Biodesign of microalgae-laden engineered living materials via 3D bioprinting: a roadmap

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engineered living materials, 3D bioprinting, alginate hydrogel, bioink optimisation, structural stability, photosynthetic activity

Abstract

Microalgae-based engineered living materials (ELMs) are increasingly explored in biodesign, yet initiating experimental work remains technically complex and parameter-sensitive. This pictorial presents a structured, experimentally grounded roadmap for extrusion-based bioprinting of microalgae-laden hydrogels, framing fabrication not as a matter of achieving geometric fidelity, but as a process of designing conditions for living systems to persist and perform. Using alginate–Pluronic F-127 formulations as a reference system, we document key stages from microalgae preparation and hydrogel characterization through printing optimization and viability assessment. Systematic evaluation across formulation composition, extrusion pressure, and printing speed shows how biological integration shifts the printable window and why geometry-based metrics alone are insufficient – print outcomes emerge from negotiating intertwined material, process, and organism constraints. Post-printing analyses confirm sustained photosynthetic activity and cell viability within the printed constructs. Translating microalgae-based ELMs from concept to practice requires coordinated decisions spanning biology, hydrogel design, rheology, and printing parameters, while most studies report only optimized end points. The presented framework supports more reproducible and design-oriented development of microalgae-based ELMs within the biodesign community.

Impact statement

By visualizing interconnected decision points and translating laboratory practice into a practical roadmap, this pictorial lowers the ELM bioprinting entry barrier for researchers and designers and invites broader design interpretations in which the algae's viability, growth temporality, and care requirements can be treated as design-relevant parameters.

Introduction

Engineered living materials (ELMs) derived from photosynthetic microorganisms are being explored across biomedical, food, and environmental applications, and increasingly in biodesign, where they occupy a space between material fabrication and ongoing biological activity. Microalgae are a practical model system: their photosynthetic activity remains functional after hydrogel immobilization and extrusion processing (Jiang et al. 2025). Extrusion-based bioprinting structures microalgae into defined three-dimensional architectures, but the hydrogel matrix is more than a form factor. It acts as a habitat, shaping light exposure, mass transport, and mechanical protection, and therefore directly influences microalgal function and viability. This makes bioprinting with living cells fundamentally different from printing inert materials: rheology, shear stress tolerance, crosslinking kinetics, and long-term viability are interdependent, and small changes in composition or printing conditions can propagate across all of them (Ouyang et al. 2016). Despite rapid growth, the field remains fragmented — formulations and strategies are typically optimized for specific setups, and most studies report endpoints rather than the decisions that led there (Chimene et al. 2019). This pictorial addresses that gap, using direct ink writing DIW as an accessible, cell-compatible platform for developing microalgae-based ELMs.

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Microalgae Bioprinting

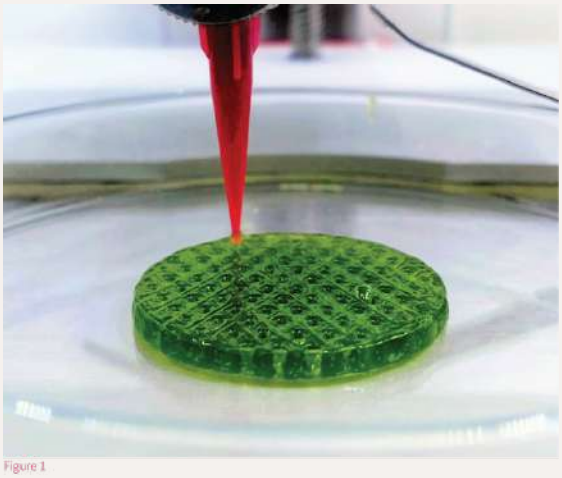


Figure 1

This work proposes an experimental roadmap for designing and 3D printing with living cells, using microalgae as a model organism [Fig. 1]. It offers a practical reference framework for designers and researchers beginning to work with living bioinks.

Based on our experience, we propose a practical approach to initiating ELM bioprinting and guiding bioink selection, crosslinking, and printing parameter optimization. We prepared alginate-based bioinks with varying microalgae content, optimized extrusion conditions, characterized their rheology, and evaluated construct viability and structural integrity using accessible methods. 3D bioprinting offers something that other fabrication approaches cannot: the ability to give precise spatial form to a living system. Unlike surface-based methods, where organisms are deposited onto a flat substrate, extrusion-based bioprinting embeds microalgae within a three-dimensional architecture that actively conditions their growth, light exposure, and metabolic activity from within. The internal geometry of the construct is not just a structural choice, it shapes how the organism lives inside it. This opens up a design space that extends well beyond fabrication: once printed, the construct continues to change, the cells grow, chlorophyll accumulates, oxygen is released. The artefact enters a trajectory of living, and the decisions made during printing determine how that trajectory unfolds (Balasubramanian et al. 2021; Zhou et al. 2022).

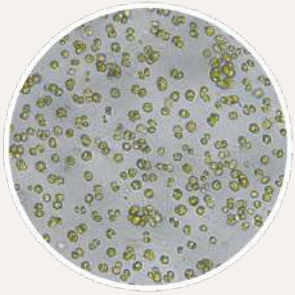
The adaptation of bioprinting for the fabrication of ELMs enables the creation of precise three-dimensional structures with complex and designed architectures [Table 1]. This may significantly extend its use beyond biomedical applications to the energy sector, urban architecture, and space exploration, and increasingly, to design practices that take seriously the agency and livingness of the organisms involved (Wakkary 2021).

Microalgae input parameters	Hydrogel input parameters	Printing input parameters
Medium selection, contamination control, pH level, photoperiod, light intensity, temperature control, optical density, dissolved oxygen, mixing/agitation rate, batch culture/ photobioreactor	Viscosity, swelling ratio, yield stress, crosslinking mechanism, crosslinking rate, shape fidelity, biocompatibility, cell viability, degradation rate, porosity, permeability, sterility, temperature/pH/ salinity sensitivity	Extrusion pressure, printing speed, nozzle geometry, nozzle diameter, printhead temperature, platform temperature, infill density, layer height, sterility

How to 3D bioprint microalgae-laden engineered living materials?

Pink: General roadmap Green: Experimental results: practical reference framework

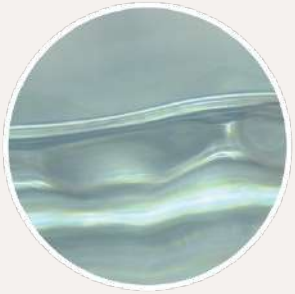
To facilitate the research process, the entire workflow may be divided into three main pillars: microalgae selection and cultivation, hydrogel selection, and printing optimization. At each of these pillars, several driving questions should be addressed [Fig. 2].



Microalgae

Do the selected microalgal species exhibit sufficient resistance to bioprinting conditions? Will the components of the culture medium affect the stability or integrity of the printed hydrogel? Which microalgal cultivation parameters are expected to be improved through 3D printing?

Key readouts: viability, growth rate, chlorophyll signal.



Hydrogel

Does the selected bioink exhibit suitable rheological properties? Are hydrogel components and crosslinking methods safe for microalgal cells? Will the hydrogel maintain sufficient stability under conditions supporting microalgal growth?

Key readouts: printability, structural stability, biocompatibility



Printing

Will the applied pressure and printing speed affect microalgal viability? Do the selected printing parameters allow for the fabrication of the desired three-dimensional geometries? Does the chosen bioink require specific temperature conditions during the printing process?

Key readouts: Pr value, filament continuity, shape fidelity.

Figure 2

Fabrication process

The fabrication of 3D bioprinted microalgae constructs is a multistep process that can be divided into six main stages, ranging from the selection of microalgae species and suitable hydrogel materials, through printing parameter optimization and 3D geometry design, to crosslinking strategy selection and subsequent cultivation of microalgae within the printed structure [Fig. 3].

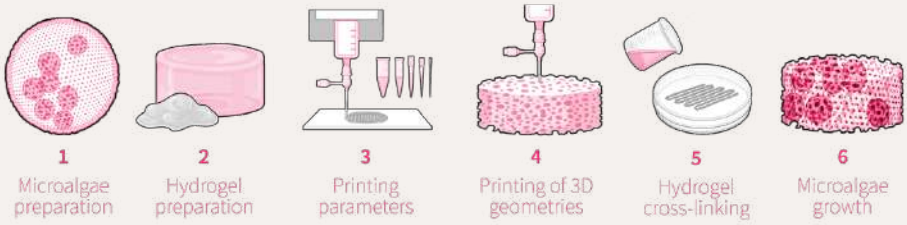
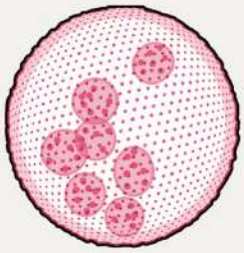


Figure 3

Behind every parameter in this roadmap lives a relationship. The pH level, light regime, and nutrient composition that define cultivation conditions are not just technical inputs. They constitute the habitat in which microalgae can grow, photosynthesize, and remain viable (Zhou et al. 2022). When these conditions are right, the organism thrives. When they shift, cell health, growth rate, and physiological state change too, and that carries forward into how the cells behave inside the printed construct. Culture age brings this entanglement into focus. Cells at different growth stages are not the same living matter. They carry different densities, different spatial arrangements, different capacities for life within the hydrogel. Deciding at which moment to work with them is not a workflow decision so much as a moment of attunement to where the organism is (Wakkary et al. 2025).

The printability window identified here marks the space where structural stability and biological viability meet. Designing within it means designing with the organism, not just for it. This roadmap is a starting point. The technical decisions it maps out are also invitations to think differently about what it means to design with living matter, where the organism is not a material to be optimised but a participant in an ongoing, shared process of making (Balasubramanian et al. 2021; Wakkary 2021).

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1 Microalgae preparation

Objective: To explore and evaluate microalgae species for 3D bioprinting applications, considering their biological requirements, environmental tolerance, and compatibility with hydrogel-based bioinks.

1.1 | Microalgae selection

The identification and evaluation of microalgae constitutes the first iterative step in the design of ELMs. The selected species is expected not only to support the intended biological function of the construct but also remain compatible with the hydrogel formulation, crosslinking strategy, and extrusion-based processing conditions. The decision cannot be treated as an isolated biological choice, as it directly interacts with material chemistry and printing stability [Fig. 4].

Several photosynthetic microorganisms can be explored in bioprinting contexts, including freshwater unicellular green algae such as Chlamydomonas reinhardtii, halophilic species such as Dunaliella tertiolecta, and filamentous cyanobacteria such as Arthrospira platensis. These organisms differ in morphology, salinity requirements, extracellular polymer production, and mechanical behavior under shear, which in turn influence both fabrication processes and the ways in which living systems behave within printed structures. This includes how these behaviours continue to develop over time as the living system responds to its environment beyond the moment of fabrication. For example, halophilic species require elevated salt concentrations that may interfere with ionic crosslinking systems, whereas filamentous cyanobacteria can significantly alter ink rheology due to their multicellular helical structure. These differences introduce design trade-offs, where material stability, biological needs, and fabrication constraints must be balanced rather than optimised independently. The choice of species emerges from a feedback loop between biological performance, material formulation, and printing conditions.

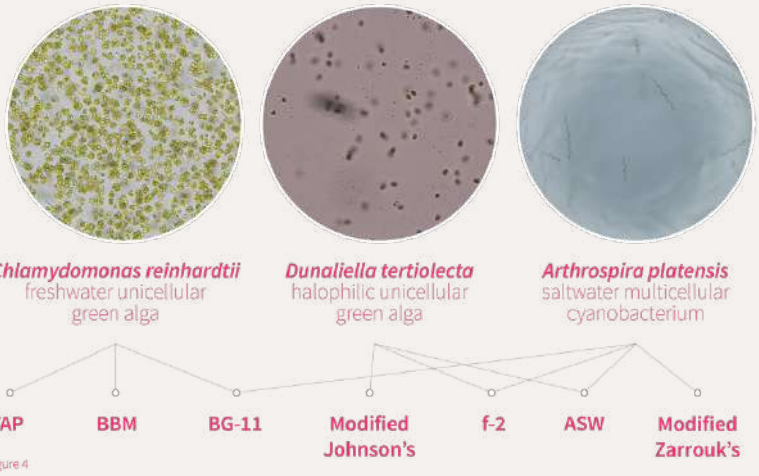


Figure 4

Chlamydomonas reinhardtii was selected as a reference organism for this study. It is a unicellular freshwater alga with a typical diameter of 5–10 µm, which supports homogeneous dispersion within hydrogel matrices and minimizes the risk of nozzle obstruction during extrusion. The cells contain a large chloroplast occupying most of the cell volume and exhibit phototactic behavior enabled by an eyespot. It can dynamically regulate light harvesting and dissipate excess energy under high illumination, which is particularly relevant in immobilized 3D constructs where light gradients and diffusion limitations are inherent to the geometry.

1.2 | Microalgae cultivation

Successful cultivation requires control over light regime, temperature, pH, nutrient composition, and mixing conditions, as these parameters directly influence growth kinetics and physiological state, while remaining responsive to the needs of the living system.

Microalgae cultivation can be performed in flexible, low-cost flask systems for laboratory studies or in precisely controlled photobioreactors that enable scalable and reproducible biomass production. While photobioreactors provide enhanced environmental regulation, flask-based systems offer operational simplicity and sufficient reproducibility for early-stage material development and exploration of printing conditions [Fig. 5].

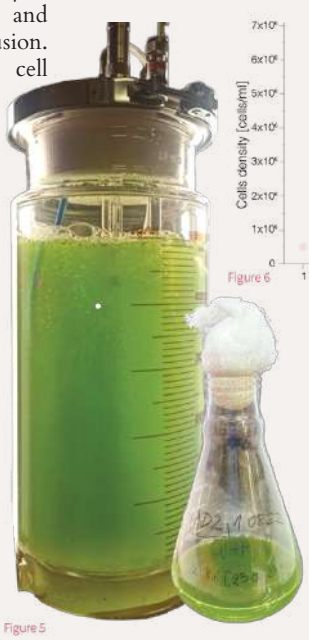


Figure 5

TAP medium was prepared under sterile conditions, pH-adjusted to 6.5–7.2, and autoclaved at 121 °C for 20 minutes. Cultures were initiated by inoculating 10 mL of existing C. reinhardtii into 250 mL Erlenmeyer flasks, supplemented to 100 mL. Cells were maintained at 22°C under a 12/12 h blue-red LED photoperiod and agitated at 135 rpm. Rapid biomass accumulation occurred over the first three days, stabilizing around day 6 and reaching ~7×10⁶ cells/mL by day 8. Growth was monitored at 550 and 680 nm; cell concentration was quantified by hemocytometer. Defined harvesting times were used to assess how physiological age influences bioink behavior and printing performance.

Cultivation times

Biomass was harvested at defined cultivation stages to evaluate the effect of physiological age on subsequent bioprinting. Microscopy images demonstrate a clear increase in cell density between day 2 and day 8, accompanied by more frequent cell clustering at later stages. Quantitative analysis of cell density indicates a progressive increase in biomass concentration over the cultivation period, with the most pronounced growth observed during the exponential phase [Fig. 6-7].

These results indicate that culture age significantly influences cell concentration and spatial distribution, which in turn may affect rheology, extrusion behavior, and post-printing performance of the bioink [Fig. 8].

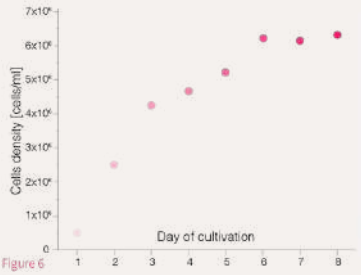


Figure 6

The timing of biomass harvesting is a relevant design parameter that influences how the living system interacts with the material during fabrication.

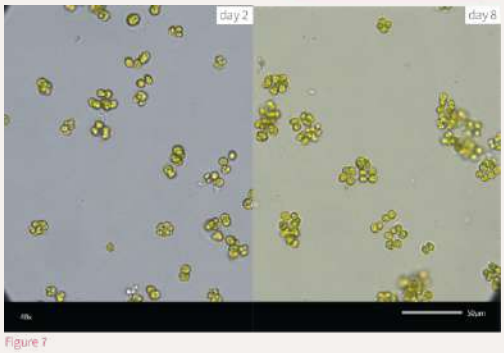


Figure 7

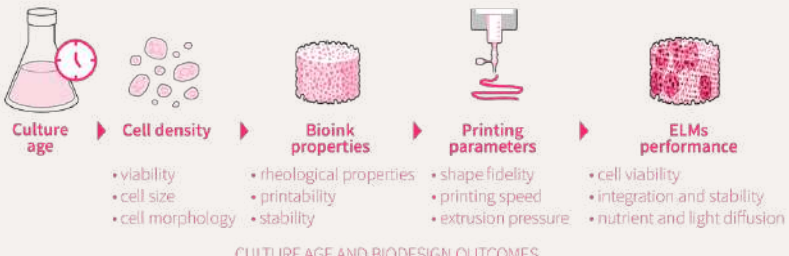


Figure 8

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Objective: To identify a hydrogel formulation suitable for 3D bioprinting that balance structural integrity with conditions supporting microalgae viability.



2 Hydrogel preparation

2.1 | Material selection

Alginate-based systems were selected as the primary matrix due to their mild ionic crosslinking and established compatibility with cell encapsulation. At the same time, pure alginate exhibits limited mechanical stability and susceptibility to ion exchange in culture media; therefore, the formulation was developed to balance printability, post-crosslinking integrity, and conditions supporting biological viability [Fig. 9].

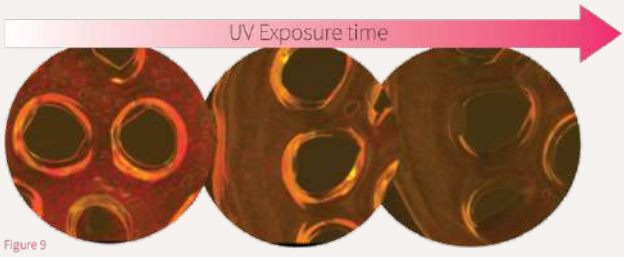


Figure 9



Figure 10

Alginate-based inks were prepared from sodium alginate (0.1 g) and Pluronic F-127 (1.25 g) dissolved in 5.0–5.5 mL of ultrapure water (Sigma-Aldrich®). Alginate served as the main microenvironment for microalgae immobilisation, while Pluronic F-127 acted as a viscosity modifier enabling DIW processing. Calcium chloride (Sigma-Aldrich®) was used as the ionic crosslinking agent. The inks were prepared by magnetic stirring in glass vials. Ultrapure water was first introduced, followed by the gradual addition of sodium alginate until complete dissolution, after which Pluronic F-127 was incorporated to obtain homogeneous hydrogel inks [Fig. 10].

Hydrogel characteristic for bioprinting

The formulation was developed to address four key criteria relevant for DIW of living materials [Fig. 11]. 1) Biocompatibility sufficient to support microalgae immobilisation in a hydrated environment; 2) Rheological behaviour enabling continuous extrusion and filament stability; 3) Mechanical integrity in the crosslinked state that maintains geometry without brittle failure; 4) Shape fidelity preserved after crosslinking and through subsequent incubation. Together, these criteria defined a working range for further ink development, balancing material properties with conditions supporting microbial viability.

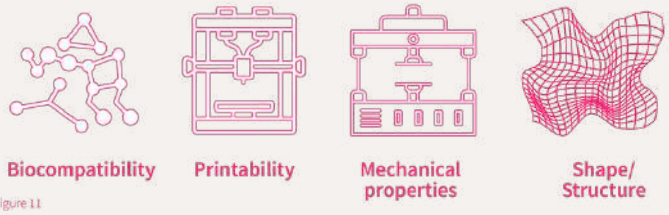


Figure 11

2.2 | Formulations development: Trial and error approach

Printability was assessed through a stepwise trial-and-error approach, combining extrusion tests with well-plate crosslinking screens. $CaCl_2$ concentration and Pluronic F-127 content were varied systematically to understand how each parameter shifted the balance between gel stiffness, extrudability, and biological compatibility [Fig. 12]. Crosslinking concentration was tested across a low-to-high range, with the understanding that higher concentrations tend to increase rigidity and geometric fidelity but risk brittleness and reduced adaptability for the living system. Lower concentrations produced softer gels, more forgiving during extrusion, but less stable post-print. Pluronic content was varied across three levels to explore how its concentration affected flow behavior and filament coherence [Fig. 13-14]. This iterative screening logic – vary, observe, adjust – allows a working formulation range to emerge from accumulated material feedback rather than from a fixed protocol [Fig. 15].

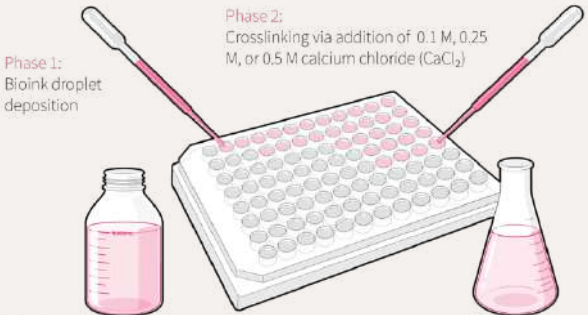


Figure 12



Figure 13

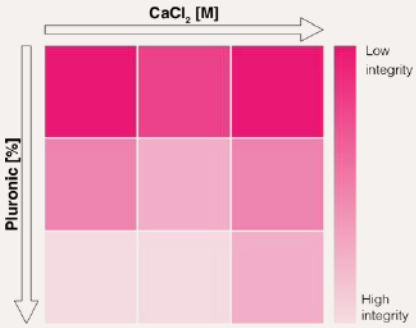


Figure 14

This exploratory phase plays a critical role in early-stage screening of material behavior and in identifying promising directions for further development.

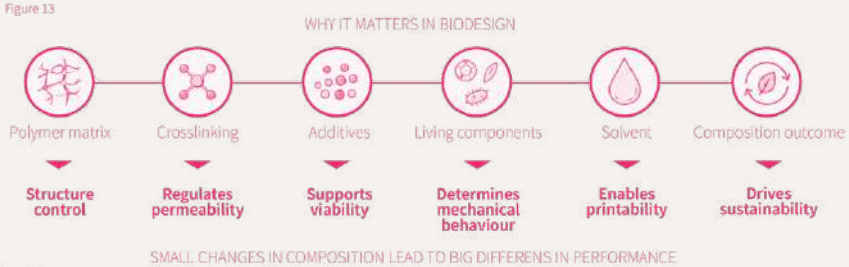
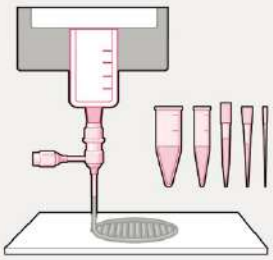


Figure 15

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3 Printing parameter exploration

Objective: To identify and evaluate printing parameters that support stable extrusion and reproducible deposition of living hydrogel inks while maintaining microalgal viability.

3.1 | Nozzle selection

A range of nozzle types and geometries can be used in direct ink writing (DIW) bioprinting, differing in diameter, shape, and material, which influence shear exposure during extrusion, extrusion fidelity, resolution and process stability, and thereby shape not only the resulting structures but also how living systems are distributed and spatially organized. In particular, excessive shear stresses in narrow metallic needles may compromise cell integrity and cause flow instabilities.

A cylindrical 27G STANDARD printing tip (needle-free) was selected to provide controlled extrusion while minimizing mechanical stress on the microorganisms [Fig. 16].



Figure 16

3.2 | Hydrogel and microalgae integration

Incorporating living cells into a preformulated ink requires careful handling at every stage, from biomass preparation to mixing strategy, to avoid compromising either the material or the organism. This step is where biological and material considerations first become inseparable. Cells were harvested by centrifugation and resuspended in ultrapure water to remove residual growth medium, which can interfere with ink rheology and crosslinking behavior. The resulting suspension was incorporated into preformulated inks at a defined volume fraction, with mixing performed via a Luer-lock connector to ensure homogeneous distribution while limiting mechanical stress on the cells. Introducing microalgae after complete ink preparation minimized exposure to polymer dissolution and intensive mixing, thereby supporting cell viability [Fig. 17]. To separate the effect of cell incorporation from simple dilution, formulations were developed in parallel: one introducing cells directly, another compensating by adjusting the water fraction [Table 2]. Both the cultivation age of microalgae and their concentration in the bioink become relevant design parameters in DIW printing, as they influence rheology, cell distribution, and the spatial organisation of living cells within the printed material.

	Water [ml]	Pluronic F127 [g]	Sodium alginate [g]	Microalgae[µl]
I	5	1.25	0.1	0
IA	5	1.25	0.1	500
IB	4.5	1.25	0.1	500
IB x3	4.5	1.25	0.1	500 x 3 cell concentration

Table 2

3.3 | Air-bubbles removal

To ensure stable extrusion in the subsequent printing stage, the cell-laden ink was transferred into a transparent 3 mL printing syringe, sealed, and centrifuged (10-20 min, 6,000 rpm) to eliminate entrapped air. This conditioning step minimizes compressibility within the cartridge and prevents pulsatile extrusion [Fig. 18]. Effective bubble removal is critical to maintain continuous filament formation and preserve the optimized rheological window during DIW of the living ink.



Figure 18

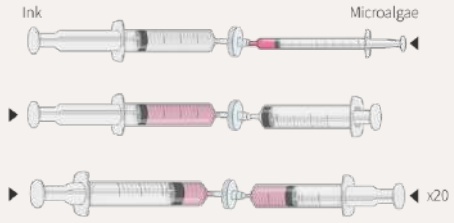


Figure 17

3.4 | Rheological characterisation

Rheological characterization was performed to determine whether the formulated inks possessed the flow and recovery behavior necessary for extrusion-based printing. This step is critical: a bioink must thin under shear to pass through the nozzle, then rapidly recover its structure upon deposition to maintain filament shape and support subsequent layers. Without this confirmation, printability remains an assumption rather than a verified material property. Both formulations, with and without microalgae, were evaluated using oscillatory and flow sweep measurements to assess viscoelastic behavior and shear-thinning response. The key questions were whether the addition of a biological component altered the ink's rheological signature, and whether the resulting properties fell within a range compatible with stable extrusion and post-print shape fidelity. Characterizing both formulations in parallel allowed material and biological contributions to be distinguished, and provided a basis for understanding how cell incorporation shifts (or preserves) the printable window of the ink [Fig. 19-20].

From a biodesign perspective, this matters beyond process validation. The rheological relationship between the hydrogel matrix and the living component reflects a broader design tension: the material must be workable, but it must also remain hospitable. Rheology is where those two demands first meet.

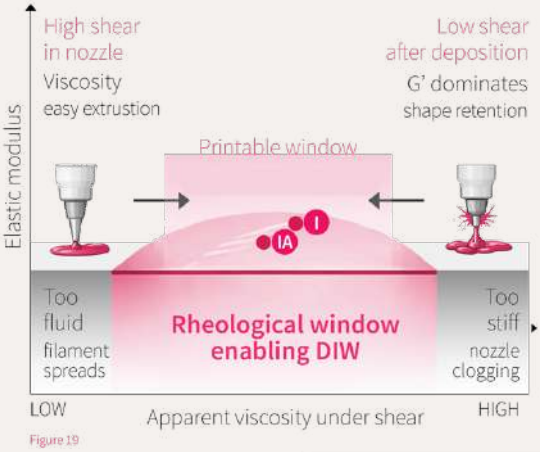
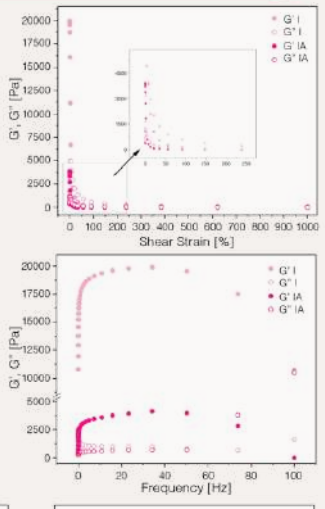


Figure 19



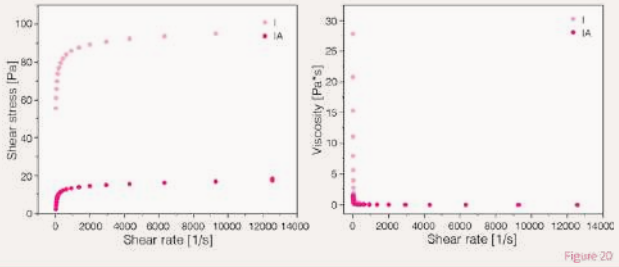


Figure 20

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3.3 | Printability assessment

In biodesign context defining reproducible printing parameters provide a stable baseline that allows designers to meaningfully explore variations in material composition and biological systems.

Printability was evaluated across a range of extrusion pressures (varied between 25 and 50 kPa) and printing speeds (ranged from 3 to 9 mm/s). For each condition, the line width (tline), printability factor (Pr), pore integrity index (POI), and normalized pore quality (POI/POImax) were determined from optical images, with POImax calculated separately for each pressure level (Marques, et al., 2023; Webb and Doyle, 2017). For the reference formulation (ink I), Pr remained within 0.86–1.02 across the tested conditions, indicating generally stable extrusion behaviour. Analysis of pore geometry showed that high geometric accuracy was observed only for specific parameter combinations. The most accurate structures were obtained at 30 kPa and 9 mm s^{-1}, where Pr reached 0.95 ± 0.01, POI = 1.00, and shape accuracy was 98%, defining a suitable printing range for subsequent ELM fabrication [Table 3].

Ink	Pressure (kPa)	Speed (mm s^{-1})	Pr (-)	POI (-)	Accuracy (%)	Strand width (mm)	Verdict
I	30	9	0.95 ± 0.01	1.00	98	0.434	I optimum
I	40	3	0.95 ± 0.03	0.31	73	-	over-extrusion
I	50	3	~0.86	0.23	-	1.83	excessive spreading
IA	25	9	0.94 ± 0.03	1.00	72	-	IA optimum
IA	40	3	0.79 ± 0.01	-	8	-	unstable
IB	30	9	0.95 ± 0.01	1.00	73	-	IB optimum

Table 3

Relying on a single metric tends to overestimate the usable process window: Pr values close to 1.00 can mask excess extrusion or incomplete path deposition, which only become visible through pore geometry analysis. For the base ink, stable and accurate structures emerged within a narrower range than Pr alone suggested, with optimal performance at moderate pressure and high speed. Introducing microalgae shifted this window; the added volume altered the flow balance, reducing geometric accuracy in the directly loaded formulation. Compensating by adjusting the water fraction largely restored printability, recovering both Pr and pore integrity to levels comparable with the base ink, though at a slightly reduced shape accuracy. This pattern: shift, compensate, re-evaluate, reflects the iterative logic that characterizes parameter optimization in living ink systems [Fig. 21-24].

From a biodesign perspective, the results show that printing parameters do not operate independently, but need to be adjusted in relation to both material composition and biological content, as small changes can shift the balance between extrusion behaviour and structural fidelity.

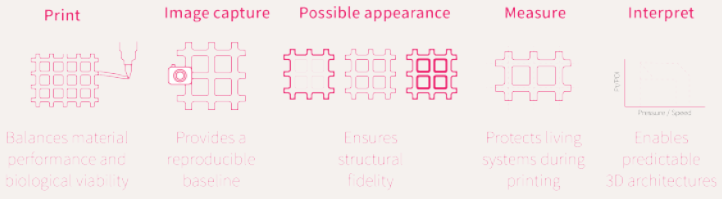
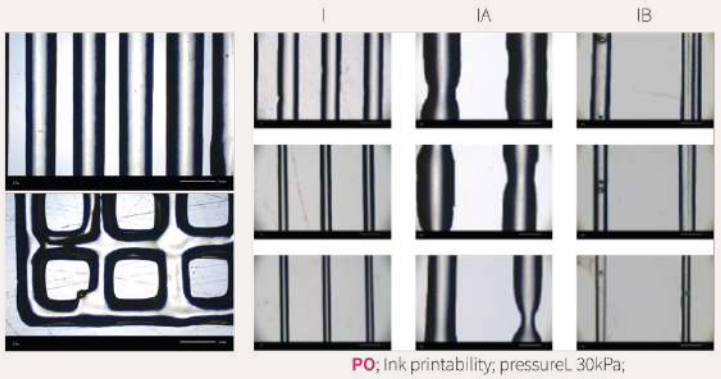
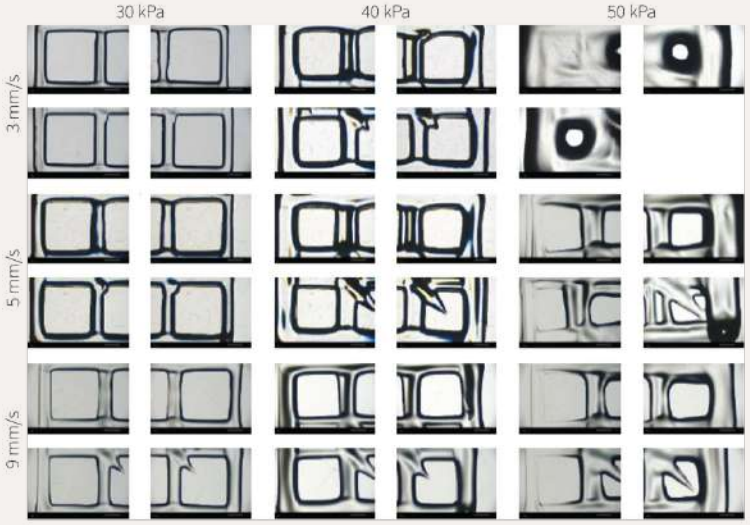


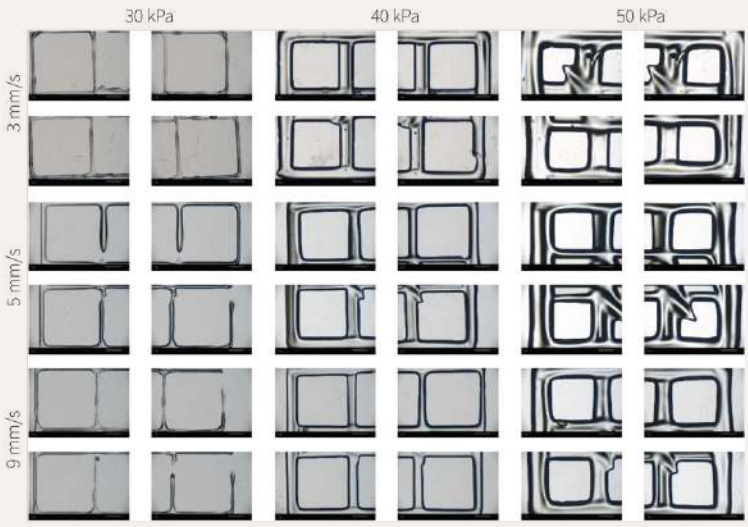
Figure 21



PO; Ink printability; pressureL 30kPa;

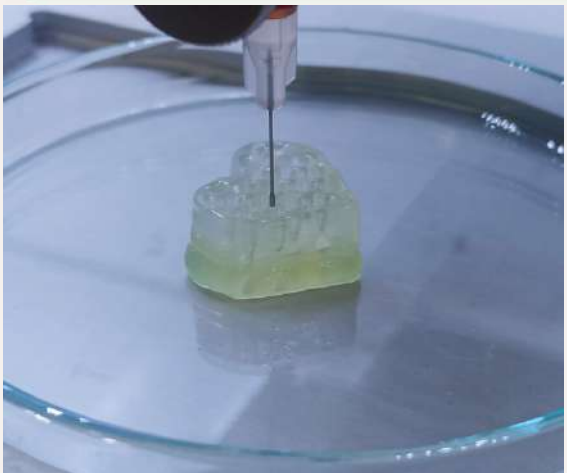


Pr: IA; Ink printability; vertical speed, horizontal pressure);



Pr: I; Ink printability; vertical speed, horizontal pressure);

Figure 22



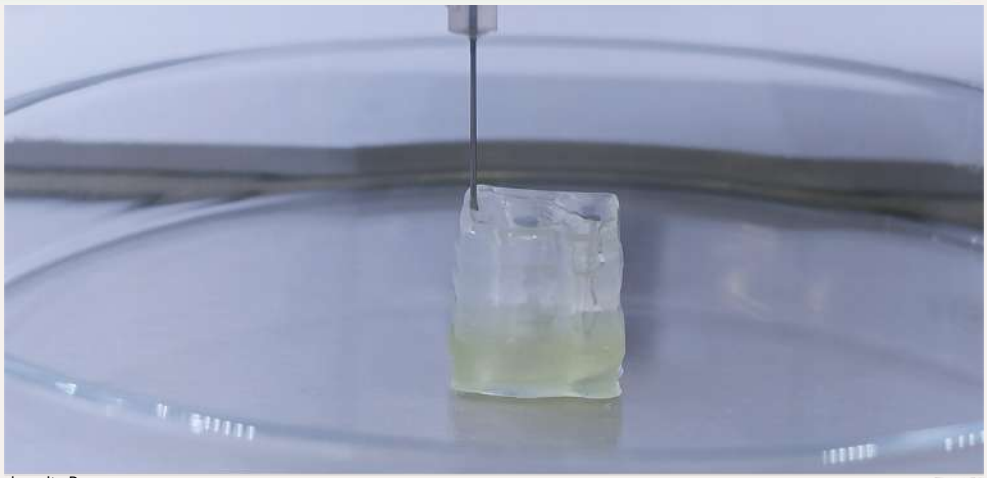
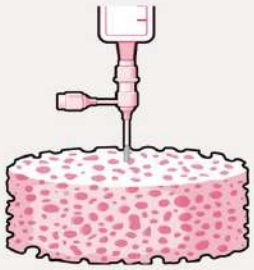


Figure 24

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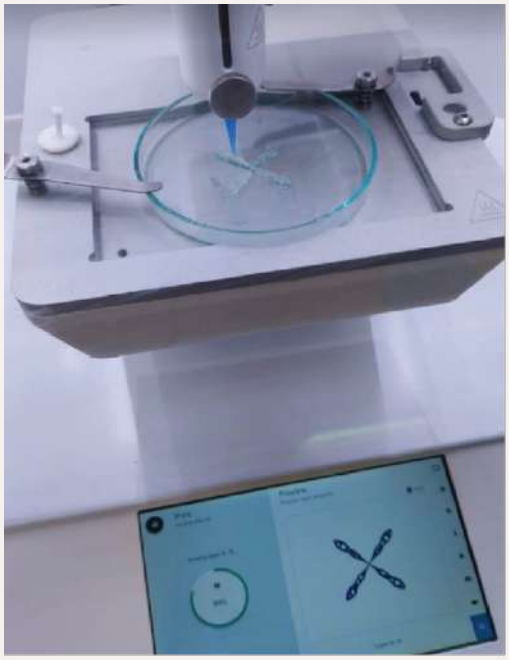
4 Printing of 3D geometries

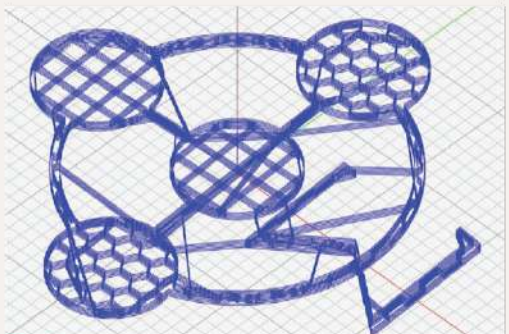
Objective: To explore how construct geometry and internal architecture influence structural stability and microalgae viability.

4.1 | Design of the geometry

Following parameter optimization, functional 3D constructs were fabricated from G-code models and printed using pneumatic DIW. Print fidelity depended on process parameters and ink rheology, allowing fabrication of delicate flower-like structures. A honeycomb pattern maintained more uniform pores across multilayer regions compared to a grid infill, which showed greater filament spreading. During incubation, constructs exhibited progressive greening: chlorophyll signal increased within 24 hours and distributed homogeneously by day 7, indicating sustained microalgal proliferation. Simpler geometries remained stable throughout; more complex structures showed partial degradation, traced to delayed crosslinking after printing. Immediate ionic crosslinking preserved geometry across the full observation period.

From a biodesign perspective, this highlights that geometry and internal architecture not only define structural properties but also influence how light, nutrients, and living cells are distributed within the construct over time. This suggests that design decisions shape both the form and the evolving biological activity of the material.





4.2 | Shaping of microalgae -laden ELMs

The developed bioinks enabled fabrication of geometries with increasing architectural complexity, from regular lattices to elongated honeycomb structures [Fig 25-27].

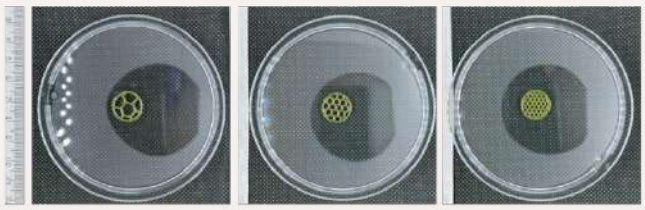
For microalgae-laden ELMs, structural stability must be maintained not only after printing but also during $CaCl_2$ crosslinking and subsequent culture. At the same time, alginate remains widely used due to its mild and hydrated microenvironment supporting microalgal survival.



Grid pattern | Honeycomb pattern | Radial infill pattern



Elongated honeycomb ELM construct



Infill density 5, 10, and 15% ink IBx3

Figure 27

Microalgae-laden constructs (ink IBx3) were printed with infill densities of 5%, 10%, and 15% (Ø 15 mm, height 3 mm). All variants were fabricated successfully, but internal architecture strongly affected structural integrity. Sparse infills preserved global geometry but lacked sufficient mechanical support, leading to local deformation. Dense infills introduced internal stress, resulting in frequent layer delamination. An intermediate density offered the most stable behavior, maintaining designed geometry in approximately two-thirds of prints with the lowest defect occurrence, reflecting a balance between structural support and material compatibility. Cell viability remained high across all conditions (>98%), with no statistically significant differences between infill densities, confirming that architecture primarily governs structural outcome rather than short-term biological performance. Live cell counts peaked early in culture and declined over time across all variants, indicating a time-dependent trend independent of geometry [Table 4]. The infill density can be selected to optimize structural fidelity without compromising microalgal survival, and that within an ELM bioprinting raodmpa, internal architecture should be treated as a design parameter with both mechanical and biological consequences.

Infill density	Shape preservation	Defect frequency	Peak live-cell count (day 2)	Viability	Statistics	Overall assessment
5%	Moderate	Local deformation observed	High	>98%	No significant difference (p=0.795)	Acceptable
10%	Highest (~2/3 prints intact)	Lowest	Highest	>98%	No significant difference (p=0.795)	Best balance
15%	Reduced	Layer delamination in 5/6 prints	Moderate	>98%	No significant difference (p=0.795)	Structurally limited

Table 4



1st day



after 24h



after 7 days

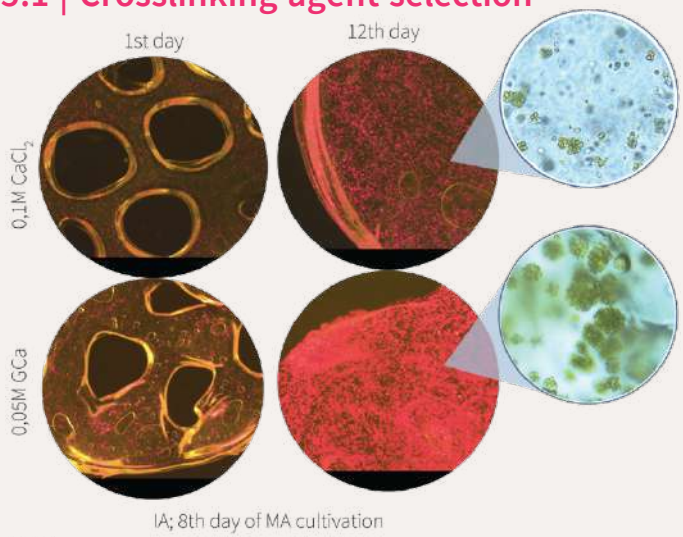
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5 Hydrogel cross-linking and material characterisation

Objective: To investigate the effect of the type and concentration of the crosslinking agent on the physicochemical properties of 3D-printed constructs.

5.1 | Crosslinking agent selection



IA; 8th day of MA cultivation

Figure 28

Selection of an appropriate crosslinking method is another critical parameter in the bioprinting of living microalga. Alginate-based hydrogels can be prepared using several physical and chemical crosslinking strategies, where the choice of agent, concentration, and timing each influence both structural outcome and biological compatibility. Calcium ions are the most widely used crosslinker for alginate due to their biocompatibility. $CaCl_2$ was used for initial printability screening, while calcium gluconate was evaluated as a milder alternative. Constructs crosslinked with calcium gluconate supported greater microalgal viability after 12 days of incubation (assessed via chlorophyll autofluorescence as an indicator of photosynthetic activity) suggesting that crosslinker choice shapes not only gel mechanics but the conditions under which immobilized cells function over time. Concentration was subsequently optimized: lower divalent cation concentrations reduce rapid surface gelation, allowing more uniform ion diffusion and a structurally homogeneous network [Fig. 28-29].

Crosslinking strategy offers a lever for tuning the balance between structural stability and biological compatibility, and should be considered from the earliest stages of ink development.



Lowering the concentration of divalent cations reduces rapid surface gelation, enhances ion diffusion throughout the hydrogel matrix, and promotes the formation of a structurally homogeneous polymer network.

Figure 29

5.2 | Material characterisation

Following the selection of appropriate crosslinking agents, comprehensive material characterization of the printed constructs should be conducted to achieve complementary structural and chemical validation of the developed material.

FTIR

FTIR analysis identified functional groups in the hydrogels (I and IBx3) by examining the positions and intensities of the observed signals. A broad band observed at 3350 cm^{-1} corresponds to O–H stretching vibrations from water molecules trapped in the microalgae and/or hydrogel. The band at 2880 cm^{-1} is attributed to C–H stretching vibrations from alginate or Pluronic. Bands at 1640 cm^{-1} and 1460 cm^{-1} are assigned to C=O stretching of carboxylic groups and O–H bending vibrations, respectively. The most intense band at 1090 cm^{-1} corresponds to C–O or C–O–C stretching vibrations, characteristic of polysaccharide structures [Fig 30].

The FTIR spectra confirmed the preservation of characteristic polysaccharide bands after printing and crosslinking, indicating that the entire process did not affect the primary chemical structure of the alginate network.

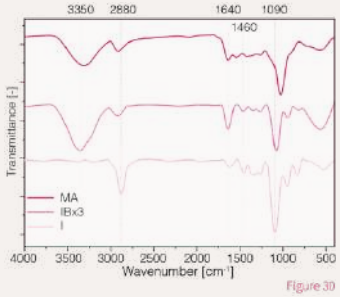


Figure 30

SEM characterisation

SEM provides essential information on material morphology and porosity, which directly influence the adhesion, distribution, and penetration of microalgal cells. Notably, hydrogels containing live microalgal cells require appropriate pre-treatment prior to SEM examination, such as cryogenic freezing and lyophilization. SEM images of hydrogel I reveal a highly porous internal architecture with a heterogeneous pore size distribution. The hydrogel surface is rough, with visible cracks and micropores. Such structural features enhance gas and fluid exchange, while providing a protective barrier for the immobilized microalgal cells [Fig. 31].

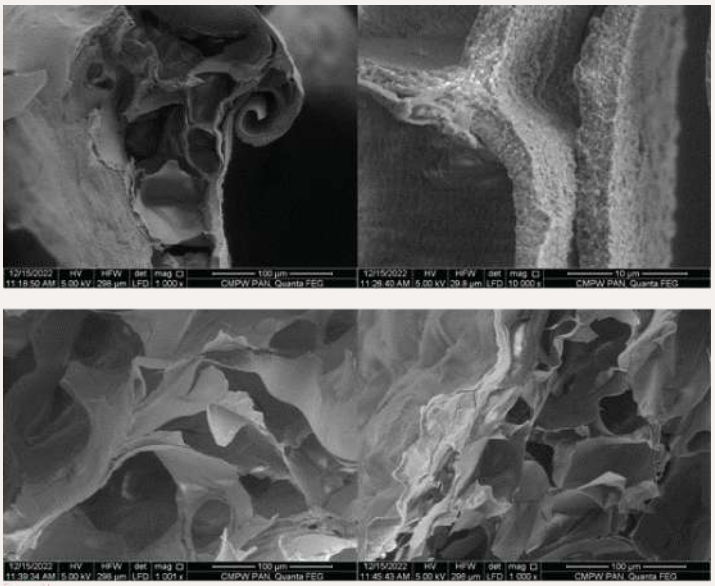
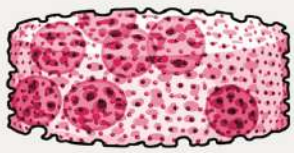


Figure 31

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Objective: To evaluate how culture age, crosslinking strategy, and structural stability influence microalgae viability and growth in 3D-printed constructs.



6 Microalgae growth and viability

6.1 | Assessment of structural integrity with respect to microalgae leakage

Cell viability and functional activity were assessed through chlorophyll autofluorescence microscopy, optical density measurements, and oxygen production analysis. A key focus was microalgal leakage from printed constructs; cells released into the surrounding medium over time, indicating gradual loss of structural containment and, with it, functional capacity within the construct. Leakage was shaped by two interacting factors: culture age and crosslinking chemistry. Constructs prepared from later-stage cultures showed consistently higher cell release, suggesting that elevated initial biomass promotes matrix overloading beyond what the hydrogel network can retain. Crosslinker choice further modulated this behavior; GCa-crosslinked constructs showed more pronounced leakage from older cultures than $CaCl_2$-based systems, reflecting differences in network homogeneity and structural integrity [Fig 32-34].

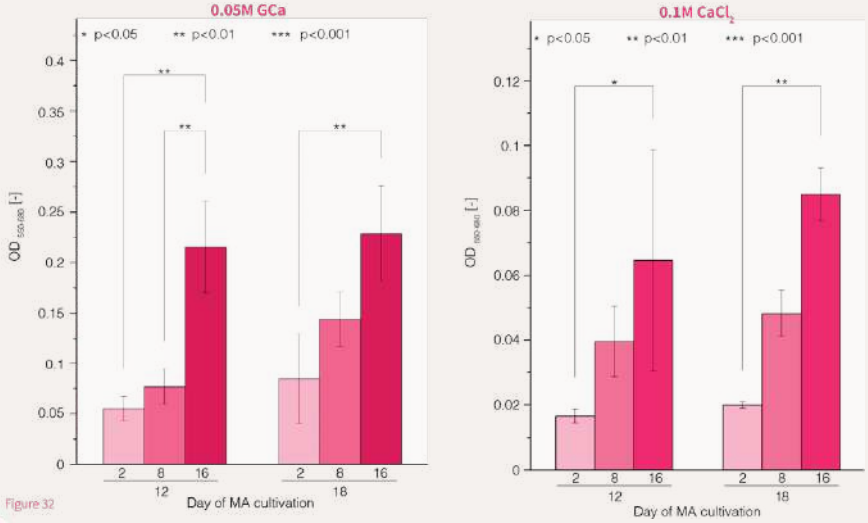


Figure 32

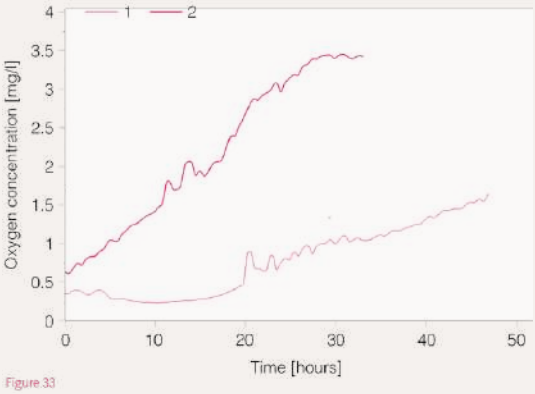


Figure 33

From a design perspective, this shows that the stability of living materials depends not only on structure, but also of how biological density and material composition interact over time. Leakage can be understood not only as a failure, but as a result of dynamic interactions between living systems and structural capacity of the material. Designing with living materials means accepting that they cannot be fully controlled and may behave unpredictably.



Figure 34

6.2 | Microalgal viability in 3D printed constructs

Viability and functional activity were assessed in parallel: fluorescence microscopy tracked cell survival over time, while oxygen evolution measurements confirmed active photosynthesis within the printed constructs. Together, these methods verify whether a construct remains both structurally stable and biologically active — the two conditions that define a functional living material [Fig. 35-36].

Oxygen accumulation was measured at 30-minute intervals across constructs crosslinked with $CaCl_2$/$BaCl_2$ and GCa. Both systems showed time-dependent oxygen release, confirming preserved photosynthetic activity after printing. $CaCl_2$/$BaCl_2$-crosslinked constructs reached approximately 3.5 mg/L; GCa-crosslinked constructs showed a slightly slower accumulation rate, though differences between systems were not substantial. Fluorescence imaging over 12 days revealed a clear influence of culture age. Constructs from 2-day cultures maintained the highest structural integrity, while 8- and 16-day cultures showed more homogeneous fluorescence distribution and higher cell abundance by day 12 — indicating greater post-print proliferation. Culture age therefore functions as a design parameter: earlier cultures favor structural confinement, later cultures favor biological development within the construct. Structural stability and biological activity cannot be optimized independently; both determine whether the living material can perform over time.

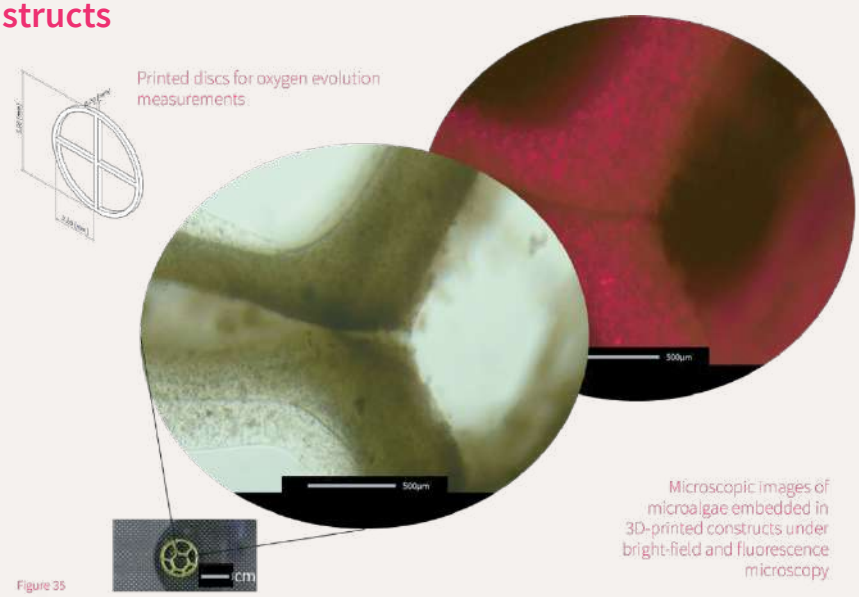


Figure 35

Microscopic images of microalgae embedded in 3D-printed constructs under bright-field and fluorescence microscopy

Oxygen profiles, autofluorescence, and leakage data define an operational window which microalgae–laden hydrogels remain mechanically reliable and metabolically functional, enabling progression toward application–oriented ILM constructs.

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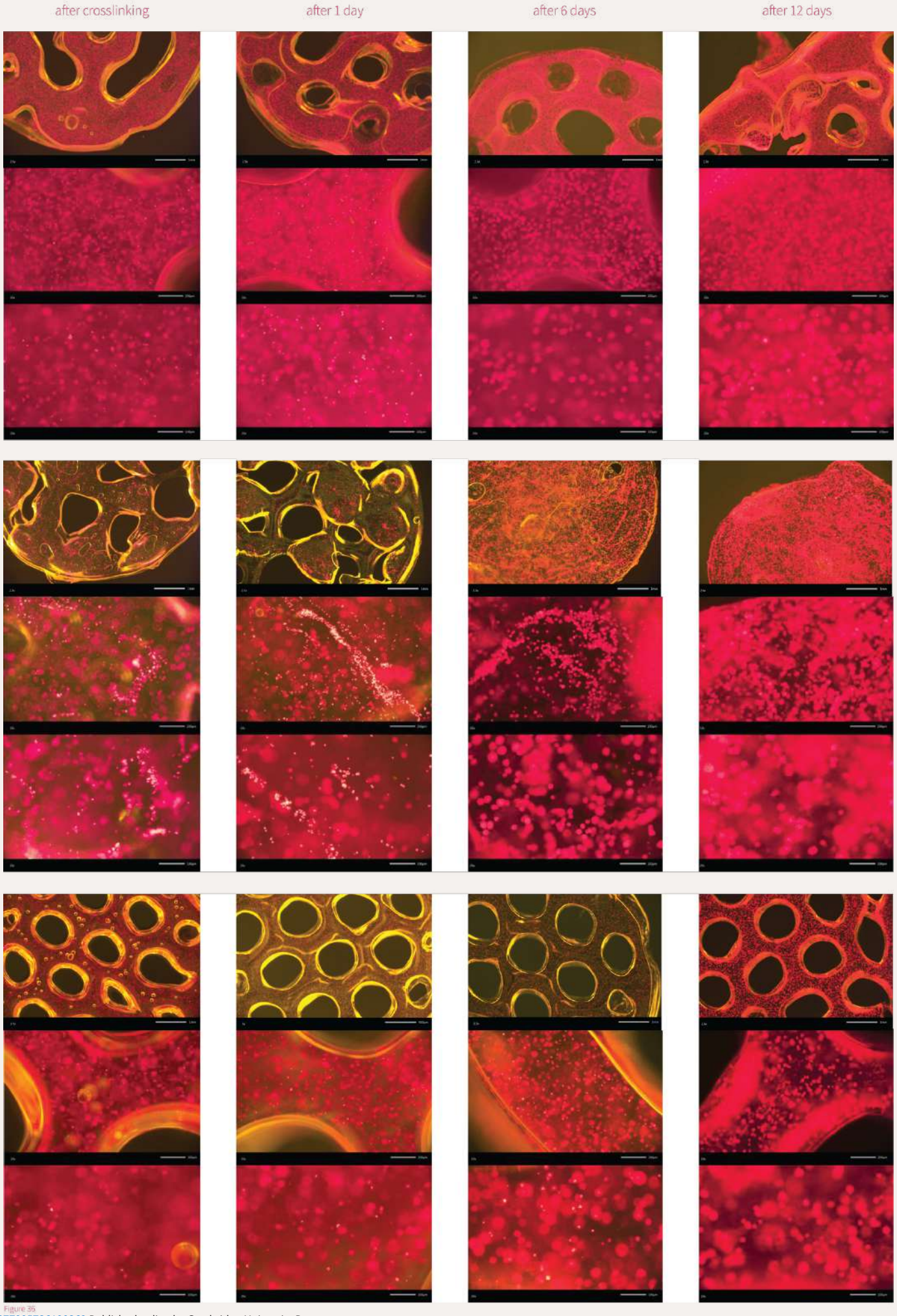


Figure 35

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BioDesign Perspectives

From optimizing to designing-with

Read through a purely technical lens, the decisions documented in this roadmap, which species, which formulation, which pressure, which geometry, are optimization problems. But each decision also describes a condition of life. The pH of the culture medium, the Pluronic content of the ink, the infill density of the construct: these parameters do not only define printability. They constitute the habitat in which microalgae grow, photosynthesize, and remain viable long after printing is complete. Recognizing this distinction is what it means to bring biodesign into conversation with more-than-human approaches to design research. As Wakkary (2021) argues, designing-with living organisms means seeing them not as materials to be shaped toward human ends, but as participants whose needs and temporalities actively shape the process. The results here already reflect this: the printable window shifts when cells enter the ink, the optimal pressure range changes, and the organism's growth stage influences how it behaves inside the construct over time. These are not complications to be managed, they are the organism's participation in the process.

The agency of the organism, imperfection, and the life of the artefact

This reframing also changes how to read the moments that fell outside the intended window: spreading at lower Pluronic concentrations, layer delamination at higher infill densities, partial structural degradation in complex geometries over time. Within an optimization logic, these are failures to be corrected. Within a more-than-human biodesign practice, they carry different meaning. Spreading changes the surface texture of the construct. Delamination opens internal architectures in unplanned ways. Leakage reveals where the organism pushes against its containment, where biomass accumulates beyond what the matrix can hold. These behaviors carry information about how microalgae respond to the conditions created for them, and that information can be as generative as the optimized result (Wakkary et al. 2025). The formulation window identified here is not only the zone where printing succeeds; it is also the zone where the organism can live well within the designed form, and its edges are worth observing, not only correcting.

Ethics of engineering living matter

Working with living organisms in designed contexts carries responsibilities that extend beyond the laboratory. The culture medium, crosslinking agents, and hydrogel matrix all have material and ecological consequences. As bioprinted living materials move toward application in medicine, architecture, or environmental remediation, questions about the conditions under which organisms live, and what happens to them at the end of a product's life, become design questions as much as technical ones. This includes the risk Ginsberg and Chieza (2018) identify in biodesign: producing living materials that are sustainable in fabrication but extractive in use, or that displace one ecological problem onto another. Knowing how microalgae behave under confinement, how their viability changes over time, and how construct geometry shapes their growth is prerequisite knowledge for designing responsibly with living organisms. Future work should address not only biological and material performance, but also ecological implications: what happens when constructs degrade, whether released cells pose environmental risks, and how the fabrication process itself can be made more sustainable.

Toward a more-than-human biodesign practice

This roadmap is primarily a technical resource, but the decisions it documents are also decisions about the conditions under which a living organism will exist. What designers do with that foundation will vary: some may work within the identified window, others may explore its edges, or focus on what the construct enables over time as the organism continues to grow within the printed form. None of these directions requires treating microalgae as merely a material to be optimized.

Key takeaways

Begin with the organism, not the material.
Microalgae are not passive ingredients. They sense, grow, and respond to their environment throughout the entire fabrication process and beyond. Understanding how they live (their light requirements, their tolerance for confinement, their growth rhythms) is where the design process begins.

The formulation is a habitat, not just a substrate.
A combination of alginate and Pluronic F-127 creates conditions where structural stability and biological viability coexist. Designing this balance is an act of mutualistic care: the material must hold form, and the organism must be able to thrive within it (Zhou et al. 2022).

Culture age reveals the organism's own timeline.
Cells at different growth stages carry different capacities for life within the hydrogel. Working with that difference, rather than standardising it away, is part of what it means to design with a living collaborator rather than from a biological material (Wakkary et al. 2025).

Geometry conditions livingness.
Geometry is not just a structural choice, it determines how much light reaches the cells, how nutrients move through the construct, how the organism continues to grow after printing. The architecture of the artefact is the architecture of the habitat.

The organism changes the system.
After microalgae integration, the printable window shifts. The cells are not simply added to an existing process, they participate in it, and the process has to be read accordingly.

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Future directions

Medicine
Bioprinted microalgae-based constructs offer possibilities for wound healing and tissue engineering that go beyond oxygen delivery. As living components embedded within therapeutic materials, they introduce a relational dimension to medical design: the organism continues to grow, respond, and eventually decline within the body's environment, raising questions about care, compatibility, and the ethics of introducing living matter into human tissue that remain largely unaddressed (Kang et al. 2026; Tang et al. 2025).

Food
In food contexts, microalgae are increasingly explored not only as nutritional ingredients but as living components of edible systems that continue to be biologically active after fabrication. This opens up questions about what it means to design food that is alive, and what responsibilities that places on designers toward the organisms involved, as well as toward the ecological systems from which those organisms are drawn (Sun et al. 2024; Marques et al. 2025).

Energy
Bioprinted photosynthetic living materials can function as components of bio-electrochemical systems, contributing to energy generation through microbial fuel cells and biophotovoltaics. Moving this work toward application requires attending not only to efficiency but to the long-term conditions under which the organisms sustain their metabolic activity, and to the broader energy and material costs of maintaining living systems in designed environments (Pu et al. 2024; Wangpraseurt et al. 2022).

Architecture
Microalgae-based living materials are increasingly imagined as components of living architecture: facades that photosynthesize, surfaces that respond to environmental conditions, building elements that are biologically active over time. Realising this potential requires moving beyond the logic of the building as a container for life, toward an understanding of architecture as habitat, designed with and for the organisms that inhabit it (Kim et al. 2025; Vajdi and Aslani 2023).

Environment
Bioprinted microalgae constructs show promise for bioremediation, air purification, and environmental monitoring. These applications position the organism as a worker within human-designed systems, raising questions about what it means to deploy living matter in service of environmental repair, and whether the conditions created for that deployment are themselves ecologically responsible (Yoon et al. 2024; Finny 2024).

Space
In space contexts, photosynthetic living materials are not isolated solutions but multifunctional infrastructure components, simultaneously supporting oxygen production, CO2 capture, and biomass generation in closed systems. Designing for these environments foregrounds questions of mutualistic cohabitation that are relevant far beyond space exploration: what does it mean for humans and microorganisms to share a closed life-support system, and how do we design for the thriving of both (Mapstone et al. 2022; Mannes Guesser De Oliveira et al. 2025)?

Conclusions

This roadmap offers a practical entry point for researchers and biodesigners beginning to work with bioprinted living microalgae, documenting key decision points across species selection, ink formulation, rheological evaluation, crosslinking, and parameter optimization. It is intended not as a fixed protocol but as a reference framework, one that grounds future work in verified material and biological behavior.

The broader potential of microalgae is well established across energy, environmental, and biomedical domains. Bioprinting makes that potential more deployable by embedding cells within controlled architectures, enabling tailored geometries, and facilitating integration with devices and infrastructure. The field is shifting from demonstrating that living materials can be printed toward ensuring they retain function over time and can be designed for defined operational contexts. Materials are moving from passive carriers toward matrices that actively support transport, protect the biological component, and enable multifunctionality within a single format.

Alongside this, biosafety and responsible implementation are becoming central concerns (containment of the living component, contamination resistance, and environmental consequence are increasingly understood as design constraints, not afterthoughts). If functional durability, biosafety, and scalability can be achieved together, microalgae-based engineered living materials have the potential to move from promising prototypes to reliable technologies, contributing practically to clean energy, water treatment, climate action, and sustainable production.

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Ethics statement
Ethical approval was not required for this study because it did not involve human participants, human tissues, personal data, or animals.

Data availability statement
The authors confirm that the data supporting the findings of this study are available within the article.

Author Contributions
Conceptualization: A.D-L., D.P., M.B-L., Methodology: A.D-L, D.P., A.K., J.K., A.K., M.N., Data curation: M.B-L., A.D-L., Data Visualisation: M.B-L., A.D-L., Investigation: A.K., J.K., A.K., M.N, A.D-L., Writing original draft: A.D-L, D.P., M.B-L., A.J., U.M, Writing review& editing: A.D-L, D.P., M.B-L., A.J., U.M, Funding acquisition: D.P. All authors approved the final submitted draft.

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Competing Interests
The authors declare that they have no competing interests.

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