

3D bioprinting of plant and animal cell-based hybrid food

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Sushila Maharjan ¹✉, Camila Yamashita ^{1,2}, Cheng Pau Lee ^{1,3}, Alejandro Villalobos Zepeda¹, Ana Karen Michel Farias¹, Andrea Duarte Rivera ¹, Francisco Javier Aguilar Rojas¹, David Sebastian Rendon Ruiz¹, Armando Martinez Hernandez¹, David Hiram Hernandez Medina¹, Carlos Ezio Garciamendez-Mijares¹, Julia Japo¹, Ludivina Bermea Jimenez¹, Sonia Golombek¹, Alessandro Bentivogli¹, Michinao Hashimoto ³ & Yu Shrike Zhang ^{1,4,5}✉

Cellular agriculture is an emerging field that leverages stem cell biology, biotechnology, and tissue engineering to produce meat and other agricultural products through cell culture techniques. One of the most promising methods within this domain is three-dimensional (3D) bioprinting, which allows for precise layering of cells to form sophisticated structures. In this study, we introduce fully automated chaotic bioprinting with a custom-built extrusion setup taking advantage of an integrated Kenics static mixer printhead to create plant and animal cell-based hybrid noodles. These bioprinted hybrid noodles are made of approximately 30–40% unicellular plant cells (*Chlamydomonas* or *Chlorella* microalgae) and 60–70% muscle cells (C2C12 or chicken myoblasts). We further 3D-bioprinted aesthetically appealing hybrid food products of various shapes and sizes, where their textures, nutritional contents, and cooking behaviors are evaluated. This proof-of-concept study demonstrates that 3D bioprinting can reliably produce a distinct category of plant- and animal cell-based hybrid foods and highlights opportunities to create complex culinary designs and explore diverse nutritional profiles with precision and efficiency.

Cellular agriculture (also known as cell-based food-production) is an emerging field that utilizes principles from stem cell biology, biotechnology, and tissue engineering to produce cultivated meat and other agricultural products from cells grown in a laboratory, instead of traditional farming^{1,2}. Among the technologies, tissue engineering holds significant potential to advance the field of cell-based food-production, especially cultivated meat, by providing essential techniques and materials for growing cells into edible food products^{3–6}. In general, cultivated meat is produced through the in vitro cultivation of

animal cells derived from tissue biopsies, cell banks, or the base of a feather in chickens⁷. These cultivating cells can be either primary cells, such as specialized muscle or fat cells, or stem cells that can be differentiated into muscle fibers, fat cells, and other essential cell types such as fibroblasts that constitute meat^{8–10}. In the process of meat-production, these cells are often seeded onto edible scaffolds that mimic the properties of muscle tissue extracellular matrix (ECM)^{11–13}. The scaffolds can then be cultured in bioreactors and supplied with growth media in controlled environments¹⁴ that support both cell

¹Division of Engineering in Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA, USA. ²São Paulo State University (UNESP), Biological Sciences Department, Assis, São Paulo, Brazil. ³Pillar of Engineering Product Development, Singapore University of Technology and Design, Singapore, Singapore. ⁴Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA. ⁵Broad Institute of MIT and Harvard, Cambridge, MA, USA. ✉e-mail: smaharjan@bwh.harvard.edu; yszhang@bwh.harvard.edu

growth and tissue maturation, ultimately converting into meat^{15–17}. Numerous efforts are underway to create cultivated meat that closely resemble traditional pork, beef, poultry, and seafood^{18–21}.

Recently, the production of hybrid meat products has been explored substituting approximately 20–30% of the meat content with plant-based proteins such as soy or wheat protein^{22,23}. Plant-based proteins, being natural biopolymers, have been developed as scaffolding materials, given their promising characteristics, including cost-effectiveness, biocompatibility, biodegradability, and structural flexibility^{24,25}. Moreover, microalgae, unicellular plants found in both fresh water and salt water, are well-recognized for their rich content of various essential nutrients and thus they are often consumed as food additives^{26–29}. For example, microalgae such as *Spirulina*^{30–32} and *Chlorella*^{33,34} are known for their abundant essential nutrients, including vitamins (particularly B12 and provitamin A), minerals (such as iron, calcium, magnesium, potassium, and zinc), carbohydrates, antioxidants, essential fatty acids (e.g., omega-3s), and dietary fibers^{26,29,35,36}. *Chlamydomonas reinhardtii* (*Chlamydomonas*) is another edible alga with a nutrient profile comparable to that of *Spirulina* and *Chlorella* on a dry-weight basis^{37–39}.

Three-dimensional (3D) bioprinting is well-established in tissue engineering for its ability to precisely deposit bioinks, comprised of cells, hydrogels, and/or biologically active molecules, to create 3D functional tissues^{40–42}. 3D bioprinting is now gradually advancing its steps into the development of cultivated meat. For example, a recent study demonstrated a method for creating steak-like tissues using bovine satellite cells and bovine adipose-derived stem cells encapsulated in fibrinogen and Matrigel to bioprint muscle, fat, and vascular fibers individually into a granular gelatin (Gel) bath with thrombin⁴³. On the other hand, microalgae-laden scaffolds were constructed by 3D bioprinting of *Chlamydomonas* and *Spirulina* separately using a bioink comprised of alginate (Alg) and carrageenan to demonstrate 3D microalgae-farming with increased yield⁴⁴.

In this study, we introduce a strategy that involves simultaneous incorporation of plant cells (microalgae) and muscle cells to create a hybrid food product using the continuous chaotic bioprinting technique^{45–47}. Typically, chaotic bioprinting enables extrusion of two or more bioinks simultaneously through the Kenics static mixer (KSM) printhead in a controlled, defined manner, creating a continuous fiber with spatially organized, internally aligned microstructures, each corresponding to a specific bioink^{48,49}. It allows for precise layering of different bioinks, forming a well-organized pattern that reflects the specific distributions of cell types within a single fiber, while preserving the characteristics of each bioink and maintaining spatial integrity. Here, we have developed a fully automated chaotic bioprinting system using a custom-designed extrusion bioprinting setup with an integrated KSM printhead. Through chaotic bioprinting using two Alg/Gel bioink formulations, we successfully demonstrated the continuous production of hybrid noodles with internally aligned lamellar microstructures of microalgae and muscle cells. By adjusting the bioink-extrusion parameters, we could precisely control the biofabrication process to create aesthetically appealing 3D food products with customizable shapes and compositions in a fully automated manner. This study presents a distinct category of cell-based hybrid food, while extending the application of chaotic bioprinting to efficiently design complex culinary products with varied nutritional profiles.

Results

Bioink design and characterizations

To facilitate the bioprinting of microalgae and muscle cells, we selected bioink blends composed of Alg and Gel and optimized the compositions depending upon their viscosities (Fig. 1a). The medium-viscosity Alg (mAlg) and Gel prepolymer solutions were combined to prepare homogenous mAlg-Gel bioink for encapsulating microalga

cells (Fig. 1b). The microalga-bioink consisted of 1% (w/v) mAlg (1mAlg) and 4% (w/v) Gel (4Gel) or 5% (w/v) Gel (5Gel). Similarly, the low-viscosity Alg (lAlg) and Gel prepolymer solutions were mixed to produce the lAlg-Gel bioink for encapsulating muscle cells (Fig. 1c). The muscle cell-bioink comprised of 2% (w/v) lAlg (2lAlg) and 5% (w/v) Gel (5Gel) or 6% (w/v) Gel (6Gel).

The rheological profile of a bioink plays a key role in determining its flow, deformation, shape fidelity, and printability. Therefore, the rheological properties of the bioinks, including changes in storage moduli (G') and loss moduli (G'') of the bioink blends, were measured at a rate of 5°C min^{-1} from 20°C – 50°C , at a fixed frequency of 1 Hz and strain 1%. The G' and G'' values provide insights into the elastic (stiffness) and viscous (flowability) properties as well as gelation profiles of the bioinks. Crossover points in temperature-sweep tests indicate the gelation point, and the G'/G'' values obtained from temperature-sweep tests showed the gelation points of bioinks to be within the range of 31°C – 37°C (Supplementary Fig. 1a). The microalga-bioink, 1mAlg-5Gel, and the muscle cell-bioink, 2lAlg-5Gel, displayed gelation properties at temperatures close to physiological temperature ($\sim 36^\circ\text{C}$). The bioink blends, 1mAlg-4Gel/2lAlg-5Gel and 1mAlg-5Gel/2lAlg-5Gel, exhibited gelation temperatures of 33.5°C and 35.2°C , respectively, while the bioink blends, 1mAlg-4Gel/2lAlg-6Gel and 1mAlg-5Gel/2lAlg-6Gel, displayed gelation temperatures of 31.1°C and 31.9°C , respectively (Supplementary Fig. 1b). Similarly, to evaluate the shear-thinning properties of the bioinks, G' and G'' values were also measured as a function of oscillatory stress of the bioink blends at constant frequency of 1 Hz over 0.1–2000 Pa, at 25°C (Supplementary Fig. 1c). The G' and G'' values for the oscillation sweep showed that the yield stress of the muscle cell-bioink comprised of 2lAlg-6Gel was higher than that comprised of 2lAlg-5Gel. Likewise, the yield stress of microalga-bioink of 1mAlg-5Gel was higher than that of 1mAlg-4Gel. The yield stress is the stress at which the biomaterial begins to flow or deform permanently, marked by the point where G' and G'' values cross over, and is crucial for assessing printability and shape-fidelity during the bioprinting. These results indicated that the bioinks, 2lAlg-6Gel and 1mAlg-5Gel, were more mechanically stable among the bioinks studied (Supplementary Fig. 1d). While the complex viscosity of the bioinks decreased with increasing temperature, the viscosity of the bioink blend, composed of 1mAlg-5Gel for microalgae and 2lAlg-5Gel for muscle cells, was higher compared to other bioinks at room temperature (Supplementary Fig. 1e).

3D chaotic bioprinting

We built a mechanical extrusion-bioprinter equipped with multiple extruders to enable the simultaneous injection of two or more bioinks through the KSM, and a magnetic holder to secure the KSM in place without movement (Fig. 1a). We then investigated chaotic extrusion of the selected Alg/Gel bioink blends for the fabrication of freestanding noodles using the three-element KSM. Based on the rheological properties of the Alg/Gel bioink blends, we examined the bioprinting capability of two bioink formulations for microalgae (1mAlg-4Gel and 1mAlg-5Gel) along with two bioink formulations for muscle cells (2lAlg-5Gel and 2lAlg-6Gel). These investigations involved varying temperatures (25°C – 37°C), flow rates (0.5 – 5 mL min^{-1}), and feeding rates (0.5 – 5 mm s^{-1}). In the flow rate-variation test, the feeding rate was kept constant while in the feeding rate-variation experiment, the flow rate was held constant. The flow rates and feeding rates were programmed into the G-code commands. These printability studies revealed that the optimal bioink formulation was the blend of muscle cell-bioink, 2lAlg-5Gel, and microalga-bioink, 1mAlg-5Gel, at a flow rate of 1.5 mL min^{-1} for continuous chaotic bioprinting of the noodles (Fig. 2a). Utilizing the three-element KSM and the two bioinks, the chaotic bioprinting process produced noodles exhibiting eight aligned internal microstriations, with four alternating layers of each bioink. This was demonstrated through the bioprinting of 1mAlg-5Gel containing red

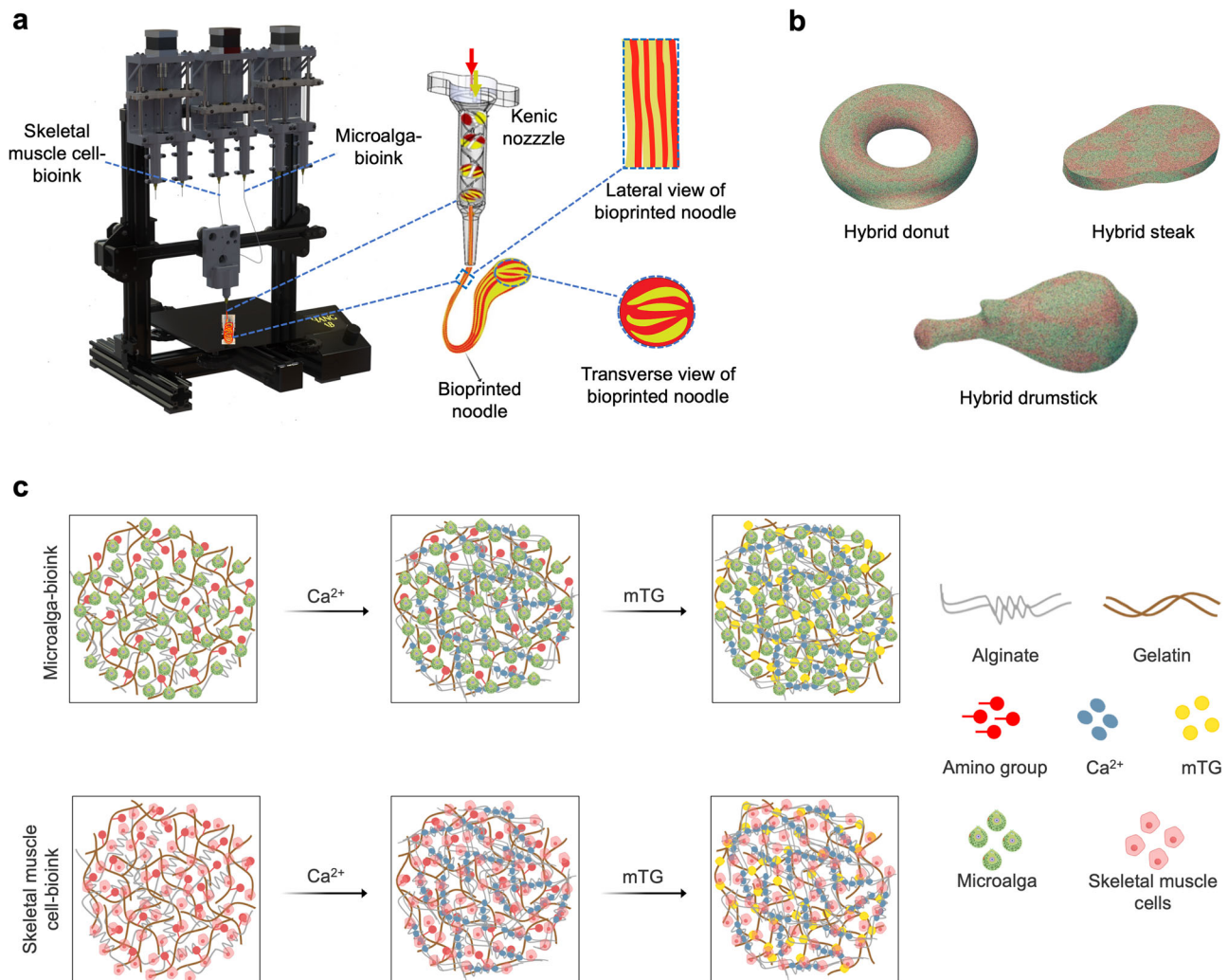


Fig. 1 | Automated continuous chaotic bioprinting. **a** Schematic illustration showing automated chaotic bioprinting of noodles with internally aligned lamellar microstructures, using the three-element KSM printhead, with highlighted lateral

and transverse views. **b** Schematics of various 3D hybrid food items, produced by simultaneous chaotic bioprinting of microalgae and muscle cells. **c** Schematic illustration showing bioink designs for microalga and muscle cell-bioprinting.

fluorescent beads and 2lAlg-5Gel, with embedded blue fluorescent beads resulting in alternating red and blue microlayers (Fig. 2a and Supplementary Movie 1).

We further optimized the chaotic bioprinting process to create 3D structures with varied designs. This optimization involved programming flow rates and feeding rates into G-codes using computer-aided design (CAD) models during the bioprinting process. In both bioprinting of freestanding noodles and 3D structures, the diameter of the bioprinted noodles varied with the flow rates (Fig. 2b). Additionally, during the bioprinting of 3D structures, the diameter of the bioprinted noodles increased gradually with the increase in the feeding rate, as shown in Fig. 2c. In addition to the optimal flow rate of 1.5 mL min^{-1} , a feeding rate of 4 mm s^{-1} was determined to be best for extruding noodles with a diameter of approximately $1000 \mu\text{m}$ during chaotic bioprinting of 3D structures using the optimized bioink blends (Fig. 2d, e and Supplementary Fig. 2). Throughout the bioprinting process, the temperature of the KSM printhead and the bioink was maintained above the gelation temperature ($\sim 37^\circ\text{C}$). The optimization process and the results revealed the reproducibility of automated chaotic bioprinting, consistently producing noodles with adjustable diameters based on the parameters used. The bioink compositions for microalgae and muscle cells, along with the bioprinting parameters used in this study, are summarized in Table 1.

Cytocompatibility of bioinks

We selected two different microalgae, *Chlamydomonas* and *Chlorella vulgaris* (*Chlorella*) to assess their growth profiles. *Chlamydomonas* cells were encapsulated separately in 1mAlg-4Gel or 1mAlg-5Gel at the concentration of $5 \times 10^6 \text{ cells mL}^{-1}$ in the precursor solution, cast in polydimethylsiloxane (PDMS) molds, and crosslinked with 1% (w/v) calcium chloride (CaCl_2) to create the *Chlamydomonas* constructs. These *Chlamydomonas* constructs were then incubated overnight in Tris-acetate-phosphate growth (TAP) medium supplemented with 1% (w/v) microbial transglutaminase (mTG) at 37°C under continuous light illumination at 2800 lux. These cultures were further maintained for up to 21 days. Similarly, *Chlorella* constructs were generated by encapsulating *Chlorella* cells in 1mAlg-4Gel or 1mAlg-5Gel at the concentration of $5 \times 10^6 \text{ cells mL}^{-1}$ in the precursor solution and cultured in Alga-Gro Freshwater (Alga-Gro) medium under similar conditions for 21 days. We evaluated the viabilities of *Chlamydomonas* and *Chlorella* throughout the cultivation period by SYTOX Orange staining for dead microalga cells, where live microalgae could be observed under fluorescence microscopy due to the presence of autofluorescence of chlorophyll. The results displayed robust growth for both *Chlamydomonas* (Supplementary Fig. 3a, b) and *Chlorella* (Supplementary Fig. 3c, d) in either 1mAlg-4Gel or 1mAlg-5Gel bioink. High cell viability values ($>97\%$) were observed throughout the period of 21 days.

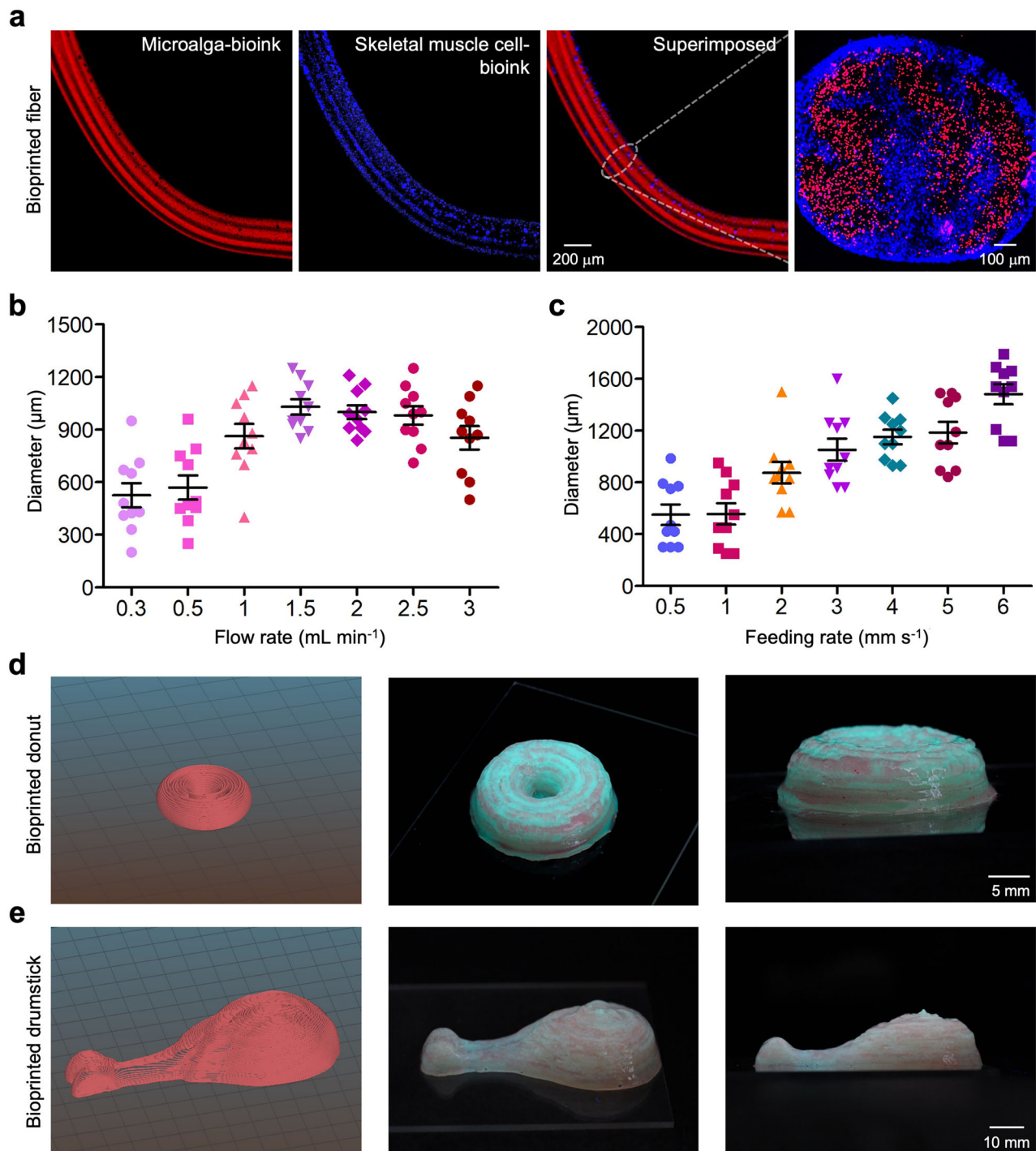


Fig. 2 | Automated chaotic bioprinting of various 3D structures. **a** Fluorescence microscopic images of bioprinted noodle with internally aligned lamellar microstructures of 1mAlg-5Gel containing red fluorescence beads and 2lAlg-5Gel with embedded blue fluorescence beads. **b** Plot showing the variations in noodle diameters with varying bioink flow rates, during chaotic bioprinting of noodles. Data are presented as the mean values $\pm$ SEMs (n=10 independent samples, each obtained from a separate printing run). **c** Plot demonstrating the changes in noodle diameter with varying bioink feeding rates during chaotic bioprinting of 3D

structures. Data are presented as the mean values $\pm$ SEMs (n=10 independent samples, each obtained from a separate printing run). **d** 3D CAD model of a donut alongside photographs showing top and side views of a bioprinted donut, utilizing microalga-bioink (green) and muscle cell-bioink (pink). **e** 3D CAD model of a chicken drumstick followed by photographs showing top and side views of the bioprinted chicken drumstick, using microalga-bioink (green) and muscle cell-bioink (pink).

Additionally, there was no significant difference in the cell viabilities of both microalgae in 1mAlg-4Gel and 1mAlg-5Gel confirming the biocompatibility and supportive nature of these bioinks for their cultivation.

Similarly, we examined the growth of murine C2C12 skeletal myoblasts (C2C12 cells) in Alg/Gel bioinks. C2C12 cells were encapsulated in 2lAlg-6Gel or 2lAlg-5Gel at the concentration of 5×10^6 cells mL^{-1} in the precursor solution, cast in PDMS molds, and crosslinked with 1%

(w/v) CaCl_2 to form C2C12 constructs. The C2C12 constructs were incubated overnight at 37 °C in high-glucose Dulbecco's modified Eagle medium (DMEM), supplemented with 1% (w/v) mTG, 10% (v/v) fetal bovine serum (FBS), and 1% (v/v) antibiotic-antimycotic solution (anti-anti). The C2C12 constructs were further cultured in DMEM, supplemented with 10% (v/v) FBS and 1% (v/v) anti-anti (DMEM growth medium), for 7 days. Then after, the culture medium was replaced with DMEM supplemented with 2% horse serum and 1% (v/v) anti-anti (DMEM differentiation medium), to stimulate myogenic differentiation. The cultures were maintained for up to 21 days with fresh DMEM differentiation medium being replaced every 2 days. We assessed the cell viabilities by Live/Dead assay and the morphology of C2C12 cells within the C2C12 constructs by F-actin staining throughout the cultivation period of 21 days. The results revealed high cell viabilities (~95%) throughout the period of 21 days and myogenic differentiation, characterized by the formation of aligned myotubes observed at 7 days post-differentiation in both 2lAlg-6Gel and 2lAlg-5Gel bioinks (Supplementary Fig. 4). There was no significant difference in the viabilities of the C2C12 cells in 2lAlg-6Gel and 2lAlg-5Gel confirming the favorable nature of these bioinks.

3D chaotic bioprinting of microalga noodles

Next, we employed automated chaotic bioprinting to produce microalga noodles by incorporating microalgae into optimized Alg/Gel blend bioinks. Based on rheological analyses, printability evaluations, and cell growth assessments, we determined 1mAlg-5Gel as the optimal bioink for microalgae and 2lAlg-5Gel for muscle cells for subsequent chaotic bioprinting of noodles and various 3D hybrid food structures.

For the chaotic bioprinting of microalga noodles, *Chlamydomonas* cell-pellets were suspended in 3 mL of the 1mAlg/5Gel precursor solution at the concentration of 1×10^7 microalga cells mL^{-1} . The *Chlamydomonas*-encapsulated microalga bioink was loaded into a syringe, whereas the acellular 2lAlg-5Gel bioink, devoid of cells, was loaded into a separate syringe. These syringes were placed within the extruder and the three-element KSM printhead was mounted in the nozzle-holder of the bioprinter. The bioprinting of microalga noodles were performed at a flow rate of 1.5 mL min^{-1} , following the optimized procedure as described above (Fig. 3a and Supplementary Movie 4). The microalga noodles, collected in a CaCl_2 bath (1% w/v), were transferred to a petri dish containing TAP medium supplemented with mTG (1% w/v) and incubated overnight at 37 °C under continuous light illumination of 2800 lux (Fig. 3b). These microalga noodles were then cultured in TAP medium for a duration of up to 21 days. The microalga noodles, approximately 1000 μm in diameter, exhibited internally aligned lamellar microstructures featuring alternating *Chlamydomonas* and acellular bioink striations, each measuring about 120–140 μm in width (Fig. 3c, d). Throughout the 21-day cultivation period, the viability assessment of bioprinted *Chlamydomonas* showed vigorous growth, exceeding 97%, which demonstrated their high viabilities within the microalga noodles (Fig. 3e). In addition, we also assessed the growth rate of the *Chlamydomonas* within bioprinted microalga noodles over the 21-day culture period (Supplementary Fig. 5a). Moreover, since the dissolved oxygen (O_2) released by microalgae and the chlorophyll content are the direct indicators of photosynthetic efficiency, the primary metabolic activity of the microalgae, we measured the dissolved O_2 released into the medium and the chlorophyll contents of the photoautotrophic *Chlamydomonas* within the microalga noodles. Consistent with the growth of the *Chlamydomonas*, the levels of dissolved O_2 (Fig. 3f) and chlorophyll contents (Supplementary Fig. 5b) were observed to also increase over the 21-day culture period.

Given that one of the objectives of automating chaotic bioprinting is to scale up food-production in a more efficient and customizable way, we have further demonstrated its scalability, revealing rapid and consistent production of *Chlamydomonas* noodles at a throughput of

Table 1 | Bioink compositions and bioprinting parameters

Bioinks	mAlg (w/v)	Gel (w/v)	lAlg (w/v)	Flow rate	Feeding rate
Microalga-bioink	1%	4%	—	1.5 mL min^{-1}	4 mm s^{-1}
	1%	5%	—		
Muscle cell-bioink	—	5%	2%		
	—	6%	2%		

approximately 12 g min^{-1} (Supplementary Fig. 6). In addition, to showcase the universal applications of our automated chaotic bioprinting to produce various nutrient-rich microalga noodles, we further bioprinted *Chlorella* noodles by incorporating *Chlorella* into the 2lAlg-5Gel bioink, following the similar procedure as described above. The *Chlorella* noodles were cultured in the Alga-Gro medium at 37 °C under continuous light illumination of 2800 lux for a period of up to 14 days (Supplementary Fig. 7a). These *Chlorella* noodles, ~1000 μm in diameter, also possessed internally aligned lamellar microstructures with alternating layers of *Chlorella* and acellular bioink (Supplementary Fig. 7b, c).

3D chaotic bioprinting of muscle and plant cell-based hybrid noodles

To produce muscle and plant cell-based hybrid noodles by chaotic bioprinting, we first used C2C12 cells as model muscle cells to form muscular microlayers alongside *Chlamydomonas* within the hybrid noodles. We initiated with the optimization of the co-culture medium to ensure the optimal growth of both C2C12 and *Chlamydomonas* cells simultaneously within the 2lAlg-5Gel constructs. We generated hybrid scaffolds by encapsulating C2C12 cells (1×10^7 cells mL^{-1}) and *Chlamydomonas* (5×10^6 cells mL^{-1}) in the 2lAlg-5Gel precursor solution, cast in PDMS molds, and crosslinked with 1% (w/v) CaCl_2 to form hybrid constructs. We explored various combinations of DMEM growth medium with TAP medium for co-culture of C2C12 and *Chlamydomonas* cells up to 21 days. After 7 days of co-culture, the DMEM growth medium was replaced with equal amount of DMEM differentiation medium in each combination studied. The combination of 3 parts of DMEM and 1 part of TAP medium (co-culture growth medium) was found to be the most effective for simultaneous growth of C2C12 and *Chlamydomonas* cells. This co-culture growth medium and co-culture differentiation medium (3:1 differentiation:TAP medium) supported the optimal growth of *Chlamydomonas* cells while simultaneously promoting the growth and differentiation of C2C12 cells within the hybrid constructs (Supplementary Fig. 8).

After optimization of the co-culture medium, we next bioprinted the C2C12-microalga hybrid noodles. To enable visualization under a fluorescence microscope, we first labeled the C2C12 cells with CellTracker Green CMFDA dye before encapsulating them in the 2lAlg-5Gel bioink. Subsequently, green CellTracker-labeled C2C12 cells were suspended in 3 mL of the 2lAlg-5Gel solution to achieve a concentration of $1.5\text{--}1.8 \times 10^7$ viable cells mL^{-1} in the bioink. Likewise, *Chlamydomonas* cell-pellets were suspended in 3 mL of the 1mAlg-5Gel solution to obtain a concentration of 1×10^7 cells mL^{-1} in the bioink. The hybrid noodles were then bioprinted at the flow rate of 1.5 mL min^{-1} , followed by immediate crosslinking in the 1% (w/v) CaCl_2 solution and overnight crosslinking in the 1% (w/v) mTG solution, using the optimized procedure as described above. These hybrid noodles, approximately 1000 μm in diameter, displayed closely aligned lamellar microstructures featuring alternating C2C12 cells (green) and *Chlamydomonas* (red) striations, each measuring approximately 120–140 μm in width, like those found in microalga noodles (Fig. 4a, b).

To test the cell growth, we again bioprinted hybrid noodles with C2C12 cells embedded within 2lAlg-5Gel and *Chlamydomonas* within 1mAlg-5Gel. These hybrid noodles were cultured in the co-culture

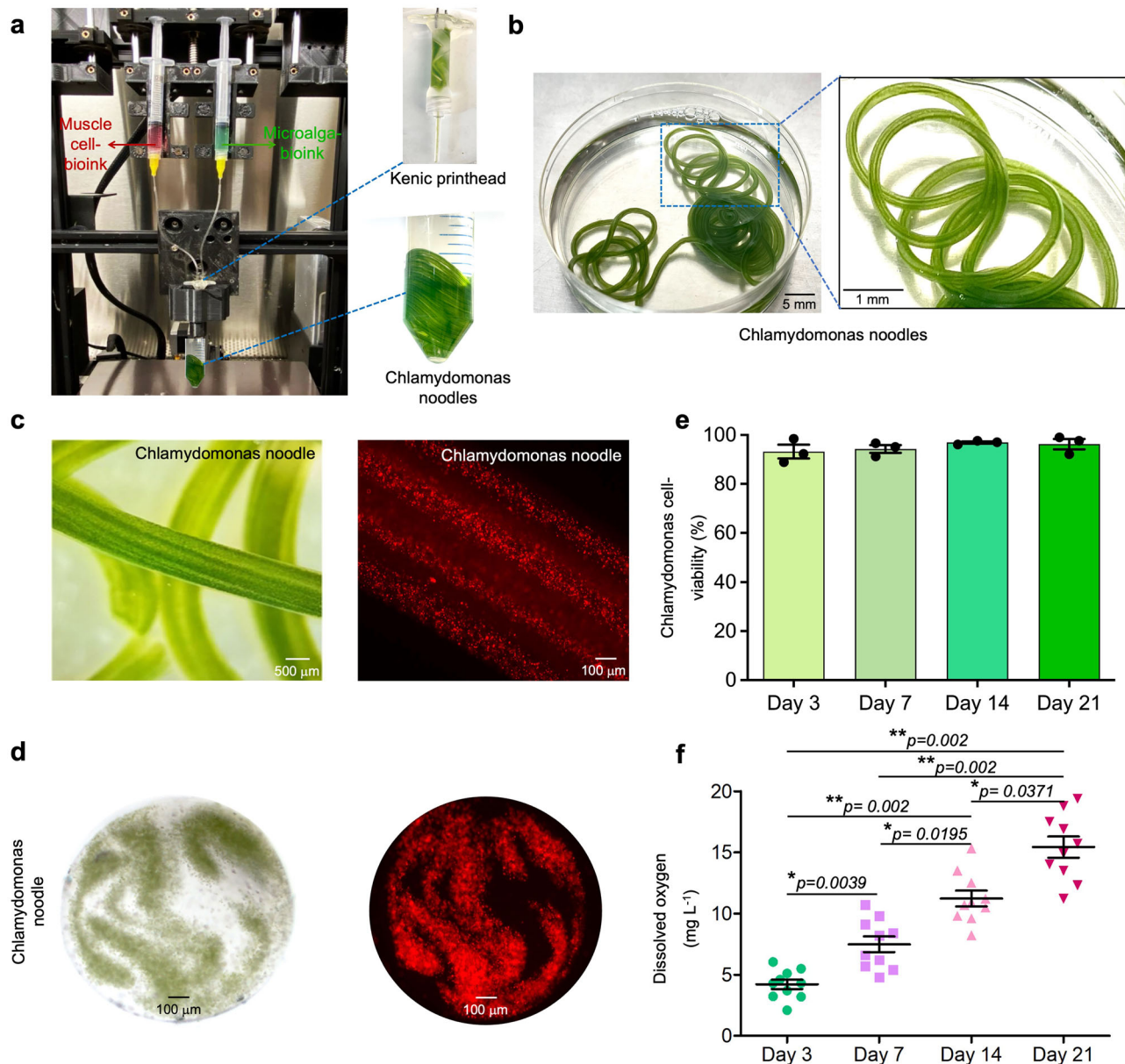


Fig. 3 | Automated chaotic bioprinting of microalga noodles. **a** Photograph of the custom-designed extrusion bioprinting setup with an integrated three-element KSM printhead for continuous chaotic bioprinting of microalga noodles, along with photographs of the KSM printhead and the collected bioprinted microalga noodles in the tube. **b** Photographs showcasing bioprinted microalga noodles, highlighting the internally aligned lamellar microstructures of microalgae (green) and muscle cells (translucent) at day 7. **c** Bright-field micrograph showing lateral view of bioprinted microalga noodles, exhibiting the natural green color of *Chlamydomonas*, followed by a fluorescence micrograph showing internally aligned lamellar microstructures of microalgae with red autofluorescence of chlorophyll at day 14. **d** Bright-field micrograph showing transverse view of a bioprinted microalga

noodle with natural green color and fluorescence micrograph showing the internally aligned lamellar microstructures of microalgae with red autofluorescence of chlorophyll at day 14. **e** Viability values of bioprinted *Chlamydomonas* assessed over the 21-day culture period at 37 °C. Data are presented as the mean values $\pm$ SEMs ($n = 3$ independent samples, each obtained from a separate bioprinting run). **f** Dissolved O_2 levels observed in medium cultured with bioprinted *Chlamydomonas* over the 21-day culture period at 37 °C. Data are presented as the mean values $\pm$ SEMs ($n = 10$ independent samples, each obtained from a separate bioprinting run) and a two-tailed paired t-test was applied to measure p values.

growth medium at 37 °C under continuous light illumination of 2800 lux, for 7 days and then replaced with co-culture differentiation medium for an additional 14 days. Both C2C12 and *Chlamydomonas* showed favorable growth patterns within the hybrid noodles (Fig. 4c) while maintaining the structures of the noodles intact (Fig. 4d). Initially, the cell viability of C2C12 was around 85%, which gradually increased to approximately 95% within the 21-day co-culture period (Fig. 4e). The viability of *Chlamydomonas* cells was roughly 90% during early days, which gradually increased to over 95% within the 21-day period (Fig. 4f). Similarly, the growth rate (Supplementary Fig. 9a) and

chlorophyll contents (Supplementary Fig. 9b) of the *Chlamydomonas* within the C2C12-microalga hybrid noodles were found to increase over the 21-day culture period. Furthermore, we evaluated the proliferation rates and lactate dehydrogenase (LDH) activities of C2C12 cells in the bioprinted noodles over the period of 21 days. The proliferation of C2C12 cells in the C2C12-microalga hybrid noodles was enhanced compared to the control C2C12 noodles without microalgae (Supplementary Fig. 9c). However, the LDH activities of the C2C12 cells in the C2C12-microalga hybrid noodles were lower than those in the control C2C12 noodles (Supplementary Fig. 9d). As the

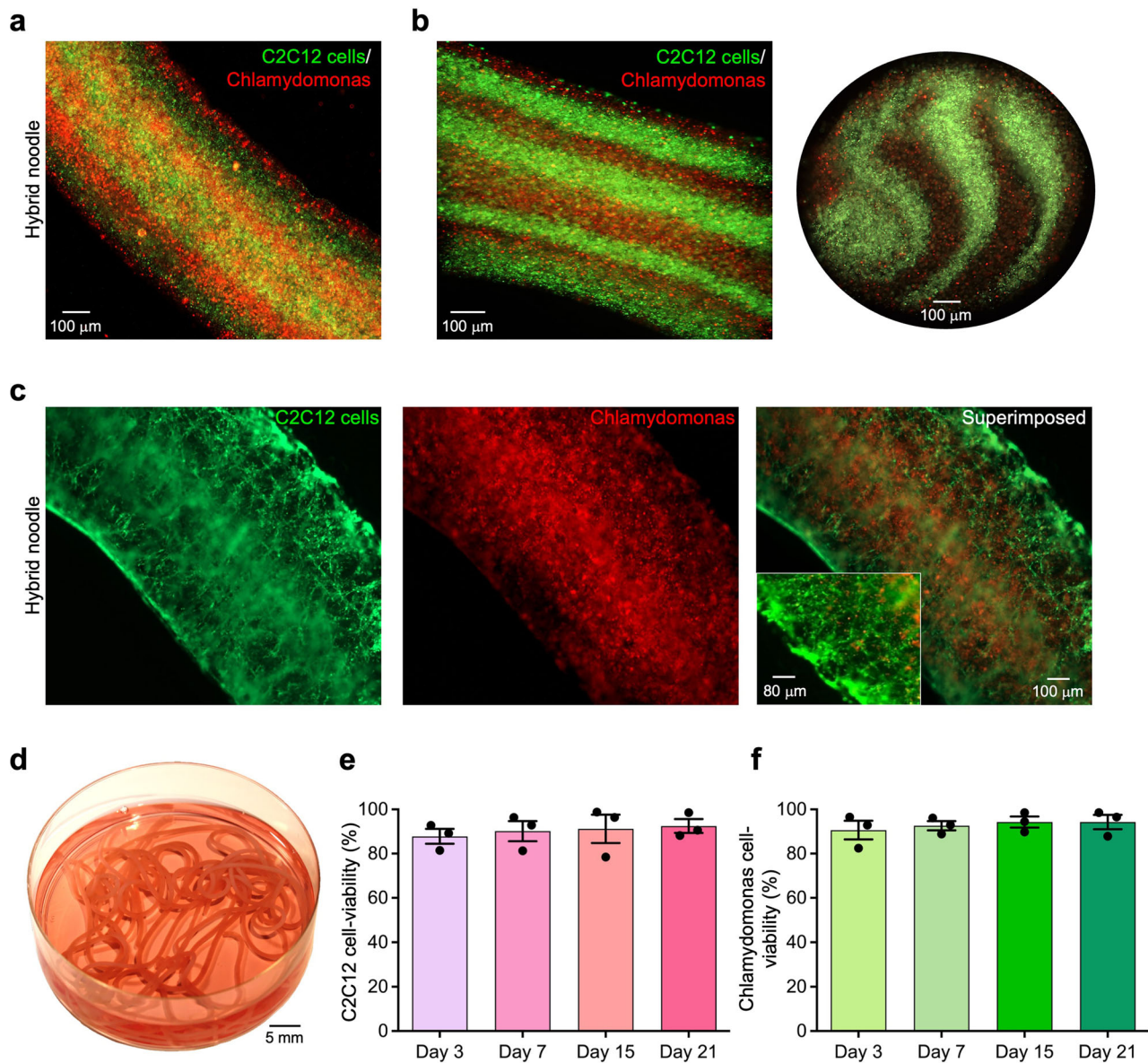


Fig. 4 | Chaotic-bioprinted hybrid noodles. **a** Fluorescence micrograph depicting the entire depth projection view of the 3D hybrid noodle, with C2C12 cells labeled with green CellTracker and Chlamydomonas cells exhibiting red autofluorescence on day 1. **b** Fluorescence micrographs showing lateral and transverse views of hybrid noodle illustrating internally aligned lamellar microstructures with green CellTracker-labeled C2C12 cells and Chlamydomonas exhibiting red autofluorescence on day 1. **c** Fluorescence micrographs showing the viability of C2C12 cells and Chlamydomonas cells on day 14. Green, live C2C12 cells; red, live

Chlamydomonas cells with red autofluorescence of chlorophyll. **d** Photograph of hybrid noodles on day 21. **e** Viability values of C2C12 cells within hybrid noodles, assessed over the 21-day culture period at 37 $^{\circ}$ C. Data are presented as the mean values $\pm$ SEMs (n = 3 independent samples, each obtained from a separate bio-printing run). **f** Viability values of Chlamydomonas cells within hybrid noodles, assessed over the 21-day culture period at 37 $^{\circ}$ C. Data are presented as the mean values $\pm$ SEMs (n = 3 independent samples, each obtained from a separate bio-printing run).

alignment of myotubes is crucial for the proper function of skeletal muscle tissues, we also examined the morphology and orientation of C2C12 cells within hybrid noodles by F-actin staining, without myogenic differentiation, on day 21. Chlamydomonas cells were visible under fluorescence microscope because of natural autofluorescence of chlorophyll. The orientation of C2C12 cells was also evaluated with the longitudinal axis along the length of the noodle designated as 0 $^{\circ}$. Despite maintaining aligned lamellar microlayers of alternating C2C12 cells and Chlamydomonas cells within the hybrid noodles, in the absence of myogenic differentiation, the C2C12 cells largely exhibited a random distribution with significant deviations from the longitudinal axis of the hybrid noodles (Fig. 5a, b and Supplementary Fig. 10a).

We subsequently assessed the morphology and orientation of the C2C12 cells within C2C12-microalga hybrid noodles after myogenic differentiation. After 7 days of proliferation, the co-culture growth medium was replaced with the co-culture differentiation medium to initiate myogenic differentiation. The myogenic differentiation and orientation of C2C12 cells within C2C12-microalga hybrid noodles were assessed by the expression of skeletal muscle myosin. Fluorescence confocal microscopy revealed strong expression of skeletal muscle myosin by C2C12 cells, with most of the cells aligned along the longitudinal axis of the noodle while maintaining the aligned lamellar microlayers of alternating C2C12 cells and Chlamydomonas cells within the C2C12-microalga hybrid noodles (Fig. 5c, d and Supplementary Fig. 10b). Besides skeletal muscle myosin, the expressions of

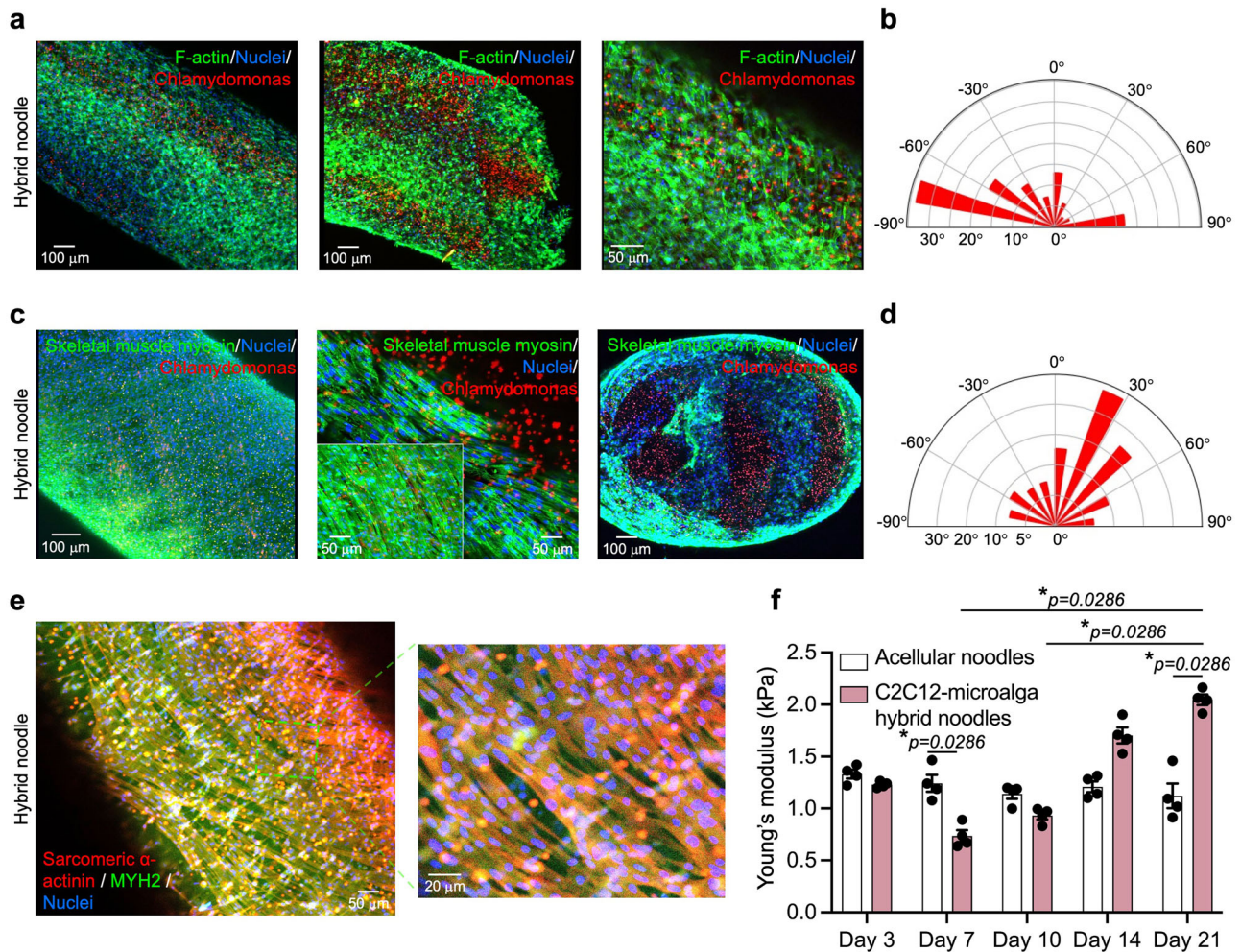


Fig. 5 | Morphological and textural assessments of chaotic-bioprinted hybrid noodles. **a** Fluorescence confocal micrographs of the entire width of the hybrid noodle showing the expression of F-actin by C2C12 cells (green) and Chlamydomonas cells with red autofluorescence of chlorophyll on day 21. **b** Polar plot depicting the angle distributions of C2C12 cells within the hybrid noodle before differentiation, on day 10. **c** Fluorescence confocal micrographs showing the expression of skeletal muscle myosin (green) by C2C12 cells and Chlamydomonas cells with red autofluorescence of chlorophyll on day 21, followed by lateral and transverse views of the hybrid noodle, revealing internally aligned C2C12 cells and Chlamydomonas cells. The inset shows C2C12 cells and Chlamydomonas cells on

the surface of the hybrid noodle. **d** Polar plot depicting the angle distributions of C2C12 cells within the hybrid noodle after differentiation, on day 21. **e** Fluorescence confocal micrographs of the hybrid noodle showing the expressions of sarcomeric α -actinin (red) and MYH2 (green) by C2C12 cells and Chlamydomonas cells with red autofluorescence of chlorophyll on day 21. **f** Young's moduli of bioprinted acellular and C2C12-Chlamydomonas hybrid noodles over the 21-day culture period. Data are presented as the mean values $\pm$ SEMs (n = 4 independent samples, each obtained from a separate bioprinting run) and a two-tailed t-test was applied to measure p values.

sarcomeric α -actinin and myosin heavy chain 2 (MYH2) are crucial for ensuring the contractility and overall functionality of skeletal muscle tissues. Thus, we further evaluated the expressions of sarcomeric α -actinin and MYH2, and found that such biomarkers were also substantially expressed by C2C12 cells (Fig. 5e and Supplementary Fig. 10c). Thus, these results indicated that the 3D C2C12-microalga hybrid noodles not only preserved the aligned lamellar microlayers of alternating C2C12 cells and Chlamydomonas cells but also facilitated the formation of aligned functional skeletal myotubes within C2C12 striations during myogenic differentiation.

To assess the adaptability of our automated chaotic bioprinting system and optimized bioinks, we utilized both two-element KSM and one-element KSM printheads to create hybrid C2C12-microalga noodles with varying numbers of micro-striations. The process involved bioprinting of C2C12 cells embedded in 2IAlg-5Gel and Chlamydomonas cells in 1mAlg-5Gel bioinks. Interestingly, chaotic bioprinting was successfully executed in both cases without altering any conditions other than the KSM printheads. With the two-element KSM,

hybrid C2C12-microalga noodles featured four aligned internal micro-striations, consisting of two alternating layers of C2C12 cells and Chlamydomonas (Supplementary Fig. 11a and Supplementary Movie 2). These C2C12- and Chlamydomonas-cell layers were intact over the period of 14 days, as demonstrated by F-actin staining without myogenic differentiation (Supplementary Fig. 11b).

Similarly, chaotic bioprinting with the one-element KSM produced hybrid C2C12-microalga hybrid noodles with two aligned internal micro-striations, comprising one half each of C2C12 and Chlamydomonas cells, with both layers remaining intact over the period of 14 days, as demonstrated by F-actin staining, without myogenic differentiation (Supplementary Fig. 12 and Supplementary Movie 3). These observations highlighted the versatility of our chaotic bioprinting system in fabrication of hybrid C2C12-microalga hybrid noodles with diverse internal compositions and micro-structures. In contrast, when C2C12 cell-encapsulated 2IAlg-5Gel bioink and Chlamydomonas-encapsulated 1mAlg-5Gel bioink were directly mixed and bioprinted through a nozzle without KSM elements, the resulting

hybrid noodles lacked aligned lamellar microlayers of alternating C2C12 and *Chlamydomonas* cells. Instead, both C2C12 and *Chlamydomonas* cells were randomly distributed within the noodles (Supplementary Fig. 13).

Textural assessments of muscle and plant cell-based hybrid noodles

The tensile and compression tests represent the simplest and most popular methods for measuring food texture. Consequently, tensile tests were conducted on the C2C12-microalga hybrid noodles throughout the 21-day culture period, with Young's moduli determined from the linear slope of the stress-strain curve between 3–15%. The C2C12-microalga hybrid noodles exhibited adequate mechanical strength to be lifted out of the solution consistently throughout the culture period. The Young's moduli initially decreased during early days of culture, from day 3 to day 7, but then they gradually increased until day 21, indicating the myogenic differentiation of C2C12 cells into aligned myotubes (Fig. 5f). Therefore, the increase in Young's moduli after day 7 can be attributed to the continued differentiation of C2C12 cells into aligned myotubes.

In addition, fracture, cyclic compression, and cyclic compression tests with increasing force, were performed on day 21 using C2C12-microalga hybrid as well as individual C2C12 and *Chlamydomonas* circular constructs. The results showed that all three types of constructs exhibited elasticities within the range of 10–60% strain. The stress-strain slopes increased moderately in the deformation range of 60–80% strain, which is a characteristic feature of viscoelastic materials. Subsequently, the stresses notably increased when the constructs were compressed beyond 80%, leading to complete collapse at about 90% strain. Of note, the compressive stress of C2C12 constructs ranged from approximately 100–500 kPa at the strain level of 90%, while those of C2C12-microalga hybrid and *Chlamydomonas* constructs ranged from ~80–200 kPa and ~80–150 kPa, respectively, at the strain level of 98% (Supplementary Fig. 14a). Similarly, the mechanical properties of all three types of constructs were assessed under cyclic loading-unloading conditions, with strain gradually increasing to a maximum of 80%. The results showed that the C2C12 constructs possessed a higher strain-recovery compared to C2C12-microalga hybrid and *Chlamydomonas* constructs (Supplementary Fig. 14b), suggesting that the mechanical strengths of C2C12-microalga hybrid constructs were lower than those of C2C12 constructs but higher than those of *Chlamydomonas* constructs.

Nutritional analyses of muscle and plant cell-based hybrid noodles

The nutritional analyses of muscle and plant-based (C2C12-*Chlamydomonas*) hybrid noodles, muscle-based (C2C12) noodles, and plant-based (*Chlamydomonas*) noodles were conducted. These analyses involved evaluating the compositions of the bioprinted noodles in terms of moisture, macronutrients (carbohydrates, proteins, and fats), micronutrients (vitamins and minerals), dietary fiber, and cholesterol levels (Table 2). The results, expressed in per gram of noodle, revealed no significant differences among the three types of noodles in terms of nutritional contents, including total calories, calories from fat, ash content, carbohydrates, total fat, and proteins.

Chaotic bioprinting of 3D muscle and plant cell-based hybrid food

In addition to scaling up food-production, another goal of automated chaotic bioprinting is to efficiently fabricate customized hybrid food. As a result, we progressed to producing 3D muscle and plant cell-based hybrid food with various shapes and sizes, following the optimal chaotic bioprinting procedure. We first bioprinted a 3D microalga-drumstick using a three-element KSM printhead with the *Chlamydomonas* bioink (Supplementary Fig. 15a) to assess the permeability of

thick, large structures. Red food dye was added dropwise using a syringe, and it was observed that the dye rapidly diffused through the microalga-drumstick immediately upon addition (Supplementary Fig. 15b and Supplementary Movie 5). Furthermore, when the microalga drumstick was immersed in the dye bath, the entire microalga-drumstick became red within 15 min (Supplementary Fig. 15c). This rapid diffusion through the spaces in between the layer-by-layer deposited fibers ensured efficient nutrient-distribution for intended cellular activities across the entire tissues.

We then successfully bioprinted hybrid drumsticks, using the three-element KSM printhead and *Chlamydomonas* cell-encapsulated 1mAlg-5Gel bioink along with C2C12 cell-encapsulated 2lAlg-5Gel bioink (Fig. 6a and Supplementary Movie 6). The bioprinted hybrid drumsticks were cultured in the co-culture growth medium for 7 days and then in co-culture differentiation medium for myogenic differentiation for additional 7–8 days. We observed high viabilities of both *Chlamydomonas* and C2C12 cells along with myogenic differentiation, throughout the entire hybrid drumsticks. Initially, the viability of C2C12 cells was about 80%, gradually increased up to approximately 90% over the 15-day co-culture period (Fig. 6b). Similarly, *Chlamydomonas* cell viability was over 82% and increased to over 95% within the same period (Fig. 6b). Immunostaining and fluorescence confocal microscopy demonstrated myogenic differentiation of C2C12 cells, as evidenced by the expression of skeletal muscle myosin in aligned C2C12 myotubes within the hybrid drumsticks (Fig. 6c and Supplementary Fig. 16).

Cooking assessments of muscle and plant cell-based hybrid food

Next, we assessed how boiling and baking influence the properties of the bioprinted hybrid foods. The hybrid cuboids were cultured for 21 days, with 7 days for proliferation and 14 days for myogenic differentiation (Fig. 6d). The maturation of the hybrid cuboids was confirmed by assessing the expression of skeletal muscle myosin by C2C12 myotubes and autofluorescence of *Chlamydomonas* at day 21 (Supplementary Fig. 17). Subsequently, the hybrid cuboid was cut into pieces of equal size, measuring $2.5 \times 2.5 \times 2.5 \text{ mm}^3$ each and subjected to boiling at 100 °C for 10 min or baking in an oven at 80 °C for 10 min (Fig. 6e). The compression analyses on raw (control), boiled, and baked pieces of hybrid cuboids exhibited the changes in Young's moduli after cooking. The Young's moduli of boiled pieces decreased significantly from an average of 0.95 kPa to 0.26 kPa, whereas those of the baked pieces increased from an average of 0.95 kPa to 1.27 kPa (Fig. 6f). We also compared weight changes in hybrid constructs, muscle constructs, and plant constructs before and after cooking, on day 21. Interestingly, no significant differences were observed in the weight of the constructs across all three groups before and after boiling. However, the weight of baked constructs decreased significantly in all three groups (Supplementary Fig. 18).

Chaotic bioprinting of 3D chicken and plant cell-based hybrid noodles

Before bioprinting hybrid noodles made of chicken cells and microalgae, we evaluated the growth of chicken skeletal muscle cells-SV40 (chicken cells) in the 2lAlg-5Gel bioink. The chicken cells were encapsulated in 2lAlg-5Gel at the concentration of 1×10^7 cells mL^{-1} , cross-linked with 1% (w/v) CaCl_2 and 1% mTG, and culture for 21 days in SuperCult immortalized chicken skeletal muscle cell (CMC) medium. However, the proliferation of chicken cells, at this density, was found to be very slow. Thus, initially, to enhance cell proliferation, we reduced the Gel concentration in the bioink from 5% to 3%, resulting in a new bioink formulation of 2% lAlg and 3% Gel, designated as 2lAlg-3Gel. The chicken cells exhibited good growth within the 2lAlg-3Gel scaffolds, both in CMC medium alone and in combination with *Chlamydomonas* in CMC:TAP (3:1) co-culture medium, under continuous light illumination of 2800 lux at 37 °C for 21 days (Supplementary Fig. 19).

Table 2 | Nutritional facts of bioprinted noodles. Results are expressed per 100 g; ND: not detected

Analyte	Microalga noodle	Hybrid noodle	C2C12 noodle	Unit	Method
Calories	11.3	13.1	16.3	cal	21 CFR part 101.9
Calories from fat	3.69	3.69	2.25	cal	AOAC
Moisture	97.6	96.4	95	g	AOAC
Ash	0.13	0.84	1.2	g	AOAC
Total fat	0.41	0.41	0.25	g	AOAC
	Saturated fat	ND	ND	g	AOAC
	Mono-unsaturated fat	ND	ND	g	AOAC
	Poly-unsaturated fat	ND	ND	g	AOAC
	Trans fatty acids	ND	ND	g	AOAC
Cholesterol	1.50	11.8	12.8	mg	AOAC
Protein	1.7	2.14	2.92	g	LECO
Carbohydrates	0.20	0.20	0.59	g	Calculation
Dietary fiber	ND	ND	ND	g	AOAC 985.29
Sugars, total*	ND	ND	0.58	g	HPLC-RI
	Fructose	ND	ND	g	HPLC-RI
	Glucose	ND	0.58	g	HPLC-RI
	Sucrose	ND	ND	g	HPLC-RI
	Maltose	ND	ND	g	HPLC-RI
	Lactose	ND	ND	g	HPLC-RI
Minerals	Sodium	30.2	268	mg	ICP-OES
	Potassium	9.57	19.2	mg	ICP-OES
	Calcium	31.7	87.3	mg	ICP-OES
	Iron	ND	ND	mg	ICP-OES
Vitamins	Vitamin D	ND	ND	mcg	HPLC

*Analytical method does not distinguish between natural and added sugars.

We therefore bioprinted chicken-microalga hybrid noodles by encapsulating chicken cells (1×10^7 cells mL^{-1}) in the 2lAlg-3Gel bioink and *Chlamydomonas* in the 1mAlg-5Gel bioink, following the chaotic bioprinting procedure (Fig. 7a). The chicken-microalga hybrid noodles were cultured in the CMC-TAP co-culture medium at 37 °C under continuous light illumination of 2800 lux, for a period of 21 days. The viability of chicken cells within the chicken-microalga noodle showed significant growth over the culture period (Fig. 7b). At the beginning, the viability of chicken cells was less than 80%, which subsequently increased up to approximately 85%, while *Chlamydomonas* maintained viabilities above 90% throughout the culture period (Fig. 7c). With the 2lAlg-3Gel bioink for chicken cells nonetheless, we were unable to observe the aligned lamellar microlayers of alternating chicken cells and *Chlamydomonas* cells within the chicken-microalga hybrid noodles. This lack of alignment was most likely attributed to the reduced mechanical properties of the bioink, which led to more dispersed cell distribution rather than forming desired layered structure. We then tested bioprinting of chicken-microalga hybrid noodles back with 2lAlg-5Gel by increasing the chicken muscle cell density to 2.5×10^7 cells mL^{-1} while keeping *Chlamydomonas* concentration the same, and slight modification of medium composition. The bioprinted chicken noodles and chicken-microalga hybrid noodles were crosslinked with 1% (w/v) CaCl_2 and 1% mTG and cultured at 37 °C under continuous light illumination of 2800 lux for 21 days in CMC:DMEM with 20% FBS (1:1, CMC/DMEM medium) and CMC/DMEM:TAP (3:1) co-culture medium, respectively. The growth rate (Supplementary Fig. 20a) and chlorophyll contents (Supplementary Fig. 20b) of the *Chlamydomonas* within the chicken-microalga hybrid noodles were found to increase over the 21-day culture period. Similarly, the proliferation of chicken cells within the chicken-microalga hybrid noodles was improved compared to the control chicken noodles without microalga (Supplementary Fig. 20c), whereas the LDH activities of the chicken cells

within the chicken-microalga hybrid noodles were lower than those within the control chicken noodles (Supplementary Fig. 20d). Moreover, the fluorescence confocal micrographs of the chicken-microalga hybrid noodles revealed aligned lamellar microlayers of alternating chicken cells and *Chlamydomonas* cells, with chicken cells expressing F-actin, MYH2, and skeletal muscle myosin while *Chlamydomonas* cells exhibiting red autofluorescence (Fig. 7d). Thus, we were able to achieve the bioprinting of chicken-microalga hybrid noodles with internally aligned chicken cells and *Chlamydomonas*.

Discussion

Given that conventional agricultural production and livestock farming heavily depend on natural resources such as land and water, utilizing traditional farming methods to meet the increasing global food demand may impose an unsustainable burden on these finite resources^{50–52}. Therefore, there is an urgent need for the development of more efficient approaches for food production to meet the demands of a growing global population while simultaneously maximizing environmental sustainability. In response to the increasing concerns relating to sustainable food engineering, in this study, we introduced a method for producing aesthetically appealing plant cell- and animal cell-based hybrid foods including noodles and various food items, such as chicken drumsticks, donuts, and more, utilizing automated chaotic bioprinting with precision and reliability (Fig. 1, Fig. 2, and Supplementary Fig. 2).

We used Alg and Gel hydrogels as bioink components due to their wide applications in the food industry and tissue engineering^{53–57}. Alg is widely used in food industries and biomedical fields for its biocompatibility, cost-effectiveness, and rapid gelation with divalent ions^{58–61}. It is used as a thickening, gelling, stabilizing, or emulsifying agent in products like sauces, dressings, and ice cream^{61–64}. Similarly, Gel, a partially hydrolyzed collagen protein, is used in the food

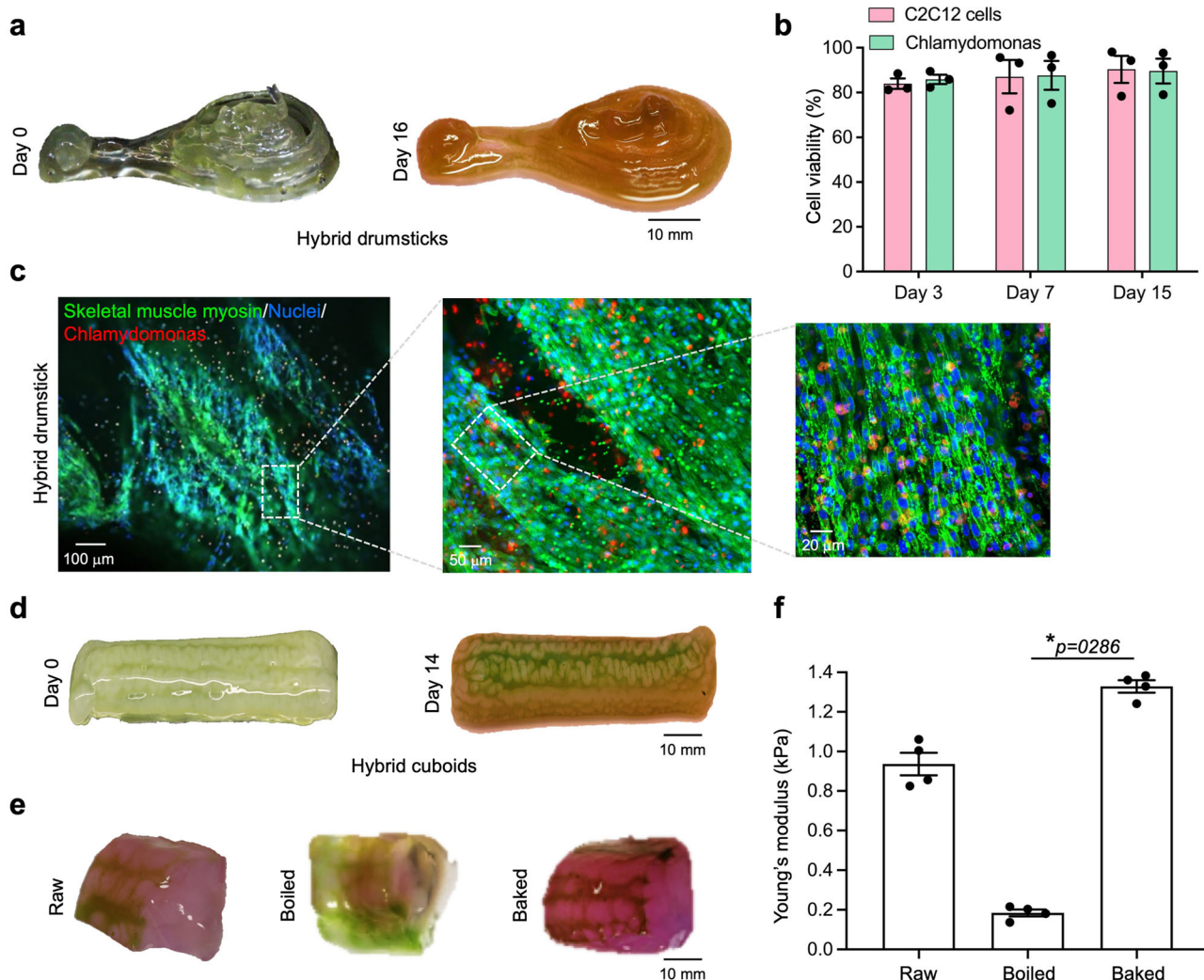


Fig. 6 | 3D-bioprinted hybrid food. **a** Photographs showing 3D hybrid drumsticks on day 0 and day 16, bioprinted with C2C12 cell- and Chlamydomonas cell-bioinks. **b** Viability values of bioprinted C2C12 cells and Chlamydomonas cells within the hybrid drumsticks, assessed over the 15-day culture period at 37 °C. Data are presented as the mean values $\pm$ SEMs (n = 3 independent samples, each obtained from a separate bioprinting run). **c** Fluorescence confocal micrographs showing the expression of skeletal muscle myosin (green) by C2C12 cells and Chlamydomonas cells with red autofluorescence of chlorophyll on day 16, within bioprinted hybrid

drumsticks. **d** Photographs of bioprinted hybrid cuboids at day 0 and day 14, using C2C12 cell- and Chlamydomonas cell-bioinks. **e** Photographs of cut pieces of bioprinted hybrid cuboids on day 21, used for cooking studies. **f** Comparison of mechanical properties of hybrid food before and after cooking on day 21. Data are presented as the mean values $\pm$ SEMs (n = 4 independent samples, each obtained from a separate bioprinting run) and a two-tailed paired t-test was applied to measure p values.

industry for its gelling, emulsifying, and stabilizing properties, enhancing texture, nutrition, and taste^{65–67}. Additionally, the Arg-Gly-Asp motifs in Gel enhance cell adhesion and proliferation, making it valuable hydrogel for ECM scaffolds in tissue engineering^{60,68–73}. Given their applications in the food industry and tissue engineering, we utilized a blend of Alg and Gel bioinks for chaotic bioprinting of plant cells and muscle cells. Furthermore, we adopted CaCl_2 and mTG for crosslinking Alg and Gel components of the bioinks, respectively, to produce the double-network hydrogels featuring good mechanical and physiochemical properties⁶¹. In fact, Alg forms an ionic network with calcium ions whereas Gel forms a covalent network when treated with mTG (Fig. 1c).

On the other hand, the automation of the bioprinting process is of utmost importance not only for technological advancement but to enhance scalability and efficiency, making it possible to produce high-quality, safe, and customized food products at an affordable cost. A typical chaotic bioprinting setup consists of a KSM printhead, syringe pump for bioprinting fibers, and a CaCl_2 bath for instant

crosslinking^{46,74–77}. This study pushes the boundaries of chaotic bioprinting into food engineering by introducing fully automated operations using a custom-built extrusion setup with integrated KSM printhead. The mechanical extrusion bioprinter was designed to streamline the bioprinting process, allowing for the simultaneous biofabrication of fibers and the precise construction of 3D structures (Fig. 1a, b). This automated setup not only enabled biofabrication of fibers but also allowed creation of well-defined 3D hybrid food items with various compositions, shapes, and sizes such as noodles and drumsticks. Nonetheless, there were challenges with using Alg/Gel bioinks in chaotic bioprinting, such as varying flow characteristics and maintaining consistent extrusion to accurately create 3D structures. We addressed these by systematically investigating the mechanical properties of the bioinks and programming optimal flow and feed rates into G-code commands, using the mechanical extrusion bioprinter and the three-element KSM printhead (Fig. 1c, d and Supplementary Fig. 1). The automated chaotic bioprinting procedure was eventually optimized using Alg/Gel bioinks at a flow rate of 1.5 mL

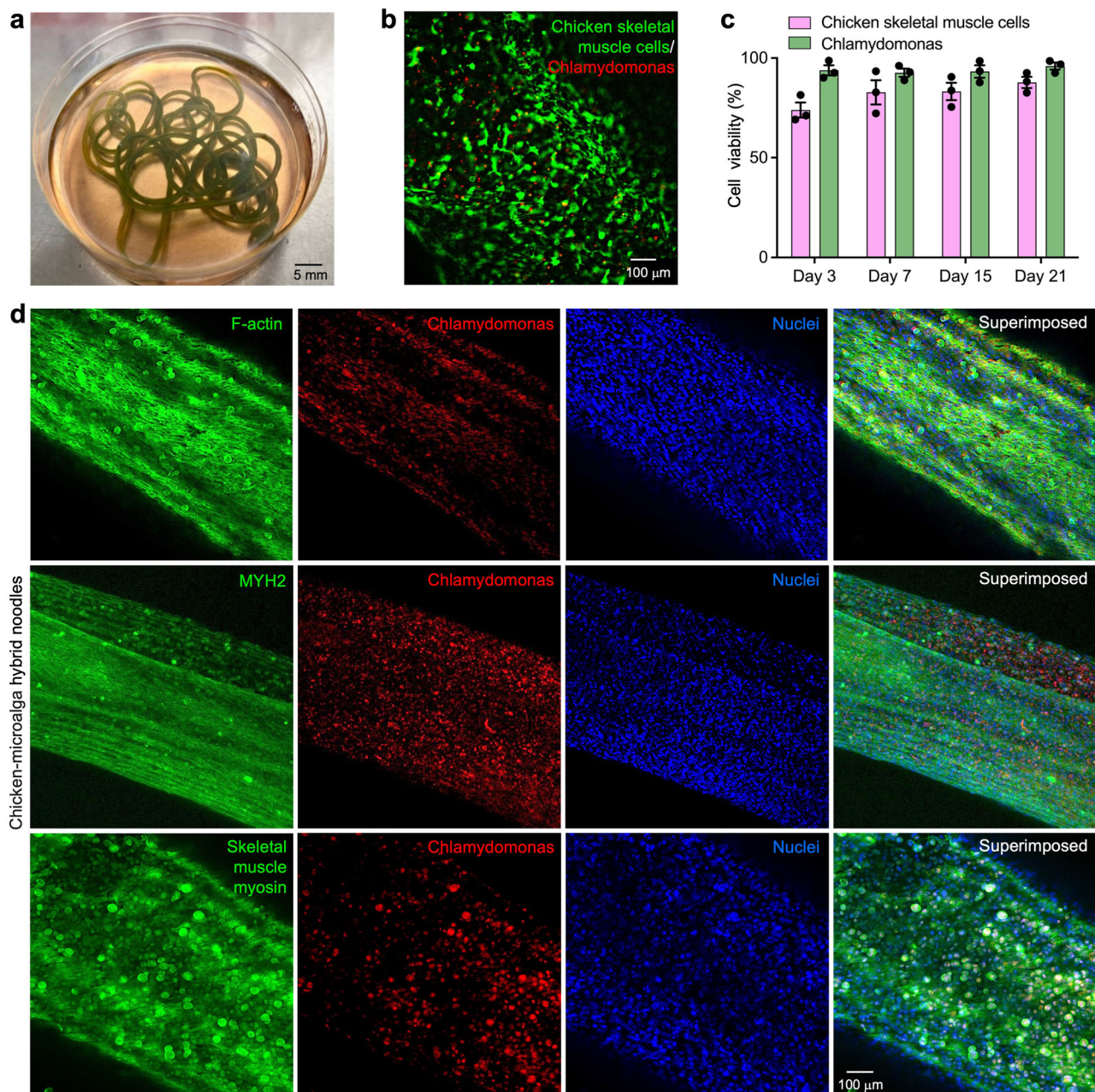


Fig. 7 | Chaotic-bioprinted chicken-microalga hybrid noodle. **a** Photograph of hybrid chicken-microalga noodles on day 21, bioprinted with chicken cell- and Chlamydomonas cell-laden bioinks. **b** Fluorescence micrograph showing live chicken cells (green) and Chlamydomonas cells with red autofluorescence of chlorophyll, within the bioprinted hybrid chicken-microalga noodles on day 21. **c** Viability values of chicken cells and Chlamydomonas cells within bioprinted

hybrid chicken-microalga noodles, assessed over the 21-day culture period at 37 $^{\circ}$ C. Data are presented as the mean values $\pm$ SEMs (n = 3 independent samples, each obtained from a separate bioprinting run). **d** Fluorescence confocal micrographs showing the expressions of F-actin, MYH2, and skeletal muscle myosin (green) by chicken cells and Chlamydomonas cells with red autofluorescence of chlorophyll, within bioprinted chicken-microalga hybrid noodles on day 21.

min^{-1} and a feed rate of 4 mm s^{-1} . This also allowed for continuous bioprinting of 3D noodles and well-defined various 3D food products (Fig. 2 and Supplementary Fig. 2).

Microalgae like Chlamydomonas, Chlorella, and Spirulina are unicellular plants, which are sustainable food sources due to their nutrient density^{37–39}, high productivity and low environmental impact^{29,35}. They are rich in proteins, essential amino acids, vitamins, minerals, omega-3 fatty acids, and bioactive compounds (e.g., antioxidants and carotenoids)^{26,29,35,36}. Moreover, microalgae are well-known as environmentally friendly, cost-efficient, and sustainable sources of photosynthetic O_2 . Chlamydomonas, in particular, is used in tissue engineering for its photosynthetic O_2 -production and is

emerging as a promising candidate in bioprinting for creating photosynthetic scaffolds^{78,79}. Its ability to thrive in hydrogels such as cellulose, gelatin methacryloyl, and Alg enhances its potential for creating photosynthetic scaffolds^{80–82}. Our previous study showed that bioprinted Chlamydomonas can produce O_2 to support the growth of human liver cells (HepG2) in engineered tissues, indicating a photosymbiotic relationship with mammalian cells⁸¹. Similarly, C2C12 cells are used in muscle tissue engineering due to their myogenic potential, proliferation, and compatibility with bioprinting techniques^{47,74}. Therefore, we used Chlamydomonas as model plant cells and C2C12 cells as the model muscle cells to produce hybrid food products.

Successful bioprinting relies on mechanical properties, biocompatibility, and printability of the bioinks used. Our results suggested that the optimal bioinks for chaotic bioprinting of hybrid food were 1mAlg-5Gel for microalgae (Supplementary Fig. 3) and 2lAlg-5Gel for muscle cells (Supplementary Fig. 4). We thus successfully bioprinted plant-based noodles using *Chlamydomonas*-encapsulated 1mAlg-5Gel and acellular 2lAlg-5Gel bioinks (Fig. 3 and Supplementary Movie 4). Over the 21-day culture period, *Chlamydomonas* exhibited increased growth and elevated oxygen production within the microalga noodles (Supplementary Fig. 5). Our automated chaotic bioprinting enabled rapid and consistent production of *Chlamydomonas* noodles, increasing throughput to approximately 12 g min⁻¹ and demonstrated scalability of the system for large-scale noodle-production (Supplementary Fig. 6). Moreover, to demonstrate the versatility of our automated chaotic bioprinting method in producing plant-based noodles, we also bioprinted *Chlorella* noodles by incorporating *Chlorella* into 1mAlg-5Gel bioinks (Supplementary Fig. 7).

Following the successful bioprinting of microalga noodles, we created C2C12-microalga hybrid noodles by optimizing the co-culture medium that supported the growth of *Chlamydomonas* and myogenic differentiation of C2C12 cells within hybrid C2C12-*Chlamydomonas* constructs (Supplementary Fig. 8). Mixotrophic green algae, such as *Chlamydomonas*, can grow photoautotrophically (using light and CO₂), heterotrophically (using organic compounds such as acetate), or mixotrophically (using both)^{83,84}. In contrast, mammalian cells depend on organic carbon sources, such as glucose, for their chemoheterotrophic metabolism. Since glucose in the medium can also be utilized by mixotrophic microalgae, studies have investigated the growth and photosynthetic activities of microalgae using mammalian cell culture media alone or adapted media combining both mammalian and microalgae media for co-cultures under mammalian cell culture conditions (37 °C and 5% CO₂). For example, *Chlamydomonas reinhardtii* and the human cell line SaOS-2 were co-cultured in a 1:1 mixture of TAP and McCoy's 5 A media (with 15% FCS, penicillin, streptomycin, and L-glutamine)⁸⁵. Similarly, *Chlorella sorokiniana* was co-cultured with the hTERT-MSC cell line in a co-culture medium using DMEM and TAP with 10% fetal calf serum⁸⁴. Based on our previous experiences⁸¹ and the published studies, we initially used a 1:1 ratio of muscle cell (DMEM) and microalga (TAP) media, along with FBS and anti-anti. However, we observed that *Chlamydomonas* overgrew and outcompeted the C2C12 cells. We then adjusted the proportions by reducing the TAP (1 part) and increasing the DMEM (3 parts), which resulted in optimal growth for both the muscle cells and the microalgae.

The C2C12-microalga hybrid noodles with micro-striations of alternating *Chlamydomonas* and C2C12 cells maintained structural integrity (Fig. 4), supported robust growth of C2C12 cells compared to those within the control C2C12 noodles without microalga. In contrast, the LDH activities of the C2C12 cells were reduced in the C2C12-microalga hybrid noodles relative to control C2C12 noodles (Supplementary Fig. 9). Since LDH activity is often measured as a marker of tissue-damage resulting from hypoxic conditions^{85,86}, the observed reduction in LDH activities in the cells within the C2C12-microalga hybrid noodles suggested that O₂ produced by the *Chlamydomonas* played a protective role, alleviating the hypoxic stress. As a result, the O₂-production likely contributed to enhanced cellular health, which in turn helped minimize cellular damage, as further evidenced by the improved proliferation rate.

It is interesting to note that undifferentiated C2C12 cells were randomly distributed during earlier stage of culture, but differentiation led to alignment along the noodle's longitudinal axis, maintaining alternating layers of *Chlamydomonas* and C2C12 myotubes (Fig. 4). This alignment was associated with strong expressions of muscle proteins including skeletal muscle myosin, sarcomeric α -actinin, and MYH2 (Fig. 5 and Supplementary Fig. 10). The expressions of these biomarkers are crucial for muscle function⁸⁷⁻⁸⁹. Myosin and actin

generates the contractile force, sarcomeric α -actinin anchors actin to Z-disks within the sarcomere, providing structural stability and contributing to muscle contraction while MYH2 is crucial for muscle fiber type-specification and plays a significant role in determining the contractile properties⁹⁰.

Furthermore, we demonstrated the versatility of our chaotic bioprinting system and bioinks by producing hybrid noodles with varying numbers of internal micro-striations, using two-element KSM (Supplementary Fig. 11 and Supplementary Movie 2) and one-element KSM printheads (Supplementary Fig. 12 and Supplementary Movie 3). In fact, chaotic bioprinting using the KSM printhead offers several advantages compared to traditional approaches, such as blending multiple different cell types into a single bioink or using separate printheads to extrude different bioinks. A key benefit of chaotic bioprinting is its ability to extrude two or more bioinks simultaneously through the KSM printhead in a controlled, defined manner, creating a continuous fiber with spatially organized, internally aligned lamellar microlayers, each corresponding to a specific bioink^{48,49}. It allowed for precise layering of different bioinks within 3D hybrid noodles, forming a well-organized pattern that reflected the specific distributions of cell types while maintaining spatial integrity and preserving the characteristics of each cell type within hybrid noodles (Fig. 5 and Supplementary Fig. 10). In contrast, bioprinting directly mixed C2C12 cell-encapsulated 2lAlg-5Gel bioink and *Chlamydomonas*-encapsulated bioink 1mAlg-5Gel through a nozzle without KSM elements resulted in hybrid noodles that possessed randomly distributed C2C12 and *Chlamydomonas* cells exhibiting no internally aligned lamellar microlayers, potentially compromising the properties of cell types such as hindering the fusion of C2C12 myoblasts to form multinucleated muscle cells (Supplementary Fig. 13). Similarly, bioprinting using separate printheads for muscle cell-bioink and microalga bioink leads to the formation of separate noodles. As a result, the C2C12 and *Chlamydomonas* cells would be isolated, limiting optimal interaction and lowering synergistic metabolic exchanges such as O₂-supply by *Chlamydomonas*. Thus, chaotic bioprinting with KSM overcomes these challenges by synchronizing bioink-extrusion, resulting in better structural fidelity and control over the formation of complex, multicellular 3D structures.

Moreover, the texture of food plays important role in quality assessment, as it significantly influences sensory experiences like biting and chewing^{91,92}. This texture is intricately linked to the physical structure and interactions of biopolymers (e.g., proteins, polysaccharides, lipids), which influence both mechanical properties and flavor^{93,94}. Thus, mechanical properties such as firmness, toughness, tenderness, and chewiness are utilized to characterize the textural characteristics of various food products⁹⁵. The bioprinted hybrid noodles exhibited adequate mechanical strength, strong enough to be lifted from the solution as supported by the increase in the Young's moduli of the hybrid noodles (Fig. 5f). Additional texture analyses, such as fracture and cyclic compression with increasing force, suggested that mechanical strength of the hybrid constructs was weaker than the muscle cell constructs but stronger than the microalga constructs (Supplementary Fig. 14). Additionally, we evaluated nutritional analyses of the bioprinted hybrid noodles such as moisture, macronutrients, micronutrients, dietary fiber, and cholesterol (Table 2). These analyses helped in evaluating the nutritional profiles of the hybrid noodles compared with individual microalga and muscle cell-based noodles.

Furthermore, we successfully bioprinted 3D hybrid food in various shapes and sizes, including hybrid drumsticks (Fig. 6a-c, Supplementary Fig. 15, Supplementary Fig. 16, and Supplementary Movie 6) and cuboids (Fig. 6d and Supplementary Fig. 17), demonstrating the production of customized hybrid foods in terms of size, shape, structure and texture. Since cooking is essential in culinary traditions to showcase the safety, flavor, and texture of foods in line

with consumer preferences⁹⁶, we cooked the bioprinted 3D hybrid cuboids by boiling and baking, and observed the resulting changes in appearances (Fig. 6e), mechanical properties (Fig. 6f), and weight (Supplementary Fig. 18).

Finally, we developed chicken-microalga hybrid noodles by bioprinting chicken cells with *Chlamydomonas* cells. The chicken-microalga noodles exhibited micro-striations of alternating chicken cells and *Chlamydomonas* cells (Fig. 7). These hybrid noodles demonstrated improved chicken cell proliferation and reduced LDH activities compared to those in the control chicken cell noodles (Supplementary Fig. 20).

In conclusion, this work served as an important proof-of-concept, showcasing the robust 3D bioprinting approach of creating eco-friendly, aesthetically appealing, and highly customizable cell-based 3D hybrid food products by combining plant and animal cell-based nutrients with precision and reliability. Given that automated chaotic bioprinting holds significant potential for customizing food items (Fig. 2, Fig. 6, and Supplementary Fig. 2) and scaling up productions, particularly in terms of increasing production efficiency (Supplementary Fig. 6), we anticipate that enhancements in the production of both animal and plant cells, along with improvements in bioink designs, will enable a more streamlined and robust large-scale hybrid food-production, as well as the creation of customizable and personalizable hybrid food.

Nevertheless, we are aware that there is significant room for improvements in the sensory properties and nutritional profiles of cell-based bioprinted hybrid food to match those of commercial products. To this end, optimizing co-culture systems, along with carefully investigating the homogeneous distributions of nutrients and growth factors particularly within the core of larger constructs, will boost these properties and overall yields of the final products, thereby paving the way for large-scale production of balanced, nutritious hybrid food, with microalgae providing essential nutrients such as proteins, omega-3s, vitamins, and minerals, while muscle cells offer lean proteins. Furthermore, health benefits can be optimized in hybrid food, as both microalgae and muscle cells can be engineered to enhance specific nutrients or reduce fat content.

It is important to note that the hybrid foods introduced in this study are not intended as direct replacements for chicken or other conventional meats. Rather, they represent a distinct category of cell-based hybrid foods designed to explore new culinary possibilities and nutritional profiles. We also acknowledge that the green color of the hybrid food may not be visually appealing to all consumers, and our objective is not to promote a specific aesthetic. Instead, the focus of our work is on the broader potential of these hybrid foods to be customized in terms of size, shape, structure, texture, and color, features that can be tailored to meet individual preferences and dietary requirements. The green coloration, derived from plant-based ingredients, serves as just one example of the many possible design variations, demonstrating the flexibility of our approach rather than presenting a finalized product.

Taken together, we believe that our proof-of-concept study, which integrates microalgae and muscle cells through a continuous chaotic bioprinting technique, represents a step forward in the development of cell-based hybrid food products.

Methods

Preparation of hydrogel precursor solutions

Each of 4% (w/v) mAlg (A2033, Sigma-Aldrich, Burlington, MA, USA), 8% (w/v) lAlg (A1112, Sigma-Aldrich, Burlington, MA, USA), and 10% (w/v) Gel (porcine skin type-A, 300 g bloom, G2500, Sigma-Aldrich, Burlington, MA, USA) stock solutions were prepared in deionized water under continuous stirring at 37 °C until components were completely dissolved. The precursor stock solutions were sterilized by subjecting them to three cold-hot cycles, alternating between 4 °C and 80 °C every 15 min.

The stock lAlg and Gel solutions were diluted in Hanks' balanced salt solution (HBSS, 14170112, Thermo Fisher Scientific, Waltham, MA, USA) to prepare bioinks for muscle cells, comprised of either 2% (w/v) lAlg and 6% (w/v) Gel or 2% (w/v) lAlg and 5% (w/v) Gel. Similarly, the stock mAlg and Gel solutions were diluted in microalga medium to prepare bioinks for microalga cells, comprised of either 1% (w/v) mAlg and 4 or 5% (w/v) Gel or 1% (w/v) mAlg and 3–5% (w/v) Gel.

Preparation of crosslinker solutions

The 4% (w/v) CaCl_2 (C7902, Sigma-Aldrich, Burlington, MA, USA) solution and 4% (w/v) mTG (1002, Modernist Pantry, Eliot, ME, USA) stock solutions were prepared individually in deionized water, followed by sterilization through vacuum filtration using a sterile 0.22- μm filter for each solution. The 1% (w/v) CaCl_2 solution was prepared by diluting stock solution in deionized water to collect the bioprinted noodles without cells, whereas the 1% (w/v) CaCl_2 solution was made in HBSS for collecting the bioprinted noodles with cells. Likewise, the 1% (w/v) mTG solution diluted in deionized water was used for crosslinking the noodles without cells, and the 1% (w/v) mTG solution in cell culture (or co-culture) medium was used for crosslinking the noodles with cells, post-bioprinting.

Rheological characterizations

The oscillatory rheometer (Discovery Hybrid Rheometer DHR-2, TA Instruments, New Castle, DE, USA) was used to measure the rheological properties of formulated bioinks. Stainless-steel parallel plates with a diameter of 20 mm and a truncation gap of 1000 μm was used for all measurements. Temperature-sweep tests were conducted at a rate of 5 °C min^{-1} from 50 °C to 20 °C, at a fixed frequency of 1 Hz and strain 1%. Crossover points in temperature-sweep tests indicate the gelation points. To assess the viscoelastic properties of the samples, stress-sweep measurements were performed with a logarithmically increasing shear stress at a constant frequency of 1 Hz over the range of 0.1–2000 Pa. The yield stresses of the bioinks were taken as the crossover points in stress-sweep tests. Excess material outside the parallel plates was removed before each measurement to prevent the edge effect.

Microalgae

Chlamydomonas (152040), *Chlorella* (152075), and *Alga-Gro* (153753) were purchased from the Carolina Biological Supply Company (Burlington, NC, USA), whereas TAP medium was obtained from Thermo Fisher Scientific (Waltham, MA, USA). *Chlamydomonas* was grown in the TAP medium⁸⁵ while *Chlorella* was grown in the *Alga-Gro* medium. These cells were separately cultured in 500 mL Erlenmeyer flasks on an orbital shaker at 120 rpm at 25 °C under continuous light illumination of 2800 lux using a relax LED bulb HD (General Electric Lighting, East Cleveland, OH, USA). For all experiments, the cultures were simultaneously grown to the mid-log phase at optical density (OD_{750}) of 0.5–0.6. The cultures were then centrifuged at 1000 $\times$ g for 5 min, the supernatant was discarded, and the cells were harvested.

Skeletal muscle cells

C2C12 mouse skeletal muscle cells (CRL-1772) were obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured in DMEM (11965092, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% (v/v) FBS (10437028, Thermo Fisher Scientific, Waltham, MA, USA) and 1% (v/v) anti-anti (15240062, Thermo Fisher Scientific, Waltham, MA, USA) at 37 °C and 5% CO_2 in a humidified incubator until 80–90% confluence. Immortalized chicken muscle cells (CSC-I9234L) and CMC medium (CM-I9234L) were purchased from Creative Bioarray (Shirley, NY, USA). Chicken muscle cells were cultured in CMC medium also at 37 °C and 5% CO_2 in a humidified incubator until 80–90% confluence. The respective culture media were replaced every 2 days.

Preparation of bioinks

Chlamydomonas or *Chlorella*, grown to OD₇₅₀ of 0.5–0.6, were harvested by centrifuging at 1000 g for 5 min. Each of the microalga pellets were washed 3 times with Dulbecco's phosphate-buffered saline (DPBS, 14190144, Thermo Fisher Scientific, Waltham, MA, USA) and suspended in 3 mL of the hydrogel-precursor solution to obtain a microalga-bioink with 1×10^7 microalga cells mL⁻¹.

The muscle cells, C2C12 myoblasts or chicken muscle cells, at 80–90% confluence, were trypsinized using trypsin-EDTA (15400054, Thermo Fisher Scientific, Waltham, MA, USA), centrifuged, and suspended in 3 mL of the hydrogel precursor solution for cells to obtain a bioink with $1\text{--}2.5 \times 10^7$ viable cells mL⁻¹. The bioinks were stored in the incubator at 37 °C to maintain temperature until bioprinting.

Automated continuous chaotic bioprinting

The bioprinting process was performed using an extrusion-based 3D bioprinter (built in-house) along with Repetier-Host (Hot-World GmbH & Co. KG, Willich, Germany). The muscle cell-bioink and microalga-bioink were individually loaded into 3-mL syringes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), which were then connected to the inlets of the KSM printhead using sterile Tygon tubing (0.5 mm of inner diameter and 1.52 mm of outer diameter, Saint-Gobain Performance Plastics, Taunton, MA, USA). These syringes were placed within the extruder, while the KSM printhead was mounted in the nozzle-holder of the bioprinter. Subsequently, the tip of the KSM printhead was immersed into 1% (w/v) CaCl₂ solution before bioprinting, to enable the immediate crosslinking of the Alg-component of the extruded noodles. The flow rate was set at 1.5 mL min⁻¹ through a manually written G-code command. The bioprinted noodles were transferred to a petri dish containing medium supplemented with 1 % (w/v) mTG solution and incubated at 37 °C overnight to allow crosslinking of the Gel component of the bioprinted noodles. After 12 h, the crosslinking medium was replaced with the fresh cell growth medium, and cultures were maintained for a duration of up to 21 days, with the medium being refreshed every 2 days. Furthermore, apart from using the KSM printhead with 3 mixing elements, we also used KSM print-heads with one and two mixing elements to bioprint the noodles.

To enable the chaotic bioprinting of various 3D cell-based foods, we made slight modifications to both bioprinting and crosslinking strategies. 3D models were obtained from Thingiverse, a public repository of 3D printable models, as standard tessellation language (STL) files⁹⁷. The STL model was then imported to Slic3r, an open-source software that slices model and generates G-codes⁹⁸. The bioprinting speed and flow rate were set at 4 mm s⁻¹ and 1.5 mL min⁻¹ for the bioprinting of 3D structures. The 3D structures were bioprinted directly onto the empty petri dish. A heater fan was placed near the bioprinter, directing warm air towards the KSM printhead to ensure that the surrounding temperature remained stable and maintain the bioink above the gelation temperature throughout the bioprinting process. Once bioprinting was completed, the medium supplemented with 1% (w/v) CaCl₂ and 1 % (w/v) mTG was added gently to the petri dish without disturbing the bioprinted structure. The dish was then incubated at 37 °C overnight. After 12 h, the crosslinking medium was replaced with the fresh cell growth medium and 3D bioprinted constructs were cultured for up to 21 days, with the medium being refreshed every 2 days.

Computational simulations

COMSOL Multiphysics version 5.4 (COMSOL, Burlington, MA, USA) was used for computational fluid dynamics simulations to understand the mixing dynamics of microalga-bioink and muscle cell-bioink during bioprinting, specifically through a KSM and co-extrusion processes. The simulation utilized a tetrahedral mesh specially calibrated for fluid dynamics and preset as normal. The simulations solved the Navier-Stokes equation for laminar flow to capture fluid velocity and

pressure in a transient state, while considering a no-slip boundary condition⁹⁹:

$$\rho(\mu \cdot \nabla)\mu = \nabla \cdot [-pI + \eta(\nabla\mu + (\nabla\mu)^T)] \quad (1)$$

$$\nabla\mu = 0 \quad (2)$$

where η represents dynamic viscosity ($\frac{\text{kg}}{\text{m}\cdot\text{s}}$), μ stands for velocity ($\frac{\text{m}}{\text{s}}$), ρ denotes fluid density ($\frac{\text{kg}}{\text{m}^3}$), and p represents pressure (Pa).

Similarly, the particle-tracing module was employed to simulate the trajectories of corresponding bioinks using the following equation:

$$\frac{d}{dt}(m_p v) = m_p F_d(\mu - v) \quad (3)$$

$$F_d = \frac{18\eta}{\rho_p d_p^2} \quad (4)$$

where v represents the velocity ($\frac{\text{m}}{\text{s}}$), ρ_p represents the density of the bioink ($\frac{\text{kg}}{\text{m}^3}$), and d_p stands for the particle diameter (m).

Cell viability assay

The viability of bioprinted muscle cells was evaluated by the Live/Dead viability/cytotoxicity kit (L3224, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Briefly, the bioprinted hybrid noodles or 3D food were washed three times with DPBS and incubated with the staining solution, containing 2 μM of calcein-AM and 4 μM of ethidium homodimer-I in DPBS, at 37 °C for 30 min. The samples were then washed three times with DPBS and imaged using an Eclipse Ti2 inverted microscope (Nikon Instruments Inc., Melville, NY, USA) or Zeiss LSM 880 confocal microscope (Carl Zeiss Microscopy, LLC, White Plains, NY, USA). The percentages of viable cells were determined using ImageJ (National Institutes of Health, Bethesda, MD, USA) Fiji¹⁰⁰.

The growth of the microalgae in the culture broth was assessed by measuring OD₇₅₀ at both 25 °C and 37 °C, using a SpectraMax M3 microplate reader (Molecular Devices, San Jose, CA, USA). Additionally, the viability of bioprinted microalgae, within hybrid noodle or 3D hybrid food, was determined using SYTOX Orange nucleic acid stain (SYTOX Orange, S11368, Thermo Fisher Scientific, Waltham, MA, USA), with excitation and emission peaks at 547 nm and 570 nm, respectively, following the manufacturer's protocol. Briefly, the samples, including bioprinted hybrid noodles or 3D hybrid food items, were incubated in medium containing 5 μM of SYTOX Orange for 5 min at 25 °C in darkness. SYTOX Orange is a fluorescent dye that permeates damaged cell membranes, staining dead microalga cells orange while viable microalga cells exhibit the red autofluorescence characteristic of chlorophyll. After washing three times with DPBS, the samples were imaged using an Eclipse Ti2 inverted microscope or Zeiss LSM 880 confocal microscope, and the percentages of viable microalga cells were determined using ImageJ Fiji.

Immunohistochemical staining

The bioprinted hybrid noodles or 3D hybrid food items were washed with DPBS and fixed in paraformaldehyde (4 % (v/v) in DPBS) for 20–30 min. Subsequently, the samples were treated with 0.2 % (v/v) Triton X-100 (T8787, Sigma-Aldrich, Burlington, MA, USA) in DPBS, for 1 h at room temperature. Following three washes with DPBS, the samples were incubated with Alexa Fluor 488-phalloidin (1:200 (v/v), A12379, Thermo Fisher Scientific, Waltham, MA, USA) in 0.1 % (w/v) bovine serum albumin (A9418, Sigma-Aldrich, Burlington, MA, USA) overnight at 4 °C. After another round of DPBS washing, nuclei were counterstained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, 1 $\mu\text{g mL}^{-1}$, D1306,

Thermo Fisher Scientific, Waltham, MA, USA) for 5 min at room temperature. After three additional DPBS washes, fluorescence images were taken using a Zeiss LSM 880 confocal microscope.

To evaluate the expression of skeletal muscle cell-specific markers, the samples were fixed as described above, followed by blocking with a blocking buffer (5 % (v/v) goat serum in DPBS) for 2 h at room temperature. Subsequently, the samples were then incubated overnight at 4 °C with intended primary antibody, including anti-skeletal muscle myosin antibody (F59) Alexa Fluor 488 (sc-32732 AF488, Santa Cruz Biotechnology, Dallas, TX, USA), anti-sarcomeric α -actinin antibody [EA-53] (ab9465, Abcam, Waltham, MA, USA), or anti-fast myosin skeletal heavy chain antibody [MY-32] (MYH2, ab51263, Abcam, Waltham, MA, USA), at 1:200 (v/v) dilution in blocking buffer. Afterwards, the noodles or constructs were washed three times with DPBS and then incubated overnight at 4 °C with the appropriate secondary antibodies (goat anti-rabbit IgG H&L Alexa Fluor 488 (ab150077, Abcam, Waltham, MA, USA), goat anti-mouse IgG H&L Alexa Fluor 488 (ab150113, Abcam, Waltham, MA, USA), goat anti-rabbit IgG H&L Alexa Fluor 594 (ab150080, Abcam, Waltham, MA, USA), and goat anti-mouse IgG H&L Alexa Fluor 594 (ab150116, Abcam, Waltham, MA, USA) at 1:200 (v/v) dilution in blocking buffer. Following DPBS washing, the samples were subjected to DAPI counterstaining and imaging as described above.

Dissolved O₂-measurement

The levels of dissolved O₂ in the culture media were evaluated utilizing a dissolved O₂ probe (Eutech DO 6+, Eutech Instruments Pte Ltd, Singapore). Prior to use, the device was calibrated in the “100 % calibration” mode in saturated air according to the manufacturer’s instructions. Following calibration, the probe was immersed into the culture medium immediately after being removed from the incubator, with steady and gentle stirring to avoid the entrapment of air bubbles in the medium. Measurements were recorded until stable readings were obtained.

Chlorophyll content-measurement

Chlorophyll contents of the bioprinted noodles were determined using an ethanol extraction method⁸¹. Bioprinted noodles (10 mg) were washed three times with DPBS and incubated with 1 mL of collagenase type IV (1 mg mL⁻¹ in DPBS, 17104019, Thermo Fisher Scientific, Waltham, MA, USA) at 37 °C for 20–30 min to facilitate the release of microalgae from the noodles through bioink-digestion. The released microalgae were centrifuged at 3000 × g for 5 min and washed with DPBS. Next, 1 mL of 95% ethanol was added and vortexed to extract green pigments at room temperature. After centrifugation at 3000 × g for 5 min, the absorption of the green solution was measured at 665 nm using the SpectraMax M3 microplate reader.

Cell proliferation assay

The MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium) assay was performed using the Cell Titer 96 AQueous One Solution Cell Proliferation Assay Kit (G3580, Promega, WI, USA) following the manufacturer’s instructions. Briefly, 10 cm of bioprinted noodles were cultured in 6-well plates at 37 °C and 5% CO₂ in a humidified incubator, under continuous light illumination of 2800 lux, for 3, 7, 14, and 21 days. At each time point, 1 mL of MTS reagent (diluted 1:4 with the respective culture medium) was added to the samples and incubated for 2 h at 37 °C, protected from light. Then, 100 μ L of MTS assay medium was transferred in triplicates to the wells of a 96-well flat-bottom plate, and the absorption values were measured at 490 nm using the SpectraMax M3 microplate reader. Muscle cell noodles without microalgae served as controls.

LDH activity-assessments

LDH activities were measured using the CyQUANT LDH Cytotoxicity Assay Kit following the manufacturer’s protocol (C20302, Thermo Fisher Scientific, Waltham, MA, USA). Briefly, the culture media were

collected from bioprinted noodles cultured at 37 °C and 5% CO₂ in a humidified incubator, under continuous light illumination of 2800 lux, for 7 and 21 days. 100 μ L of medium was transferred in triplicates to the wells of a 96-well flat-bottom plate and 100 μ L of reaction mixture was added to each well, followed by gentle mixing by tapping the plate. The plate was then incubated at room temperature for 30 min, protected from light. Thereafter, 100 μ L of stop solution was added to each well, and it was gently tapped to mix well. Absorbance was measured at 490 and 680 nm using the SpectraMax M3 microplate reader. Muscle cell noodles without microalgae served as controls.

Textural analyses

All the mechanical tests were carried out with an Instron universal testing machine (Instron 3342, Norwood, MA, USA). For textural analyses, bioprinted noodles, cultured for 10 days, were cut into segments of approximately 3 cm in length for subsequent tensile testing. The tests were performed at a strain rate of 1.5 mm min⁻¹, utilizing a load cell rated at 100 N. The Young’s modulus was calculated according to the linear slope of the stress-strain curve between 3–15%.

In addition, three different types of compression tests, fracture, cyclic compression, and cyclic compression with increasing force, were performed on day 21. Circular constructs with about 6 mm in thickness and 9 mm in diameter were used. The fracture tests were performed with a load cell of 100 N and the compression rate of 95% min⁻¹. Additionally, we conducted a cyclic compression test where a strain of 150% min⁻¹ was applied repeatedly for 100 cycles. Furthermore, successive loading-unloading tests were carried out, gradually increasing the applied maximum strains from 30–90%, each applied for 5 cycles.

Cooking

Hybrid cuboids with a dimension of 65 × 15 × 6 (l × w × h) mm³ were bioprinted and cultured for 21 days. The bioprinted hybrid cuboids were cut into cubes with the dimension of 2.5 × 2.5 × 2.5 (l × w × h) mm³. Two methods of cooking were performed, boiling the samples in a water bath at 100 °C on a hot plate for 10 min and baking them in an oven at 80 °C for 10 min. They were then subjected to post-treatments to observe the changes in modulus and weight. Compression tests were conducted on the cubes using an Instron universal testing machine with a load cell of 100 N. The Young’s modulus was determined by analyzing the linear slope of the stress-strain curve within the range of 3–15%. Weight changes were calculated based on the initial weight ($W_{initial}$) and final weight (W_{final}), before and after cooking, using the following formula:

$$W_c = \frac{W_{final} - W_{initial}}{W_{initial}} \times 100 \quad (5)$$

Statistics and reproducibility

All the experiments were independently repeated at least three times. Each result was presented as mean value $\pm$ standard error of the mean (SEM) of at least three independent experiments (n). Statistical analyses were performed by two-tailed paired t-test using GraphPad Prism (GraphPad Software, Boston, MA, USA) and $p \leq 0.05$ was considered statistically significant (* $p \leq 0.05$, ** $p \leq 0.01$).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All data generated in this study are available within the article and its Supplementary Information files as well as Source data provided with this paper. Source data are provided with this paper.

Code availability

No custom codes central to the study were used in the research.

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Author contributions

S.M. and Y.S.Z. conceived and designed the experiments; S.M., C.Y., C.P.L., A.V.Z., A.K.M.F., A.D.R., D.H.H.M., S.G., J.J., A.B. and L.B.J. performed the experiments; A.M.H. and F.J.A.R. performed computational simulation and analysis; D.S.R.R. and C.E.G.M. helped with printing of KSM printhead and bioprinter setup; Y.S.Z. supervised the study and provided financial support; S.M. analyzed all the data and wrote the initial manuscript; S.M. and Y.S.Z. edited the manuscript; M.H. provided technical input. All authors read and approved the final manuscript.

Competing interests

YSZ consulted for Allevi by 3D Systems; cofounded, consults for, and holds options of Linton Lifesciences; and sits on the scientific advisory board and holds options of Xellar. The relevant interests are managed by the Brigham and Women's Hospital. The other authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Sushila Maharjan or Yu Shrike Zhang.

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