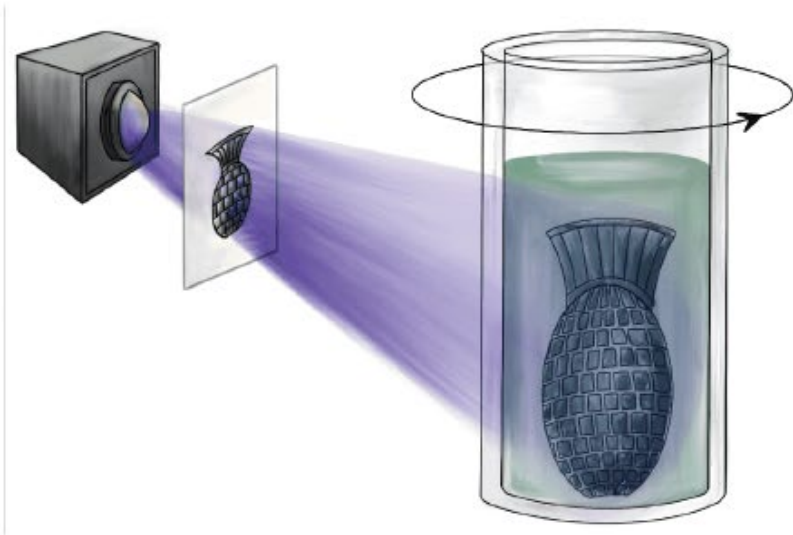
# Now onto the results (a schematic?)

a

Lattice structure design



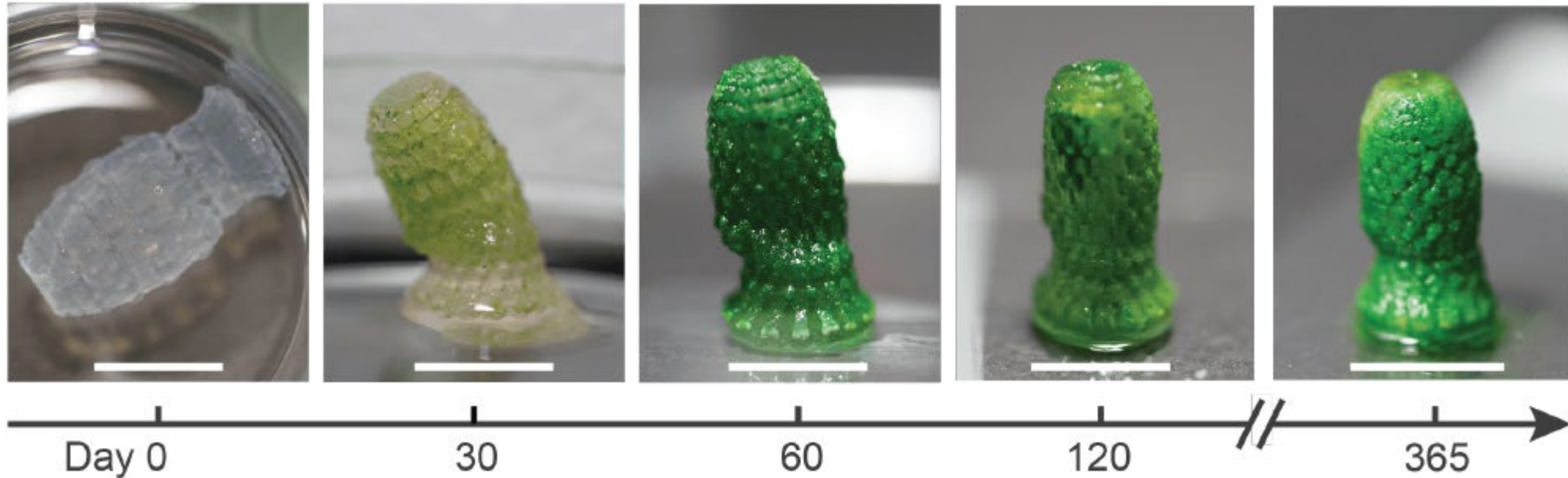
Volumetric printing



- Design to improve CO<sub>2</sub> sequestration and long term viability
- lattice structures with strut sizes between 0.15 mm and 0.70 mm to facilitate gas and nutrient transport
- Inspired by cellular fluidics, growth media passively transported by capillary action, full immersion not needed
- Volumetric printing for 1 step printing of object (not layer by layer)

# Now onto the results (yes!)

b

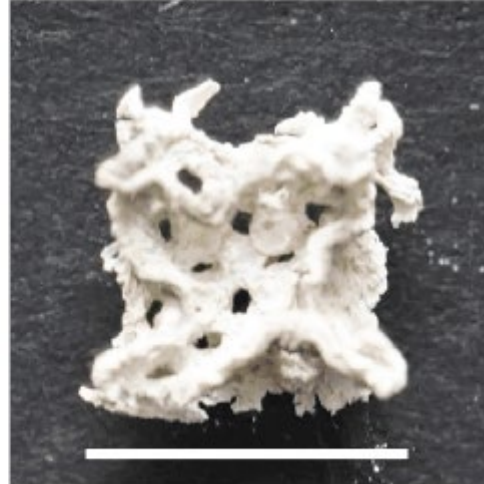
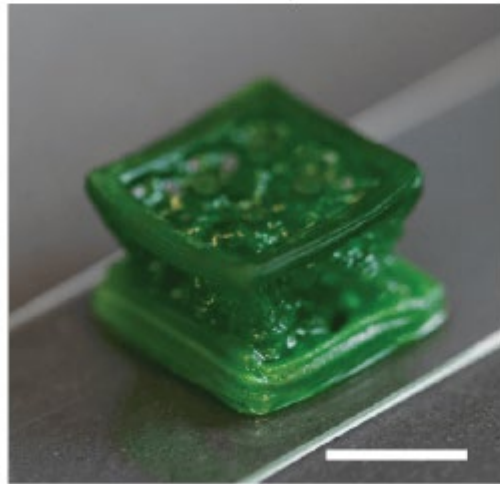


- Scale bar = 1 cm
- Volumetric printing for cm-scaled objects with complex geometries and an optical resolution of  $28 \times 28 \mu\text{m}$  within tens of seconds
- Viable for 1 year
- After 30 days, could stand upright and liquid actively drawn up
- Further stiffening with time

c

Thermal decomposition

600°C



- Thermally degraded after 60 days
- Remaining carbonates retained shape of porous structure
- Thus carbon sequestration deemed homogeneous, despite only partial immersion



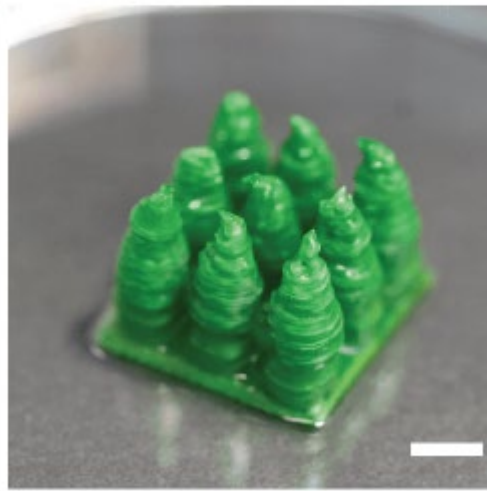
# Now onto the results (yes!)

## Textured surface

Day 0



Day 20



- 3 by 3 pillar array on a 2 by 2 cm base to “minimize self shielding)
- With this design, increased volume by 150%, without compromising viability
- Highlight synergy between living materials and design of living structures to increase carbon sequestration efficiency

## Writing a "good" discussion section

This is usually the hardest section to write. You are trying to bring out the true meaning of your data without being too long. Do not use words to conceal your facts or reasoning. Also do not repeat your results, this is a discussion.

Goals:

- Present principles, relationships and generalizations shown by the results
- Point out exceptions or lack of correlations. Define why you think this is so.
- Show how your results agree or disagree with previously published works
- Discuss the theoretical implications of your work as well as practical applications
- State your conclusions clearly. Summarize your evidence for each conclusion.
- Discuss the significance of the results



Positions their work in the larger context of ELMs and their potential e.g., growth, bioremediation

Recaps their work:

- printable photosynthetic ELMs for carbon sequestration via biomass growth and inorganic carbonate precipitation
- design strategies to enhance sequestration
- After 400 days, total CO<sub>2</sub> sequestered was  $26 \pm 7$  mg per gram of photosynthetic living material (12 times more than after day 30 – *seems almost linear?*)

# Discussion (it's 1 page)

Frames their work in a larger context:

- Chemical mineralization, e.g., carbonation of recycled concrete aggregates can sequester 6.7 mg recycled aggregate – claim their method with 26 g/g is competitive; *what about time?*
- However, both chemical and biological (this study) sequestration are less efficient than CCS, but CCS requires a concentrated CO<sub>2</sub> source and controlled conditions of T and P – their system works under ambient conditions
- *Back to MSE 341...*

# Discussion (it's 1 page)

What could be better?

- Needs improved “usability” and upscaling
- Gives ideas to how this can be done – using larger scale porous or granular scaffolds
- Further optimizing light harvesting
- Make it better by genetic modification or microorganism consortia

# Discussion (it's 1 page)

Where do we see this technology one day?

- As surface coatings for green building materials or bioreactors in sequestration plants – bio remediating CO<sub>2</sub> emissions and supporting carbon negative or carbon neutral infrastructure
- Simple requirements and easy maintenance enable installation in various environments for long-term sequestration

# Conclusion (UCI has no template for this – now onto nature.com)

Papers that report experimental work are often structured chronologically in five sections: first, *Introduction*; then *Materials and Methods*, *Results*, and *Discussion* (together, these three sections make up the paper's body); and finally, *Conclusion*.

- The *Introduction* section clarifies the motivation for the work presented and prepares readers for the structure of the paper.
- The *Materials and Methods* section provides sufficient detail for other scientists to reproduce the experiments presented in the paper. In some journals, this information is placed in an appendix, because it is not what most readers want to know first.
- The *Results* and *Discussion* sections present and discuss the research results, respectively. They are often usefully combined into one section, however, because readers can seldom make sense of results alone without accompanying interpretation — they need to be told what the results mean.
- The *Conclusion* section presents the outcome of the work by interpreting the findings at a higher level of abstraction than the *Discussion* and by relating these findings to the motivation stated in the *Introduction*.

- Way more concise!
- There are no fixed rules
- But their needs to be a supported logic and story throughout

- New cyanobacteria-laden photosynthetic living material for dual carbon sequestration
- Performed this sequestering over a long lifetime > 400 days with light and atmospheric carbon as its energy and carbon source
- Base formulation enabled DIW and light-based additive manufacturing, enabling structure design for photosynthetic efficiency
- Something about “*spatiotemporal control provided by 3D printing allows scale up for potential applications in disparate fields, such as civil engineering and architecture*”...OK



# I skipped the methods

Often at the end of papers, not always read, but SO CRITICALLY important. If you are writing a paper, now is the time to include all details and not restrict yourself!

## Writing a "good" methods section

The purpose is to provide enough detail that a competent worker could repeat the experiment. Many of your readers will skip this section because they already know from the Introduction the general methods you used. However careful writing of this section is important because for your results to be of scientific merit they must be reproducible. Otherwise your paper does not represent good science.

Goals:

- Exact technical specifications and quantities and source or method of preparation
- Describe equipment used and provide illustrations where relevant.
- Chronological presentation (but related methods described together)
- Questions about "how" and "how much" are answered for the reader and not left for them to puzzle over
- Discuss statistical methods only if unusual or advanced
- When a large number of components are used prepare tables for the benefit of the reader
- Do not state the action without stating the agent of the action

**Did you like this paper?**  
**Why or why not?**



**YES**



**NO**

# Lesson takeaways



- An even better idea of ELMs
- Using ELMs to help solve an important problem
- A general understanding of cyanobacteria, its role in oxygenating the atmosphere, and its potential role in decarbonizing the atmosphere
- Stopping emissive activities is critical, capturing CO<sub>2</sub> when emissive activities occur is critical, actively lowering CO<sub>2</sub> in atmosphere is also important
- Not one answer, most likely (and hopefully soon or now), different technologies will be implemented at scale