

A 3D-Bioprinted Artificial Coral Platform for Investigating Structural Effects on Microalgal Photophysiology

Yazhi Sun, Netanel Kramer, Mary K. Melarkey, Ashley K. Altera, Martin Tresguerres, Daniel Wangpraseurt, and Shaochen Chen*



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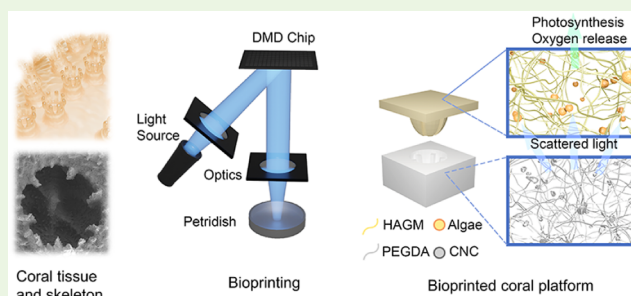
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ABSTRACT: Coral skeletal morphology and optical properties play critical roles in regulating light distribution to symbiotic dinoflagellates and shaping their growth and photosynthetic performance. However, existing experimental approaches lack precise control over skeletal microgeometry and optical scattering, limiting comprehensive studies of coral photophysiology. Here, we present a 3D bioprinted artificial coral platform integrating engineered hydrogel-based tissue with tunable skeletal structures to investigate coral–algal light interactions. Diffusion-optimized hyaluronic acid glycidyl methacrylate (HAGM) hydrogels supported robust growth and photosynthesis of encapsulated dinoflagellates. Using natural coral skeletons from shallow and mesophotic environments, we demonstrate that algal growth within the HAGM tissue layer is regulated by the underlying skeletal morphology. We further fabricated artificial coral skeletons with fine-scale corallite geometries by incorporating cellulose nanocrystals to enhance light scattering. Evaluation under varying light intensities revealed photosynthetic performance trends consistent with those observed under natural conditions. This platform provides a controllable in vitro model for studying coral–algal photophysiology.

KEYWORDS: coral, algae, bioprinting, microbial life, artificial coral



1. INTRODUCTION

Coral reefs serve as critical biodiversity hotspots that support a wide range of marine life. Despite thriving in nutrient-poor waters, their remarkable productivity is largely attributed to mutualistic relationships with symbiotic dinoflagellates.¹ These microalgal partners reside within the coral's endodermal tissues, transferring a substantial proportion of their photosynthetically fixed carbon to the host, thereby meeting most of, and sometimes surpassing the coral's metabolic demand for carbohydrates.² Corals exhibit sophisticated photoadaptive strategies, including morphological and physiological arrangements of the tissue layers that host and surround the symbiotic dinoflagellates, to optimize light capture and sustain photosymbiosis, while the highly organized calcium carbonate skeleton contributes to internal light regulation.^{3,4} This intricate 3D skeleton plays a vital role in promoting photosynthesis by reflecting and scattering light, effectively increasing the availability of photons to the symbiotic algae.^{4,5} While the physiological responses of the tissue and optical contributions of the skeleton have been recognized individually, the coupled interactions between these two components remain incompletely understood. Bridging this gap is essential for predicting coral photophysiology under changing environmental conditions and for guiding the design of bioinspired artificial reef systems.

Variations in coral skeletal structures, including corallite diameter and depth, septal width and length, play a critical role in determining how light is propagated, scattered, and redistributed within the overlying tissue, thereby modulating the photosynthesis efficiency.^{4,6,7} Corals from different depths exhibit skeletal adaptations to their environmental light regime. For instance, *Stylophora pistillata* colonies in shallow, high-light environments tend to develop larger and deeper corallites with more pronounced septa, which increases self-shading and helps prevent photodamage. In contrast, mesophotic *S. pistillata* typically exhibits smaller, shallower corallites with reduced septal height, improving the light scattering for photon delivery under low-light conditions.^{6,8} These variations in skeletal geometry play an essential role as an adaptive optical element, directly shaping the internal photic environment experienced by the symbiotic algae.

However, isolating the functional roles of specific skeletal features in natural coral systems remains challenging due to the

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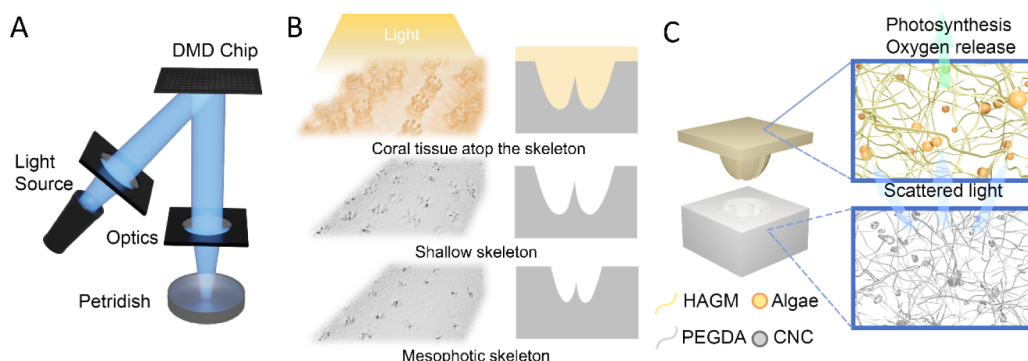


Figure 1. 3D-bioprinted coral models replicating natural coral features for the investigation of coral photophysiology. (A) Schematic representation of the DLP printing. (B) Schematic of a coral tissue and underlying skeleton from shallow and mesophotic corals, perspective view (left) and cross-section (right), showing differences in corallite morphology for both the distribution and polyp dimensions. Complex coral polyp and gastric structures within the tissue are omitted in the cross-sectional view. The illustrated corallite dimensions with the tissue are representative and not specific to either shallow or mesophotic morphologies. (C) Schematic of the engineered coral replicating the biological structures, enabling investigation of their responses to diverse environmental stimuli.

coupled effects of tissue optics, symbiont distribution, and skeletal microstructure, as well as fluctuating environmental conditions.^{3,5,9,10} As a result, developing artificial coral models becomes essential, as they provide a controlled alternative by decoupling these variables. By controlling skeletal geometry and material properties, these models enable the assessment of how specific structural features influence light scattering, and symbiont photosynthetic performance. Furthermore, artificial systems can maintain stable optical and environmental conditions over extended periods, enabling reproducible testing across a wide range of light regimes, flow conditions, and symbiont densities, which are difficult or impossible to achieve in living corals *in situ*.

Recently, bioprinted microalgae constructs have been developed for various applications, such as for facilitating oxygen delivery in hypoxic tissue environments,^{11–13} for efficient biomass or biofuel production,^{14,15} and for environmental remediation applications.^{16–18} Among 3D bioprinting techniques, digital light processing (DLP) can achieve a rapid fabrication speed through the fashion of projecting digital masks to photo-cross-linkable bioink in a layer-by-layer fashion.^{19,20} Current 3D bioprinted coral models have captured the morphological features of living coral tissue and the light properties of natural skeletons.^{21,22} However, current models primarily reproduce macro-scale morphological traits, such as sinuous valleys and bowl-shaped corallites.⁷ They have not been designed to systematically tune fine-scale skeletal structures, such as corallite diameter and depth, which play critical roles in modulating the internal light fields.^{4,10} Previous studies have also been constrained by printing limitations associated with high concentrations of scattering materials, which hinder the fabrication of physiologically relevant microscale skeletal structures that influence symbiont growth. As a result, the ability to decouple and investigate the roles of tissue matrix properties and skeletal morphology in regulating algal growth has remained limited. This limitation restricted the investigation into the factors such as the tissue matrix, skeleton morphology that influencing the growth. Therefore, the development of engineered coral models incorporating these microscale skeletal features offers opportunities to investigate the relationship between skeletal architecture and the regulation of photosynthetic efficiency in coral–algal symbiosis.

To address this challenge, we utilize a multimaterial DLP bioprinting approach (Figure 1A) to create a biomimetic coral platform that mimics the key morphological features of the coral species *S. pistillata* (Figure 1B). To replicate a soft coral tissue and rigid skeleton, we choose biocompatible hydrogels, hyaluronic acid glycidyl methacrylate (HAGMA) and poly(ethylene glycol) diacrylate (PEGDA), respectively, to construct the platform. In this design, the HAGMA tissue layer supports symbiont growth by enabling efficient nutrient and gas exchange, while the PEGDA skeletal component incorporates tunable geometric traits that influence optical properties (Figure 1C). This multilayer, multimaterial design allows systematic control over corallite geometry, including diameter and depth, thereby providing a well-defined experimental system to investigate how skeletal morphology modulates internal light distribution and, consequently, algal photosynthetic performance.

In this study, we first optimized the HAGMA artificial tissue using a diffusion-limited model and evaluated algal proliferation and photosynthetic efficiency as performance metrics. The optimized tissue layer was then applied to natural coral skeletons to assess how variations in skeletal geometry influence biological outcomes. To enable systematic control over the optical properties of engineered skeletons, we simplified the complex architecture of natural coral skeletons into parameterized models based on the measured dimensions. By integrating up to 10% (w/v) cellulose nanocrystals (CNCs) within the PEGDA matrix, we achieved enhanced light scattering while maintaining high printing fidelity, enabling the fabrication of physiologically relevant microscale skeletal features with fine resolution. Our artificial coral systems achieved dinoflagellate growth and photosynthetic performance comparable to those of natural coral reefs, providing a versatile platform for investigating coral physiology with precisely engineered structural controls and offering valuable insights for future reef restoration and conservation strategies.

2. RESULTS AND DISCUSSION

2.1. A Nutrient Diffusion-Limited Construct to Guide the Bioprinting Condition

Efficient nutrient and gas transport is critical for maintaining algal viability and photosynthetic activity within hydrogel-based constructs. In this study, we employed HAGMA as the

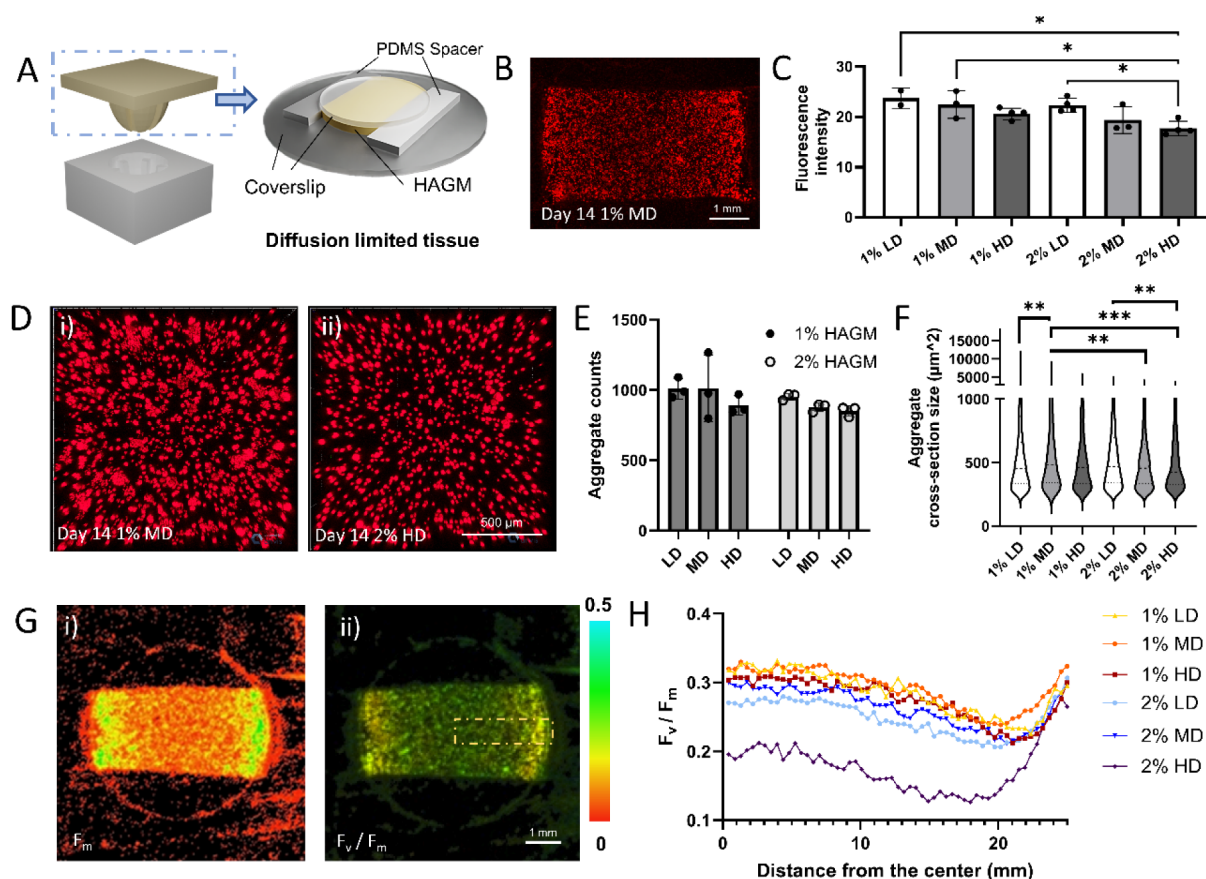


Figure 2. Optimization of tissue layer composition and printing conditions for algal proliferation and photosynthesis. (A) Schematic of the double coverslip structure constructed by two coverslips and bonded PDMS spacers. (B) Fluorescence images (single-plane epifluorescence) of algal proliferation in the double coverslip chamber after 14-day culture, showing a radial gradient after culture. Red: chlorophyll a autofluorescence. Scale bar = 1 mm. (C) Quantified chlorophyll a autofluorescence intensity based on grayscale analysis after 14-day culture, $n = 2-4$, exact values indicated in the figure. (D) Top view of 3D reconstruction of algal aggregates from confocal z-stacks from two representative groups: (i) 1% MD group and (ii) 2% HD group after 14-day culture. Scale bar = 500 μm . (E) Aggregate counts for each condition ($n = 3$). (F) Violin plot showing distribution of cross-sectional areas of cell aggregates ($<250 \mu\text{m}^2$), $n = 3$. Statistical significance was assessed using Dunn's multiple comparisons test with Bonferroni correction. (G) Visualization of the spatial distribution of (i) maximum fluorescence (F_m) and (ii) quantum yield (F_v/F_m) values from a 1%HA MD sample after 14-day culture. Dashed box indicates the radial area used for quantification of F_v/F_m values. (H) F_v/F_m values plotted along the radial direction from the center to the edge, $n = 3$. Statistical significance was assessed using Tukey's post hoc test for multiple comparisons.

tissue-mimicking material due to its biocompatibility and its ability to form a hydrated network that supports mass transport.²³

In our previous study, methacrylated alginate (AlgMA) was used to construct the tissue layer.²² However, in the preliminary experiments for the current study, algal growth in the AlgMA matrix was not superior to that observed in HAGM. More importantly, the AlgMA matrix exhibited weaker optical transparency, which interfered with imaging by attenuating the chlorophyll a autofluorescence signal from the algae (Figure S1). Therefore, HAGM was selected as the hydrogel matrix for all subsequent experiments in this study.

To investigate the effect of diffusion limitation on algal growth within the hydrogel, we developed a custom nutrition diffusion-limited chamber (Figure 2A). In this configuration, the top and bottom glass coverslips blocked convective transport and nutrient diffusion across the surfaces, whereas the PDMS spacers confined the HAGM hydrogel and restricted lateral exchange with the surrounding medium. Because PDMS is effectively impermeable to ionic nutrients but permeable to gases, the nutrient supply to the hydrogel is

dominated by diffusion from the two open ends of the chamber, creating a well-defined and reproducible nutrient gradient across each sample. We intentionally maintained gas permeability at the side walls to avoid introducing hypoxia or CO_2 accumulation as additional limiting factors, which would complicate the interpretation of algal growth responses. By minimizing gas limitation while imposing controlled nutrient restriction, this design ensured that nutrient diffusivity was the primary variable influencing algal performance, enabling a clear comparison of hydrogel formulations under consistent environmental constraints. Therefore, this nutrient-focused diffusion assay provides a robust platform for selecting hydrogel compositions with superior solute transport properties.

We selected the dinoflagellate *Symbiodinium spp* (strain Purpflex) as the photosynthetic symbiont for its ecological relevance in coral systems and its robust photosynthetic activity and adaptability to controlled light and nutrient environments, making it particularly suitable for assessing how structural and material parameters influence algal growth and photosynthetic performance. Furthermore, its role as a representative *Symbiodiniaceae* species in natural coral reefs

ensures that the results from our engineered system can be meaningfully compared to *in situ* coral–algal interactions.

The extent of diffusion limitation is influenced by the physical properties of the hydrogel matrix.^{24,25} Since the polymerization of photo-cross-linkable polymers proceeds via a light-induced free radical chain reaction, both the hydrogel concentration and light exposure parameters can affect cross-linking density and, consequently, the diffusion characteristics of the material. To facilitate diffusion, a loosely cross-linked network is expected to enhance nutrient transport within the hydrogel matrix.²⁶ However, reduced cross-linking density may also compromise cell attachment and confinement, and result in insufficient mechanical stability during long-term culture.²⁷

To assess these effects, we tested two HAGM concentrations (1% and 2% w/v) selected to ensure efficient nutrient diffusion while maintaining structural integrity. The properties of hyaluronic acid-derived photo-cross-linkable bioinks can vary due to factors such as batch-to-batch differences in the source material, molecular weight distribution, and degree of substitution, leading to variations in cross-linking behavior and mechanical properties. As a result, the optimal concentration range is system dependent. In our case, 1% and 2% HAGM were identified as suitable concentrations that yield mechanically stable hydrogels after printing. In addition, the cross-link density could be easily tuned by adjusting the UV exposure. We chose three light exposure conditions for bioprinting: 20 mW cm⁻² for 40 s, 20 mW cm⁻² for 20 s, and 13 mW cm⁻² for 20 s. The three conditions applied the exposure dosage 800 mJ cm⁻², 400 mJ cm⁻², and 260 mJ cm⁻² to the HAGM slab, and are labeled as high dosage (HD), medium dosage (MD) and low dosage (LD) in the following, respectively. In practice, we found that exposure dosage under the LD condition may be underpolymerized after the printing, failing to result in consistently successful printing for the culture. Therefore, this condition was chosen as the lower boundary of our exposure dosage.

The UV dosage affects both matrix stiffness and cell viability. As expected in DLP bioprinting, a higher light intensity and longer exposure time increased polymer cross-linking, producing stiffer matrices. Compression testing revealed that the 2% HD group exhibited the highest stiffness at ~3 kPa, whereas all other conditions produced softer hydrogels with stiffness values below 1 kPa (Figure S2). Although UV light exposure in bioprinting has previously been shown to be nondetrimental to cell viability,^{28,29} UV- and free radical-induced cytotoxicity from high UV dosage is primarily acute and typically manifests within the first few days after exposure.^{30,31} We confirmed that algal viability was unaffected for 3-day culture under our conditions (Figure S3).

We evaluated the algal proliferation in the diffusion-limited design under different exposure conditions. As shown in Figure 2B, after culturing for 14 days, the algal cells showed an obvious fluorescence gradient from the edge to the center, indicating the limited diffusion of nutrient and gases could impair the cell growth of the center area. Figure S4 shows the fluorescence images of the samples in each condition, indicating the gradual formation of the cell density gradient during the 14-day culture. The quantified fluorescence intensity (Figure 2C) showed that the 2% HD group exhibited significantly lower fluorescence intensity in comparison to groups with lower exposure dosage (2% LD, $p < 0.05$) and lower concentration (1% LD, $p < 0.05$, and 1% MD, $p < 0.05$), indicating that the denser hydrogel network could limit cell

growth and migration, which could be caused by the limited diffusion.

To further examine algal aggregate proliferation, we performed confocal microscopy to obtain 3D reconstructions of the cell aggregate morphology in the central region of the samples, thereby characterizing the proliferation in the most diffusion-limited area. Over the 14-day culture period, the size of the aggregates increased and the differences were observed between experimental groups. The confocal 3D reconstruction (Figure 2D) showed the 2% HAGM HD group exhibited visually smaller aggregates compared to the 1% HAGM MD group. Aggregate counts were quantified for each group (Figure 2E). Although 1% HAGM conditions visually appeared to contain larger aggregates with more dispersed red fluorescence, the quantified aggregate size distribution revealed the opposite trend: 1% HAGM groups contained a higher proportion of smaller aggregates (Figure S5C). To investigate the contradiction between the visual evaluation and the quantified results, we applied a size filter. After filtering out aggregates with a cross-sectional area less than 250 μm^2 , the distribution of the aggregate sizes is shown in Figure 2F. These results indicate that the aggregate size distribution of the 1% HAGM group before the filtration was dominated by the small aggregates. To further investigate this phenomenon, that visually denser aggregates showed smaller average aggregate sizes in the quantitative analysis, we compared the aggregate sizes at the center versus the edge of the double-glass setup. At the edge area where algal cells had easier access to nutrients, we expected more proliferation than the center area where the nutrients were limited. As expected, aggregates at the edge appeared visually larger than those at the center (Figure S5A). In the quantification of aggregate sizes, the distribution also showed a higher proportion of smaller aggregates at the edge, leading to a smaller average value. However, after the filtration, the average value of aggregate sizes at the edge of the coverslip was larger than those in the center (Figure S5B). This result confirmed our assumption that the visually denser groups contained more spread and smaller aggregates, and also larger maximum aggregate sizes.

Post hoc pairwise comparisons were conducted (Figure 2E) and identified significant differences between 1% LD and 1% MD ($p < 0.01$), 1% MD and 2% MD ($p < 0.01$), and 2% LD and 2% HD ($p < 0.01$). A highly significant difference was observed between 1% MD and 2% HD ($p < 0.0001$). These results suggest that the 1% HAGM composition supported a broader distribution of aggregate sizes, enabling both the formation of very large aggregates and the migration of single cells through the hydrogel network. In contrast, the 2% HAGM formulation appeared to restrict cell mobility, promoting the formation of relatively uniform, *in situ* aggregates. These differences are likely due to variations in cross-linking density. Within the same HAGM concentration, stronger light exposure for bioprinting had a similar effect, further confirming the role of matrix stiffness in modulating algal aggregation behavior. Notably, the 1% HAGM LD group exhibited more isolated single cells or small aggregates after 14-day culture, suggesting partial network degradation that allowed increased cell mobility.

We further evaluated photosynthetic efficiency using Imaging Pulse-Amplitude-Modulation (PAM) fluorometry. In this technique, a weak measuring light detects the steady-state fluorescence (F_0) from chlorophyll, and brief saturating light pulses determine the maximum fluorescence (F_m) when all

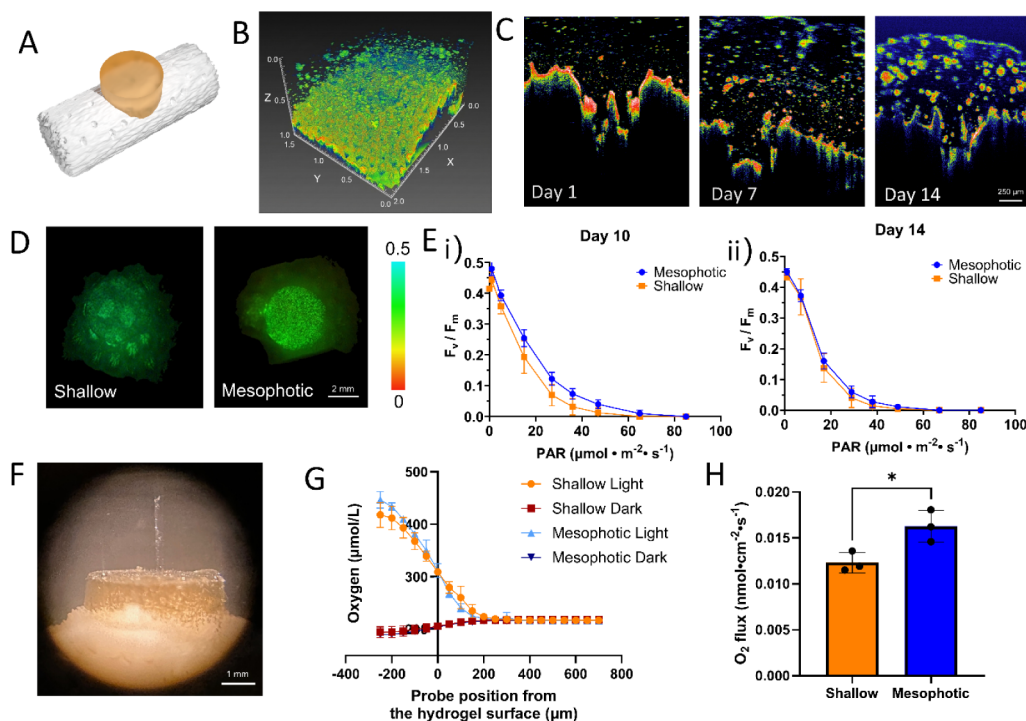


Figure 3. Artificial tissue grown on natural coral skeletons exhibiting different geometric features. (A) Schematic showing a cylindrical hydrogel printed atop a natural coral skeleton. (B) OCT 3D reconstruction of encapsulated algae cells on top of a corallite from a shallow coral skeleton after 7-day culture. The axes are labeled in mm. (C) OCT cross-sectional images for corallite areas from a shallow coral skeleton and the HAGM layer encapsulating algal cells. (D) Colormap of F_v/F_m from HAGM layer atop natural coral skeletons from Imaging-PAM. (E) Light curve showing photosynthesis efficiency of the artificial tissue layer on (i) day 10 and (ii) day 14 during the culture, $n = 4$. (F) Digital image of microsensor measurement with a probe inserting into the HAGM slab. (G) Oxygen profile along the vertical direction, $n = 3$. (H) Plot of calculated oxygen flux from Figure 3G, $n = 3$. Student's t test conducted for group comparison.

photosystem II reaction centers are closed. The effective quantum yield, F_v/F_m , is then calculated as $(F_m - F_0)/F_m$, representing the proportion of absorbed light energy used for photochemistry. As shown in Figure 2G, F_v/F_m distribution within the double-coverslip system revealed higher F_v/F_m values in the central region compared to the edge, where cell density was significantly higher. Despite the easy access to nutrients at the edge, the overgrowth of algal cells and the formation of dense colonies in this region likely resulted in multilayered arrangements that caused self-shading. In such structures, incident light is attenuated as it penetrates through the outer layers, so that deeper cells receive weaker excitation and operate at lower photosystem II efficiency. These shaded cells still contribute to the fluorescence signal detected by imaging-PAM, but with a reduced quantum yield, thereby lowering the apparent F_v/F_m value for that area. Light scattering and reabsorption within the aggregate can further distort excitation and emission, compounding the reduction in local photosynthetic efficiency. F_v/F_m values were quantified along the radial direction, as the dashed box shown in Figure 2G (Figure 2H). In particular, post hoc multiple comparisons of the group factor revealed that the 2% HD group was significantly lower than all other groups ($p < 0.05$), indicating reduced overall photosynthetic activity of the cells. This was likely due to the dense hydrogel network in the 2% HAGM HD group, which constrained photosynthetic activity.

Based on these results, we found the 2% HAGM HD group showed significantly worse performance in both cell proliferation and photosynthesis. Considering the degradation of 1% HAGM LD during the culture, material consumption, and less

UV exposure, we selected the 1% HAGM MD for subsequent experiments as it offers a favorable balance between structural integrity, cell viability, and photosynthetic efficiency.

2.2. Artificial Coral Tissue on Natural Coral Skeletons

To assess whether dinoflagellates encapsulated in the HAGM bioink could mimic the growth and photosynthetic performance of those in natural coral tissues, we printed a hydrogel slab directly onto natural coral skeletons. Skeletons originating from shallow and mesophotic zones were selected, as they exhibit markedly different morphologies. As shown in Figure 3A, a cylindrical hydrogel slab (4 mm diameter, 1 mm thickness) was printed onto a coral skeleton rod. The diameter was chosen to cover at least one polyp on the skeleton surface, while the 1 mm thickness falls within the natural coral tissue range,³² and ensures sufficient signal intensity for subsequent Imaging-PAM measurements.

Due to the opaque nature of the natural skeletons, traditional microscopy was not suitable for visualizing tissue adhesion and cell distribution. Instead, we employed optical coherence tomography (OCT) to characterize tissue layer attachment and monitor algal growth. As shown in Figure 3B and C, the HAGM tissue layer adhered to a natural shallow coral skeleton without any air bubbles, and the size of algal aggregates increased over the course of the culture. It is noticeable that due to the spiky morphology of the natural skeleton, the thicknesses of the printed hydrogel slab vary across the skeleton surface.

We further examined the photosynthetic efficiency of the artificial tissue layer. Figure 3D shows the colormap of F_v/F_m values from both shallow and mesophotic groups, with the

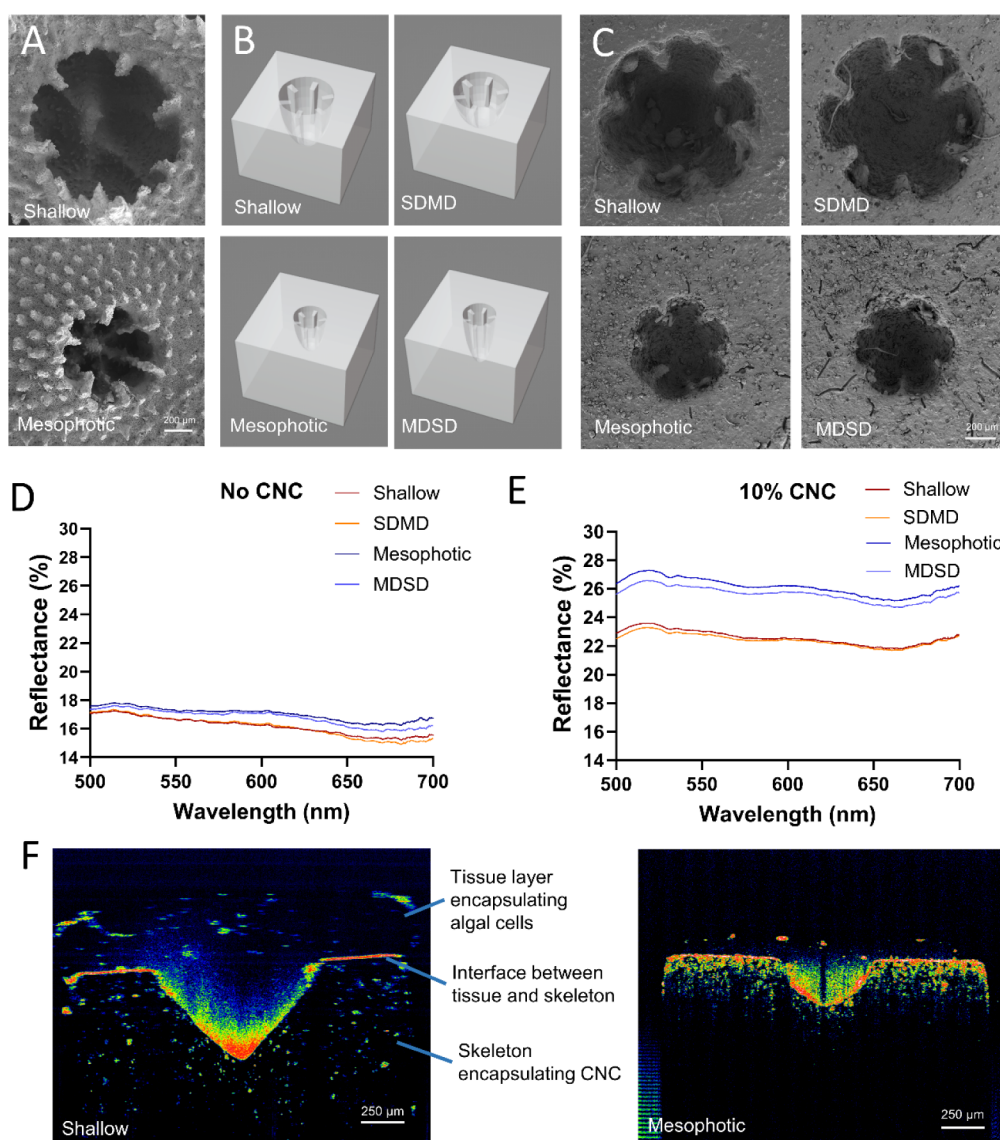


Figure 4. Engineered artificial skeleton mimicking natural structural influence on the tissue layer. (A) SEM images of natural coral corallite. (B) Schematic of the artificial skeleton design based on measured parameters of natural coral skeletons. SDMD: shallow diameter mesophotic depth. MDSD: mesophotic diameter and shallow depth. (C) SEM images of 3D-printed artificial skeletons containing 10% (w/v) CNC. Scale bar = 200 μm . (D) Reflectance of artificial PEGDA skeleton without CNC, $n = 3$. (E) Reflectance of artificial skeletons with 10% CNC, $n = 3$. (F) OCT scan showing a cross-section of the artificial coral structure (left, shallow; right, mesophotic). Scale bar = 250 μm .

circular shape of the hydrogel slab and the natural skeleton surface visible. The signal observed from the coral skeleton was attributed to algal cell adhesion to the surface, and the corallite morphology was more clearly visible in the shallow skeletons due to their larger and deeper corallites. We measured the F_v/F_m values under different photosynthetically active radiation (PAR) conditions. During culture, we found that the photosynthetic performance between the two groups was time sensitive. Within the first week, the photosynthetic performance measured on days 3 and 7 was similar between the groups (Figure S6). The difference between groups became more pronounced by day 10. Post hoc analysis showed that mesophotic samples exhibited significantly higher quantum yield ($p < 0.05$) at intermediate light intensities (PAR = 5–47), while no significant differences were observed at very low or high light levels. The enhanced quantum yield observed in mesophotic samples at intermediate light intensities (PAR = 5–47) is consistent with their adaptation to low-light

environments, characterized by higher light-use efficiency and delayed onset of photochemical saturation. This difference diminished by day 14 (Figure 3E). The observed decline in photosynthetic efficiency in the later stage of the culture was likely due to increased cell density, which can lead to self-shading. The rapid proliferation of algal cells during the culture was confirmed by the fluorescence intensity during the Imaging-PAM measurement (Figure S7), as samples on day 14 showed stronger fluorescence signal. These findings suggest that during the initial phase of algal proliferation, the mesophotic skeleton provided a more favorable light environment, but it took time for algal cells to response to the environmental difference with measurable results. Due to the fast proliferation rate of algal cells, its advantage diminished as both groups reached saturated cell densities. This observation is consistent with previous studies reporting that mesophotic corals exhibit higher photosynthetic efficiency than their shallow-water counterparts.³³

To evaluate the actual oxygen generation, the oxygen production profile was collected along a vertical range extending from 250 μm below the hydrogel surface to 700 μm above the surface (Figure 3F). The results showed that the mesophotic coral skeleton exhibited a higher oxygen production capacity (Figure 3G). Based on the oxygen profile curves, the quantified oxygen flux values indicate the oxygen generation was significantly greater ($\sim 30\%$) from the mesophotic coral skeleton group than those measured for the shallow skeleton (Figure 3H).

The photosynthetic performance and the oxygen generation results demonstrated that the artificial tissue layer could be influenced by natural coral skeletons, in a manner that the mesophotic skeletons facilitate the photosynthesis. This highlights the artificial tissue's value as a controllable platform for constructing artificial coral models to investigate how skeletal geometry influences algal growth and photosynthetic activity. Despite the same hydrogel composition and fabrication conditions being applied, the intricate geometry feature of the natural coral skeleton surface prevented the formation of an artificial layer with uniform thickness, leading to challenges for systematic evaluation. Therefore, an engineered model with precise systematic control is need.

2.3. Construction of Artificial Coral Skeleton and Tissue

Based on dimensions of natural skeletons from shallow and mesophotic corals (Figure 4A), we simplified corallite-scale geometry (diameter, depth, septa) into engineered models (Figure 4B). According to previous studies, the polyp size, which could be represented by the calyx diameter and the theca depth, plays a more dominant role in influencing coral function than other structural features.⁴ Therefore, we designed the engineered coral skeletons based on diameter and depth measurements obtained from natural *S. pistillata* samples. In addition to replicating natural morphologies, we also created artificial variants by swapping the diameter and depth values, enabling systematic investigation of the functional roles of these geometric parameters. Four groups are labeled as shallow, shallow diameter mesophotic depth (SDMD), mesophotic, and mesophotic diameter shallow depth (MDSD), respectively.

To mimic the scattering effect of natural coral skeletons, we embedded CNCs into PEGDA. PEGDA is biocompatible and was utilized as the skeleton material to construct coral skeleton in the previous studies.^{21,22} CNCs were chosen due to its suitable light-scattering properties and mechanical strength.²¹ In addition to the previous study where the CNC concentrations were ranged up to 7% (w/v),²¹ we preferred a higher CNC concentration to better mimic the scattering properties of natural coral skeletons. However, the challenging part of DLP printing with CNC is that light scattering caused by CNCs in the bioink hampers the printing quality of fine structures. Therefore, a balance needed to be found between the scattering effect and printing quality. We tested the CNC concentration of 10% (w/v) and achieved good printing quality while capturing the main geometric features. The printing quality with 10% CNC was demonstrated using PEGDA (MW 250). Scanning electron microscope (SEM) images of the printed 10% CNC-doped coral skeleton showed structures well aligned with the design dimensions, including key feature sizes, such as the polyp and septa (Figure 4C), even compared to pure PEGDA prints (Figure S8).

The reflectance of the polyp region was investigated for both natural and artificial skeletons. Natural carbonate skeletons exhibited high reflectance (60%–75%), with mesophotic skeletons showing greater reflectance than their shallower counterparts, which allowed more light to be reflected toward the top of the polyp (Figure S9). For the artificial skeleton without CNCs, the reflectance values were much lower, and the difference between the groups was not statistically significant (Figure 4D). For the 10% CNC-doped artificial skeletons, similar to the trend in the natural skeletons, groups mimicking mesophotic architectures exhibited higher reflectance (Figure 4E). Post hoc multiple comparisons revealed that the mesophotic group exhibited significantly higher reflectance compared to all other groups ($p < 0.05$). Additionally, engineered geometries (SDMD and MDSD) showed significantly improved reflectance compared to the shallow group ($p < 0.01$), while no significant difference was observed between SDMD and MDSD configurations.

In the 10% CNC doped artificial skeletons, although the overall reflectance (between 21% to 28%) was lower than that of the natural skeletons (between 60% to 75%), a similar pattern was observed between artificial and natural skeletons, and this concentration can still provide optical properties comparable to those of natural corals. In our previous work,²¹ CNCs were shown to aggregate into microparticles (1–10 μm) within the printed skeleton matrix, producing efficient broadband white light scattering similar to natural coral structures. This aggregation enhances multiple scattering events within the skeleton, which are more relevant to light transport to the overlying tissue than surface reflectance alone. In scattering-dominated media such as coral skeletons, bulk optical performance for light redistribution is governed primarily by the transport scattering coefficient rather than absolute reflectance. The measured lower reflectance in the artificial skeleton may therefore reflect differences in surface microtexture or porosity, without substantially impairing the multiple scattering processes that promote light delivery to symbiotic algae. Thus, a 10% CNC loading is expected to maintain sufficient scattering efficiency to emulate the light-enhancing function of natural coral skeletons.

We further incorporated an artificial tissue layer atop the artificial skeleton, through the multimaterial bioprinting approach. The bioprinting process enabled the alignment of the small tissue piece to the previously printed PEGDA skeleton. OCT cross-sectional imaging confirmed that the artificial tissue layer adhered well to the artificial skeleton, without the presence of air bubbles (Figure 4F). Since both HAGM and PEGDA undergo photo-cross-linking via a free-radical mechanism, HAGM can covalently bond to the surface of the PEGDA skeleton through reactions with residual unreacted methacrylate groups. However, the strength of this bonding is influenced by the exposure intensity. In practice, we observed that the HAGM layer could detach from the PEGDA skeleton when directly printed on top of it. To enhance adhesion, the PEGDA surface was pre-exposed prior to building the HAGM layer, as described in the Methods section.

2.4. Photosynthesis of the Artificial Coral Model under Different Conditions

We evaluated the photosynthetic performance of the artificial coral models with different skeleton designs. It is noticeable that coral tissues can dynamically expand or contract in response to environmental cues, resulting in different light-

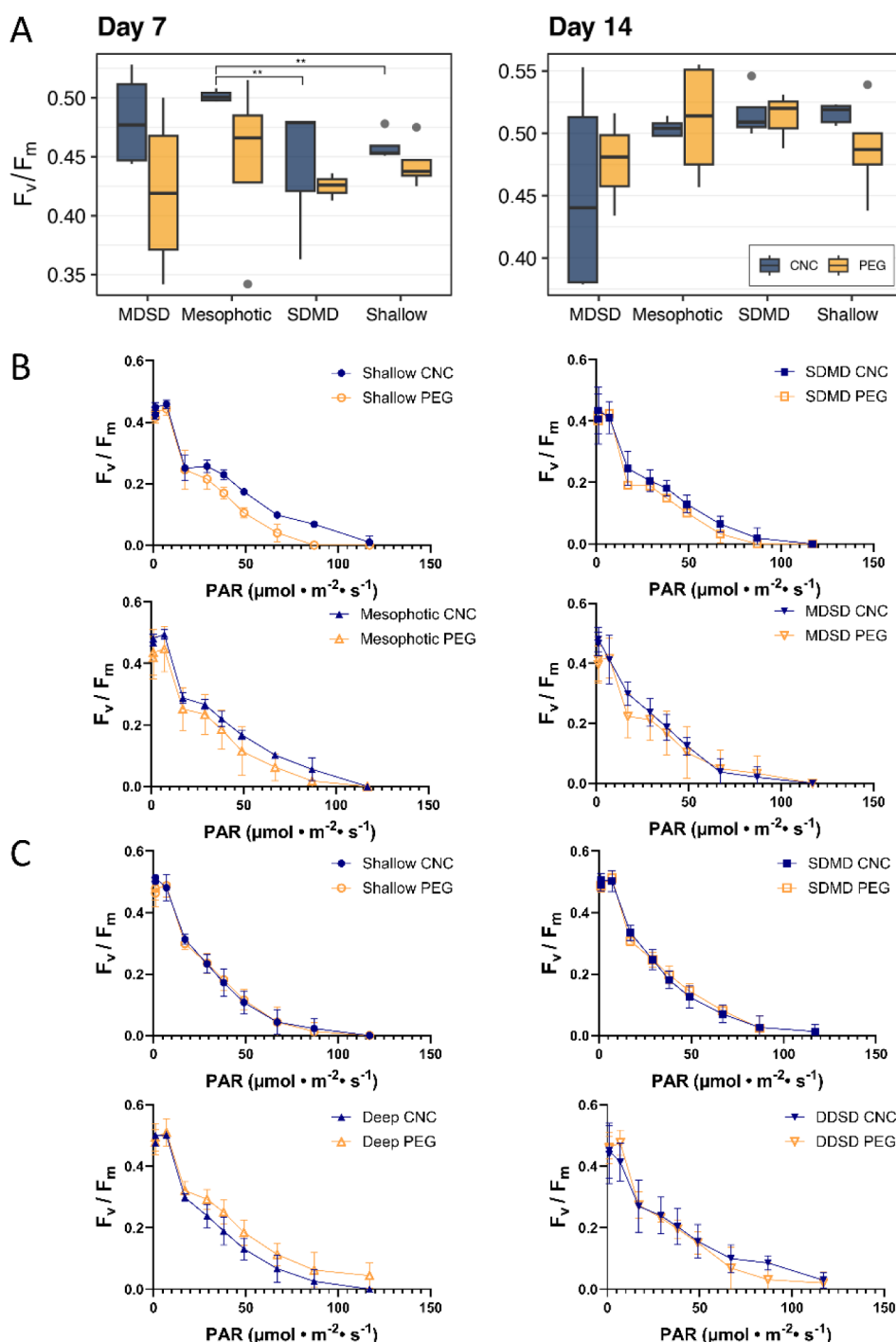


Figure 5. Photosynthesis efficiency of artificial corals under different light conditions. (A) Boxplot of F_v/F_m at PAR = 0 under 25% nutrient culture condition on Day 7 and Day 14. Horizontal lines depict the median, box height depicts the interquartile range, whiskers depict $\pm 1.5 \times$ interquartile range, and dots represent outliers. (B) Light curve (F_v/F_m) of artificial skeletons on Day 7 and (C) Day 14.

regulation behaviors.³⁴ In this study, the artificial coral was intentionally designed to represent a contracted tissue state, in which coral polyps exhibit morphology with retracted tentacles. Compared to the expanded state, this contracted configuration features reduced surface exposure, enabling controlled and reproducible measurements. This approach isolates the role of skeletal light management while minimizing confounding effects from tissue dynamics.

To better resolve differences in algal growth and photosynthetic performance within the artificial coral platform, the

nutrient concentration of the culture medium was adjusted during the experiment. This modification was necessary because the artificial constructs contained a smaller tissue volume ($2 \times 2 \times 0.5 \text{ mm}^3$) compared to the HAGM slab printed in Section 2.3. The small XY dimension was restricted to the dimension of printable high-resolution artificial corallite. The thinner thickness was restricted by the lower reflectance of the artificial skeleton, which may cause nondistinguishable scattering between groups inside the thicker gels along the vertical direction. Therefore, reducing the nutrient concen-

tration allowed us to prolong the observation window and better capture temporal variations in photosynthetic activity, while acknowledging that the two systems are not directly comparable.

The F_v/F_m values measured under a PAR of 1 on culture days 7 and 14 are shown in Figure 5A. On day 7, tissues cultured on CNC-containing skeletons exhibited higher photosynthetic efficiency than those on PEG skeletons. Notably, effect size analysis of F_v/F_m revealed a statistically significant difference between mesophotic and shallow morphologies on day 7 under the CNC condition, as the mesophotic group showed around 10% higher than the shallow group. In the PEG groups, differences between geometry designs were less pronounced, likely because the lower scattering efficiency of PEG reduces the influence of structural variations on light distribution within the tissue layer. On day 14, the mesophotic groups exhibited lower F_v/F_m values, which could be caused by self-shading of overgrowth dinoflagellates, while the shallow groups were still going through the proliferation stage.

The light-response curves measured on day 7 and day 14 are shown in Figure 5B and C. Geometry-dependent effects were evaluated but did not show statistically significant differences under the tested conditions, likely due to the small sample size and the detection limitation. In contrast, the difference between CNC and PEG conditions was more pronounced. Across light intensities, CNC skeletons supported better photosynthetic performance estimated from F_v/F_m , with the difference becoming more pronounced at higher PAR levels. This trend is supported by generalized additive model (GAM) analysis, which revealed a significant difference between CNC and PEG conditions on day 7 ($p < 0.05$), indicating that the CNC skeletons provided a more favorable photosynthetic environment during the early culture stage. This suggests that the dinoflagellates in CNC skeletons acclimated to stronger light conditions more effectively, possibly through enhanced light distribution within the tissue and improved photoprotection. This is consistent with the observation that symbionts in shallow-water corals are generally adapted to high and fluctuating light, possessing greater photochemical capacity and photoprotective mechanisms to dissipate excess energy. In contrast, symbionts from mesophotic-water corals are adapted to low-intensity, spectrally filtered light and may be more vulnerable to photoinhibition when exposed to high irradiance. The larger surface area of shallow corallites may amplify reflectance differences between CNC and PEG skeletons, further enhancing light availability in CNC groups and supporting better performance under strong illumination.

By day 14, however, the GAM analysis indicated that the difference between CNC and PEG conditions was no longer significant ($p = 0.460$), except for the shallow morphology. This convergence suggests that, over time, the symbionts were able to physiologically acclimate to the distinct optical microenvironments imposed by each skeleton type and corallite geometry. We hypothesize that by day 14 the microalgae reached a photosynthetic equilibrium across most morphologies and material conditions through internal photoacclimation processes, resulting in comparable efficiencies despite initial differences in skeletal light-scattering properties. These results indicate that the artificial coral model captures both early stage, skeleton-dependent light regulation and longer-term symbiont acclimation behaviors observed in

natural coral systems, highlighting its potential for investigating coral physiology under more complex and dynamic conditions.

While all morphologies exhibited approximately linear light–photosynthesis relationships on day 7 (effective degrees of freedom (edf) ≈ 1), only the MDSD geometry developed a significantly nonlinear response by day 14 (edf = 2.05, $p < 0.001$). This nonlinearity likely reflects increased optical complexity within the narrow, deep corallites, where heterogeneous light distributions and localized saturation require enhanced physiological regulation by the symbionts, consistent with a more optically demanding microenvironment. These results indicated that the artificial model resembles the photosynthetic pattern on the natural skeleton, suggesting the potential application of our model for investigating coral physiology in more complex conditions.

We further added a control group with a skeleton with a flat plane and used it to examine if the skeleton morphology would affect the algal performance. In this setting, the PEGDA skeletons were a cube shape with the same height, without the engineered corallite shape. As shown in Figure S10, there is no statistically significant difference between the light curves on day 7 and day 14. Notably, the increase in F_v/F_m values over time was more gradual compared to the structured geometries, suggesting a reduced capacity to modulate the local light environment in the absence of corallite features.

This observation provides important context for the results in Figure 5B. While geometry-dependent effects (e.g., shallow vs mesophotic) were not statistically significant under the tested conditions, clear differences between CNC and PEG materials were observed within the structured geometries. In contrast, such material-dependent differences were not detected in the flat control. Together, these results suggest that the influence of material optical properties on photosynthetic performance is coupled with the presence of corallite-inspired geometry, which likely enhances light redistribution within the tissue. In the absence of such structural features, the advantage of CNC-based light scattering is diminished.

Our combined results indicate that our CNC-doped artificial skeleton can emulate the optical influence of natural coral skeletons on the overlying tissue layer, affecting its growth and photosynthetic performance. However, it should be noted that our sample sizes were limited and the sensitivity of Imaging-PAM restricted our ability to detect subtle differences arising from fine adjustments in corallite geometry. In future work, we aim to scale up the artificial coral models using DLP bioprinting, for example, by fabricating organized corallite arrays to support larger tissue constructs, thus enabling a more systematic evaluation of skeletal design effects and provide a comparable result to the natural coral skeleton system. In addition, the current design includes homogeneously distributed CNCs to enhance the scattering in the PEGDA skeleton. It is expected that increasing the skeleton thickness would enhance light scattering due to extended light paths. In this study, we maintained a constant material composition and printing configuration and did not independently vary skeleton thickness. A systematic investigation of thickness-dependent optical behavior would be valuable for future optimization of light management. Moreover, the current system represents a simplified biomimetic model rather than a fully bioartificial coral, as the current HAGM layer primarily functions as a static and supportive matrix for algal growth, lacking the dynamic biological responses with an active host. Therefore, while the term “artificial coral” is used to describe the structural and

functional mimicry achieved in this platform, future efforts could focus on incorporating living host cells, enabling responsive behaviors to better recapitulate native coral systems. As a proof of concept, our current model demonstrates the potential of engineered tissue-skeleton systems as experimental platforms to study coral physiology, with possible extensions to applications in coral reef restoration and environmental remediation.

3. CONCLUSIONS

In this study, we developed and validated a bioprinted artificial coral platform integrating engineered tissue and skeleton components. First, we optimized the hydrogel formulation and diffusion conditions in HAGM to support the growth and photosynthetic activity of dinoflagellates. We then demonstrated that the HAGM tissue layer, when cultured on natural coral skeletons, could reflect differences in underlying skeletal morphology. Building on this, we fabricated artificial skeleton structures with fine-scale corallite features using DLP bioprinting, incorporating cellulose nanocrystals to enhance light-scattering properties. The resulting artificial coral models supported algal growth and photosynthetic performance comparable to those on natural skeletons. Together, these results highlight the potential of our artificial coral system as a versatile platform for investigating coral physiology and as a foundation for future applications in coral reef restoration and environmental remediation.

4. METHODS

4.1. HAGM Polymer Synthesis

HAGM was prepared as described previously.²³ Briefly, 1 g of hyaluronic acid was dissolved in 100 mL of 50% acetone solution at room temperature overnight. Then, 7.2 mL triethylamine (Sigma) and 7.2 mL glycidyl methacrylate (Sigma) were added dropwise until fully mixed. The solution was stirred at room temperature overnight, then dialyzed against DI water with 3.5 kDa dialysis tubing (Spectrum Laboratories) for 48 h. The DI water was changed at 2, 4, 12, and 24 h. The HAGM solution was lyophilized at -80°C in a freeze-dryer (Freezone, Labonco) for 1 week to remove the solvent. The HAGM stock solution was prepared by mixing lyophilized HAGM and filtered seawater without nutrients to reach 4% w/v.

4.2. Natural Coral Skeleton Treatment

Fragments from intact adult coral colonies (approximately 20–25 cm in diameter) were collected from the northern Gulf of Eilat/Aqaba (GoE/A), Red Sea, during recreational and closed-circuit rebreather dives at shallow (4–5 m) and upper mesophotic (45–50 m) depths, corresponding to 40–45% and 3–8% of midday surface PAR, respectively.³⁵ Conspecific coral colonies were sampled at least 5 m apart to avoid collecting clone mates, and fragments were taken from the center of each colony to minimize within-colony variation. The samples were submerged in 5% sodium hypochlorite (NaClO) for 24 h to dissolve the soft tissue, rinsed with distilled water, and air-dried at room temperature.

4.3. Dinoflagellate Cell Culture

B. psygmophilum (Purpflex) cultures were grown in the f/2 medium supplemented with antibiotics (pen/strep 1:1000, kanamycin 1:1000) in sterile culture flasks at 25°C controlled by a growth incubator (model: MIR-154, PHCbi, UK). The incident downwelling irradiance was $\approx 100\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ photons, provided by white LED light panels (AL-H36DS, Ray2, Finnex). For bioprinting experiments, stock cultures were sampled during the active growth phase (about 3–5 days after subculturing). Cells were sampled and if needed spun down at a low centrifugal force of 1300 rcf for 5 min to achieve a desired seeding cell density.

4.4. Bioink Preparation

For the tissue layer bioink, Purpflex culture suspension was centrifuged at $1300 \times g$ for 5 min, and the cell pellet was resuspended in a HAGM stock solution, diluted with filtered seawater to achieve the final printing composition: HAGM at either 1% or 2% (w/v), lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP; Tokyo Chemical Industry) at 0.4% (w/v), photoabsorber tartrazine (Sigma-Aldrich) at 0.1% (w/v) and 2×10^6 Purpflex cells per milliliter. The bioink for the artificial skeleton layer used for SEM imaging was prepared with PEGDA (MW 250; Sigma), photoabsorber tartrazine at 0.1% (w/v) and the photoinitiator Irgacure 819 (Sigma) at 1% (w/v). The bioink for the artificial skeleton with the algal culture atop was composed of 50% (w/v) PEGDA (MW 700; Sigma), photoabsorber tartrazine at 0.1% (w/v), and LAP at 0.4% (w/v).

4.5. Nutrient Diffusion-Limited Chamber Fabrication and Culture

Twelve mm and 5 mm round coverslips were functionalized with methacrylate groups prior to use. Briefly, clean coverslips were incubated overnight at room temperature on a shaker in a solution containing 1.75% (v/v) 3-(trimethoxysilyl)propyl methacrylate (Sigma-Aldrich), 10.50% (v/v) acetic acid:ethanol (1:10), and 87.75% (v/v) absolute ethanol. Following incubation, the coverslips were rinsed twice with absolute ethanol and twice with Milli-Q water, then air-dried at room temperature. The treated coverslips were stored in the dark at room temperature until further use.

To construct the nutrient diffusion-limited chamber, 500 μm -thick PDMS sheets were manually cut into rectangular strips. Both the PDMS strips and the methacrylated coverslips were treated with oxygen plasma for 1 min. Following plasma treatment, the PDMS strips and coverslips were manually assembled to form the designated diffusion-limited chambers. The assembled devices were then incubated at 60°C to promote robust covalent bonding, thereby preventing detachment during the subsequent handling and culture.

The photo-cross-linkable bioink encapsulating algal cells was injected into the narrow gap between two glass coverslips separated by PDMS spacers and polymerized by UV exposure using the designated exposure conditions for each group: 20 mW cm^{-2} for 40 s, 20 mW cm^{-2} for 20 s, and 13 mW cm^{-2} for 20 s. The constructs were cultured in a 24-well plate with 1.5 mL f/2 media in each well. The media was changed every 3 days during the culture.

4.6. Bioprinting

Bioprinting was performed using an in-house-built 3D bioprinter consisting of a 385 nm LED light source, focusing optics, a digital micromirror device (DMD) chip (Texas Instruments, USA), a build platform, and a three-axis motorized stage (Zaber, Canada). The DMD chip contained over 4 million micromirrors (2560×1600 array), each of which could be digitally controlled to project designed masks. The printer was connected to a computer running custom-written software for digital mask input and build-platform movement control.

During printing, the 3D model was sliced into multiple cross sections by the software, and each cross-section was sequentially loaded onto the DMD. The LED light, modulated by the DMD, projected each cross-section pattern onto the photocurable bioink. A thin layer of bioink was cross-linked within a few to several tens of seconds of exposure. After exposure, the motorized stage lifted the cured sample by one layer thickness, allowing uncured bioink to refill the gap between the cured sample and the substrate. The next layer was then formed by exposing the subsequent cross-section. Repeating this process resulted in the fabrication of the complete 3D object.

To print the HAGM tissue layer on natural skeleton, the natural skeleton was secured on the printer probe with a piece of clay. The clay allowed manual adjustment of the skeleton orientation to align the flattest surface suitable for the DLP printing on each skeleton and ensure that the highest point of the skeleton made direct contact with the bioink vat. When loading the bioink, directly printing bioink onto the skeleton often resulted in air bubbles being trapped inside the

polyp cavities (Figure S11). To resolve this issue, all skeleton pieces were presoaked in 1% HAGM solution at 37 °C overnight on a shaker to facilitate the removal of trapped air bubbles. The residual 1% HAGM on the skeleton surface was sucked up through a pipet, while keeping the surface moisturized. Bioink with algal cells was manually injected into the corallite. After loading the skeleton to the probe, the probe lifted up 500 μm to print with the designated exposure dosage. This step was repeated to achieve a total of 1 mm height of HAGM gel. After printing, the skeleton was gently removed from the clay and soaked in a 24-well plate for culture under the incubator light.

To construct the artificial coral, a methacrylated coverslip was first glued to the printer probe using UV glue. To ensure adhesion of the printed structure to the coverslip, the coverslip was exposed to 20 mW cm^{-2} for 5 s. The artificial skeleton was then sequentially printed in a layer-by-layer fashion, with an exposure of 20 mW cm^{-2} for 1 s per layer and a layer height of 100 μm . After printing was completed, the probe was removed. The printed PEGDA skeleton was carefully rinsed with filtered seawater and dried with Kimwipes. Residual rinsing solution may cause weak attachment of the tissue layer. Instead of directly printing the HAGM layer, 10 μL of HAGM bioink was manually pipetted into the corallite within the PEGDA skeleton to prevent air bubble trapping. The probe was then reloaded onto the printer, and HAGM bioink encapsulating algal cells was added to the bioink reservoir. Upon lowering the printing probe to the previous height where printing stopped, the manually pipetted HAGM bioink merged with the bioink in the reservoir due to surface tension. To ensure good adhesion between the HAGM layer and the skeleton, the skeleton surface was exposed using the tissue layer mask for 5 s at the designated exposure intensity for each group. The HAGM layer was then continuously printed by lifting the probe up to 500 μm under the designated exposure conditions. After printing was completed, the coverslip was carefully cut off from the probe and placed into a 24-well plate for culture in the incubator under the same light conditions as the cell culture. F/2 medium with 25% nutrient concentration was added to each well and replaced every 3 days.

4.7. UV Cytotoxicity Assay

A 500 μL Purpflex suspension at a cell density of 2.5×10^5 cells per well was seeded into a 48-well plate and allowed to adhere overnight. The entire well was then exposed to the designated UV conditions using a photomask covering the full well area: 20 mW cm^{-2} for 40 s, 20 mW cm^{-2} for 20 s, or 13 mW cm^{-2} for 20 s. Dead cells were stained using Sytox Green (Invitrogen). Live and dead cells were quantified from fluorescence images using ImageJ.

4.8. Mechanical Measurement

Mechanical properties were measured with a commercial device MicroSquisher (CellScale). Hydrogel cylinders with the height of 1 mm and the diameter of 1 mm were printed. Samples were compressed by a cantilever at the speed of 3 $\mu\text{m/s}$ to reach the strain rate of 18%, and was hold for 2 s, then recovered to the original size at the speed of 6 $\mu\text{m/s}$. Each sample was compressed three times. The first two compression cycles removed hysteresis caused by internal friction. The elastic modulus was calculated using an in-house MATLAB code with the force and displacement data of the third compression cycle from the CellScale software.

4.9. Imaging and Morphological Characterization

Fluorescence images for the double coverslip models were obtained using Leica fluorescent microscopy (Leica DMI6000B, Leica Microsystems, Germany). Fluorescence images were captured at the middle plane of the gel slab under a consistent emission fluorescence intensity throughout the time points. ImageJ was used to quantify the grayscale value of the center area of the slab with a square shape (600 $\mu\text{m} \times 600 \mu\text{m}$).

To quantify the aggregate size in the double coverslip nutrient diffusion-limited models, fluorescence imaging was performed using a confocal microscope (Zeiss LSM 800 with Airyscan). The imaging was conducted in the center area of the HAGM gel slab. The z-stacked scan with a dimension of 1200 $\mu\text{m} \times 1200 \mu\text{m} \times 400 \mu\text{m}$ with

a z step of 10 μm was captured under the 10 $\times$ magnification. The 3D reconstruction images were captured through ZEISS ZEN software.

The microstructure of PEGDA skeleton was observed through scanning electron microscope (SEM, Zeiss Sigma 500). The samples were coated with iridium to enhance conductivity.

4.10. Imaging-PAM Measurement

To noninvasively monitor photosynthetic efficiency we imaged variable chlorophyll a fluorescence using Imaging-PAM (mini-version, Heinz Walz GmbH, Germany). For each measurement, individual samples were placed in a single well dish and dark-adapted for 30 min prior to measuring the maximum quantum yield of photosystem II (F_v/F_m). During the measurement, the minimal fluorescence (F_0) of dark-adapted reaction centers in photosystem II was measured under a light source that would not lead to photosynthesis, and the maximal fluorescence (F_m) was measured under the oversaturating light pulse when all the reaction centers were closed. The difference between F_m and F_0 could show the optimal quantum yield, which represents the amount of absorbed photons that can be utilized for photosynthesis.

For double coverslip structures, the F_v/F_m images were exported. A rectangular area with a dimension of 0.6 mm $\times$ 3 mm was selected along the radial direction. Values were read from the grayscale images according to the manufacturer's instructions and averaged along the shorter-axis direction. For the samples with natural or artificial skeletons, the F_v/F_m was calculated for the area covering the circular slab using the ImagingWinGigE freeware by Walz.

4.11. Microsensor Measurement

Clark-type O_2 microsensors (tip size 50 μm , Unisense, Aarhus, Denmark) were used to measure O_2 microenvironments and photosynthesis of the 3D bioprinted HAGM slab on natural coral skeletons. Sensors were mounted to a motorized micromanipulator (MU1, PyroScience GmbH, Germany) that was attached to a vibration-free optical table. Samples were mounted to a small piece of clay, then placed in a custom-made laminar flow system that provided slow laminar flow (flow rate = 1 cm s^{-1}). Illumination was provided at defined levels of incident downwelling irradiance (90 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photons) by a fiber optic halogen light source (ACE, Schott GmbH). O_2 profiles were performed on day 10. The sensor tip was carefully adjusted to the hydrogel surface under a microscope, then was inserted to a depth of 250 μm . During the measurement, the sensor was slowly pulled out with a step size of 50 μm . The oxygen concentration was recorded at each position. Three samples were measured for each group (mesophotic and shallow), and on each sample 3 replicate profiles were measured. For each sample, the diffusive O_2 flux was calculated using Fick's first law of diffusion using a diffusion coefficient of $D_{\text{O}_2} = 2.2417 \times 10^{-5}$ based on the linear region from the oxygen profile curve, between depths from -150 to $150 \mu\text{m}$.

4.12. OCT Measurement

An OCT system (GAN311 Ganymede OCT Base Unit, Thorlabs, Inc.) equipped with a Thorlabs LSM03 objective scan lens was used to capture the 3D structure of artificial tissue cultured on natural coral skeletons. Cross-sectional regions of interest were manually selected by defining a line on the top-view images of the samples. For imaging on natural coral skeletons, corallite regions were selected randomly due to the opaque HAGM gel and complex surface topology. Scans were performed in air to maximize signal contrast. The sampling rate was set at 40 kHz. The effective Numerical Aperture (NA) is 0.075, and the OCT's coherence length is 5.5 μm in air and 4.1 μm in water.

4.13. Reflectance Measurement

The samples were placed in a black acrylic chamber and illuminated with homogeneous diffuse light provided by a hemisphere coated with barium oxide (BaO) and a LED lamp (CRI-MAX PAR30, Yuji Lighting). Reflectance was measured using a flat-cut fiber-optic reflectance probe (diameter = 0.23 cm, Ocean Insight) connected to a miniature spectrometer (Flame, Ocean Insight; $n = 5$ scans per measurement, boxcar width = 2 nm, resolution = 0.2 nm). The probe was in the center of the corallite on the skeletal surface at a 45° angle

relative to the surface. Three to four samples were measured for each group, and the experimental outcomes were normalized against a 99% diffuse reflectance standard (Spectralon, Labsphere).

4.14. Statistical Analysis

All data were presented as mean $\pm$ standard deviation. All graphs were generated from GraphPad software. Unless otherwise stated, the statistical significance was evaluated using unpaired Student's *t* test between two groups, and using one-way ANOVA for multiple group comparisons. For non-normally distributed data, nonparametric tests were applied. Specifically, algal aggregate sizes in Figure 2F were analyzed using Mann–Whitney tests with Bonferroni correction for multiple comparisons. For Figures 2H, 4D,E, statistical significance was assessed using Kruskal–Wallis test followed by Dunn's post hoc test with Bonferroni correction.

To characterize symbiont light responses, we employed Generalized Additive Models (GAMs) using the {mgcv} package in R, allowing for the quantification of nonlinear physiological trends through effective degrees of freedom (*edf*). To determine the magnitude of differences between corallite geometries and materials (CNC vs PEG), we calculated Hedges' *g* standardized effect sizes and 95% confidence intervals (CI). This approach was chosen for its robustness with small sample sizes, where a difference was considered statistically significant only if the CI did not overlap zero. Comparisons were performed pairwise within each condition to track physiological convergence or divergence between Day 7 and Day 14.

Results are considered significant when **p* < 0.05, ***p* < 0.01, and ****p* < 0.001.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsbiomaterials.6c00243>.

3D reconstruction of confocal images of algal aggregates from the previous and current studies (Figure S1); mechanical characterization of the HAGM matrix (Figure S2); cell viability following UV exposure (Figure S3); fluorescence imaging of the double-coverslip structure (Figure S4); aggregate size distribution within the double-coverslip structure (Figure S5); photosynthetic light curves of HAGM on natural skeletons at days 3 and 7 (Figure S6); Imaging-PAM fluorometer images showing fluorescence intensity during HAGM tissue culture on natural skeletons (Figure S7); SEM images of pure PEGDA 3D-printed skeletons (Figure S8); reflectance spectra of natural shallow and mesophotic skeletons (Figure S9); photosynthetic light curves of flat-surface controls with CNC and PEG (Figure S10); OCT cross-sectional images of the HAGM tissue layer on skeleton surfaces (Figure S11) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Shaochen Chen — Department of Chemical and Nano Engineering, University of California San Diego, La Jolla, California 92093, United States; Email: shc064@ucsd.edu

Authors

Yazhi Sun — Department of Chemical and Nano Engineering, University of California San Diego, La Jolla, California 92093, United States; orcid.org/0000-0002-7525-8327

Netanel Kramer — Marine Biology Research Division, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92037, United States

Mary K. Melarkey — Department of Chemical and Nano Engineering, University of California San Diego, La Jolla, California 92093, United States

Ashley K. Altera — Department of Chemical and Nano Engineering, University of California San Diego, La Jolla, California 92093, United States

Martin Tresguerres — Marine Biology Research Division, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92037, United States

Daniel Wangpraseurt — Marine Biology Research Division, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92037, United States;

orcid.org/0000-0003-4834-8981

Complete contact information is available at:

<https://pubs.acs.org/doi/10.1021/acsbiomaterials.6c00243>

■ Author Contributions

Y.S. contributed to the conceptualization, performed the experiments, and analyzed data. N.K. performed the experiments and analyzed data. M.K.M. and A.K.A. performed the experiments. Y.S. and N.K. wrote the manuscript. S.C., D.W., and M.T. participated in the overall conceptualization, experimental design, data analyses, interpretation, manuscript review, and obtained funding for the project.

■ Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Roth, M. S. The Engine of the Reef: Photobiology of the Coral–Algal Symbiosis. *Front Microbiol.* **2014**, *5*, 422.
- (2) Muscatine, L. The Role of Symbiotic Algae in Carbon and Energy Flux in Reef Corals. *Ecosyst. World* **1990**, *25*, 75–87.
- (3) Murthy, S.; Picioreanu, C.; Kühl, M. Modeling the Radiative, Thermal and Chemical Microenvironment of 3D Scanned Corals. *Front. Mar. Sci.* **2023**, *10*, 1160208.
- (4) Kramer, N.; Guan, J.; Chen, S.; Wangpraseurt, D.; Loya, Y. Morpho-Functional Traits of the Coral Stylophora Pistillata Enhance Light Capture for Photosynthesis at Mesophotic Depths. *Commun. Biol.* **2022**, *5* (1), 861.
- (5) Enríquez, S.; Méndez, E. R.; Prieto, R. I. Multiple Scattering on Coral Skeletons Enhances Light Absorption by Symbiotic Algae. *Limnol. Oceanogr.* **2005**, *50* (4), 1025–1032.
- (6) Kaniewska, P.; Magnusson, S. H.; Anthony, K. R. N.; Reef, R.; Kühl, M.; Hoegh-Guldberg, O. Importance of Macro- Versus Microstructure in Modulating Light Levels Inside Coral Colonies. *J. Phycol.* **2011**, *47* (4), 846–860.

- (7) Swain, T. D.; Lax, S.; Lake, N.; Grooms, H.; Backman, V.; Marcelino, L. A. Relating Coral Skeletal Structures at Different Length Scales to Growth, Light Availability to Symbiodinium, and Thermal Bleaching. *Front. Mar. Sci.* **2018**, *5*, 450.
- (8) Kramer, N.; Tamir, R.; Eyal, G.; Loya, Y. Coral Morphology Portrays the Spatial Distribution and Population Size-Structure Along a 5–100 m Depth Gradient. *Front. Mar. Sci.* **2020**, *7*, 615.
- (9) Wangpraseurt, D.; Polerecky, L.; Larkum, A. W. D.; Ralph, P. J.; Nielsen, D. A.; Pernice, M.; Köhl, M. The in Situ Light Microenvironment of Corals. *Limnol. Oceanogr.* **2014**, *59* (3), 917–926.
- (10) Kramer, N.; Galindo-Martínez, C. T.; Jacques, S. L.; Tresguerres, M.; Loya, Y.; Wangpraseurt, D. Depth-Dependent Microskeletal Features Modify Light Harvesting in Turbinaria Reniformis Corals. *iScience* **2025**, *28* (8), 113137.
- (11) Maharjan, S.; Alva, J.; Cámara, C.; Rubio, A. G.; Hernández, D.; Delavaux, C.; Correa, E.; Romo, M. D.; Bonilla, D.; Santiago, M. L.; Li, W.; Cheng, F.; Ying, G.; Zhang, Y. S. Symbiotic Photosynthetic Oxygenation within 3D-Bioprinted Vascularized Tissues. *Matter* **2021**, *4* (1), 217–240.
- (12) Liu, H.; Mei, H.; Jiang, H.; Jiang, L.; Lin, K.; Jiang, M.; Ding, N.; Li, X.; Gao, Z.; Liu, B.; Lin, W.; Li, J.; Zhou, J. Bioprinted Symbiotic Dressings: A Lichen-Inspired Approach to Diabetic Wound Healing with Enhanced Bioactivity and Structural Integrity. *Small* **2025**, *21* (4), 2407105.
- (13) Wang, X.; Yang, C.; Yu, Y.; Zhao, Y. In Situ 3D Bioprinting Living Photosynthetic Scaffolds for Autotrophic Wound Healing. *Research* **2022**, *2022*, 9794745.
- (14) Zou, Y.; Yi, J.; Dai, Y.; Lei, I. M. Architected Microalgae-Based Matter via 3D Printing: Properties, Printing Techniques, and Applications. *Adv. NanoBiomed Res.* **2025**, *5* (7), 2500023.
- (15) Borges, M. E.; Hernández, L.; Ruiz-Morales, J. C.; Martín-Zarza, P. F.; Fierro, J. L. G.; Esparza, P. Use of 3D Printing for Biofuel Production: Efficient Catalyst for Sustainable Biodiesel Production from Wastes. *Clean Technol. Environ. Policy* **2017**, *19* (8), 2113–2127.
- (16) Boons, R.; Siqueira, G.; Grieder, F.; Kim, S.-J.; Giovanoli, D.; Zimmermann, T.; Nyström, G.; Coulter, F. B.; Studart, A. R. 3D Bioprinting of Diatom-Laden Living Materials for Water Quality Assessment. *Small* **2023**, *19* (50), 2300771.
- (17) Zhao, S.; Guo, C.; Kumarasena, A.; Omenetto, F. G.; Kaplan, D. L. 3D Printing of Functional Microalgal Silk Structures for Environmental Applications. *ACS Biomater. Sci. Eng.* **2019**, *5* (9), 4808–4816.
- (18) Liu, X.-L.; Wang, X.; Wang, Y.; Huang, D.; Li, K.-W.; Luo, M.-J.; Liu, D.-F.; Mu, Y. 3D Bioprinting of Engineered Living Materials with Extracellular Electron Transfer Capability for Water Purification. *Environ. Sci. Technol.* **2024**, *58* (38), 16905–16914.
- (19) Yu, C.; Schimelman, J.; Wang, P.; Miller, K. L.; Ma, X.; You, S.; Guan, J.; Sun, B.; Zhu, W.; Chen, S. Photopolymerizable Biomaterials and Light-Based 3D Printing Strategies for Biomedical Applications. *Chem. Rev.* **2020**, *120* (19), 10695–10743.
- (20) Hwang, H. H.; You, S.; Ma, X.; Kwe, L.; Victorine, G.; Lawrence, N.; Wan, X.; Shen, H.; Zhu, W.; Chen, S. High Throughput Direct 3D Bioprinting in Multiwell Plates. *Biofabrication* **2021**, *13* (2), 025007.
- (21) Wangpraseurt, D.; You, S.; Azam, F.; Jacucci, G.; Gaidarenko, O.; Hildebrand, M.; Köhl, M.; Smith, A. G.; Davey, M. P.; Smith, A.; Deheyn, D. D.; Chen, S.; Vignolini, S. Bionic 3D Printed Corals. *Nat. Commun.* **2020**, *11* (1), 1748.
- (22) Wangpraseurt, D.; Sun, Y.; You, S.; Chua, S.-T.; Noel, S. K.; Willard, H. F.; Berry, D. B.; Clifford, A. M.; Plummer, S.; Xiang, Y.; Hwang, H. H.; Kaandorp, J.; Diaz, J. M.; La Jeunesse, T. C.; Pernice, M.; Vignolini, S.; Tresguerres, M.; Chen, S. Bioprinted Living Coral Microenvironments Mimicking Coral-Algal Symbiosis. *Adv. Funct. Mater.* **2022**, *32* (35), 2202273.
- (23) Baier Leach, J.; Bivens, K. A.; Patrick, C. W., Jr.; Schmidt, C. E. Photocrosslinked Hyaluronic Acid Hydrogels: Natural, Biodegradable Tissue Engineering Scaffolds. *Biotechnol. Bioeng.* **2003**, *82* (5), 578–589.
- (24) Tokuyama, H.; Nakahata, Y.; Ban, T. Diffusion Coefficient of Solute in Heterogeneous and Macroporous Hydrogels and Its Correlation with the Effective Crosslinking Density. *J. Membr. Sci.* **2020**, *595*, 117533.
- (25) Wu, Y.; Joseph, S.; Aluru, N. R. Effect of Cross-Linking on the Diffusion of Water, Ions, and Small Molecules in Hydrogels. *J. Phys. Chem. B* **2009**, *113* (11), 3512–3520.
- (26) Baker, B. M.; Chen, C. S. Deconstructing the Third Dimension – How 3D Culture Microenvironments Alter Cellular Cues. *J. Cell Sci.* **2012**, *125* (13), 3015–3024.
- (27) Caliri, S. R.; Burdick, J. A. A Practical Guide to Hydrogels for Cell Culture. *Nat. Methods* **2016**, *13* (5), 405–414.
- (28) Xu, H.-Q.; Liu, J.-C.; Zhang, Z.-Y.; Xu, C.-X. A Review on Cell Damage, Viability, and Functionality during 3D Bioprinting. *Military Med. Res.* **2022**, *9* (1), 70.
- (29) Adhikari, J.; Roy, A.; Das, A.; Ghosh, M.; Thomas, S.; Sinha, A.; Kim, J.; Saha, P. Effects of Processing Parameters of 3D Bioprinting on the Cellular Activity of Bioinks. *Macromol. Biosci.* **2021**, *21* (1), 2000179.
- (30) Nichol, J. W.; Koshy, S. T.; Bae, H.; Hwang, C. M.; Yamanlar, S.; Khademhosseini, A. Cell-Laden Microengineered Gelatin Methacrylate Hydrogels. *Biomaterials* **2010**, *31* (21), 5536–5544.
- (31) Fairbanks, B. D.; Schwartz, M. P.; Bowman, C. N.; Anseth, K. S. Photoinitiated Polymerization of PEG-Diacrylate with Lithium Phenyl-2,4,6-Trimethylbenzoylphosphine: Polymerization Rate and Cytocompatibility. *Biomaterials* **2009**, *30* (35), 6702–6707.
- (32) Qin, Z.; Yu, K.; Liang, Y.; Chen, B.; Huang, X. Latitudinal Variation in Reef Coral Tissue Thickness in the South China Sea: Potential Linkage with Coral Tolerance to Environmental Stress. *Sci. Total Environ.* **2020**, *711*, 134610.
- (33) Kramer, N.; Tamir, R.; Ben-Zvi, O.; Jacques, S. L.; Loya, Y.; Wangpraseurt, D. Efficient Light-Harvesting of Mesophotic Corals Is Facilitated by Coral Optical Traits. *Funct. Ecol.* **2022**, *36* (2), 406–418.
- (34) Spicer, G. L. C.; Eid, A.; Wangpraseurt, D.; Swain, T. D.; Winkelmann, J. A.; Yi, J.; Köhl, M.; Marcelino, L. A.; Backman, V. Measuring Light Scattering and Absorption in Corals with Inverse Spectroscopic Optical Coherence Tomography (ISOCT): A New Tool for Non-Invasive Monitoring. *Sci. Rep.* **2019**, *9* (1), 14148.
- (35) Tamir, R.; Eyal, G.; Kramer, N.; Laverick, J. H.; Loya, Y. Light Environment Drives the Shallow-to-Mesophotic Coral Community Transition. *Ecosphere* **2019**, *10* (9), No. e02839.

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