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Chia seed mucilage widens the thermal processing window of GelMA biomaterial inks

Running title: Chia widens GelMA thermal print window

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Abstract

Gelatin methacryloyl (GelMA) is among the most widely used bioinks because it retains the cell-adhesive and protease-sensitive motifs of gelatin and photo-crosslinks on demand. At printable concentrations, however, its viscosity is governed by the gelatin coil–helix transition near ambient temperature, so ink rheology becomes a steep function of print-head temperature and extrusion is difficult to control. Here we report a physically blended biomaterial ink in which microwave-synthesized GelMA (10% w/v) is combined with mucilage extracted from chia (*Salvia hispanica* L.) seeds at 0.5%, 1%, and 2% (w/v) and photo-crosslinked with 0.3% (w/v) lithium phenyl-2,4,6-trimethylbenzoylphosphinate under a 395–480 nm curing light. ^1H NMR gave a degree of methacrylation of 81.9% for the microwave-synthesized GelMA, determined from consumption of the lysine methylene resonance against an aromatic internal reference. The principal finding is thermal: chia mucilage decouples ink viscosity from temperature. Pristine GelMA lost a factor of 3838 in viscosity between 15 and 45 °C and 63% of its remaining viscosity per degree at the 30 °C print head, whereas the 1% and 2% blends lost only 49- and 41-fold overall and 32% and 15% per degree. The mucilage itself is nearly athermal (258 $\rightarrow$ 186 mPa·s over the same range), identifying it as the component responsible. Mechanical reinforcement, by contrast, is not a dose response but a threshold effect. Relative to pristine GelMA (55.7 ± 5.8 kPa), 0.5% chia gave a modestly lower compressive modulus (42.6 ± 6.2 kPa) that did not reach significance ($P = 0.056$), whereas 1% and 2% raised it 17.5- and 30.0-fold (974.8 ± 101.3 and 1671.0 ± 173.8 kPa; $P = 0.004$). Scanning electron microscopy showed a fibrous polysaccharide network that is sparse and discontinuous at 0.5% and becomes continuous between 0.5% and 1% (w/v), and we interpret the mechanical threshold as rigidity percolation of this network — an interpretation consistent with the microstructure rather than a mechanism established by it. Fourier-transform infrared spectroscopy showed dose-dependent growth of the pyranose C–O–C and carboxylate bands together with a broadened O–H/N–H envelope, indicating hydrogen bonding between the mucilage and the gelatin amide groups, while X-ray diffraction confirmed that the mucilage remains amorphous and well dispersed. Filament geometry measured across nine pressure–speed combinations gave optimal spreading ratios of 1.158 for GelMA, 1.164 for the 1% blend and 1.016 for the 2% blend. Chia mucilage therefore acts less as a stiffener than as an agent that decouples ink viscosity from temperature, widening the thermal processing window of GelMA at low cost from a renewable plant source. Because no cell-laden printing or cytocompatibility assay was performed, the cell-free formulations described here are termed biomaterial inks rather than bioinks, following the consensus biofabrication nomenclature; the study establishes the rheological and engineering foundation for subsequent cell-laden translation.

Keywords: Gelatin methacryloyl; Chia mucilage; Blend biomaterial ink; Extrusion bioprinting; Thermal decoupling; Percolation; Printability; Rheology

1. Introduction

Three-dimensional bioprinting builds cell-laden constructs by depositing biomaterials with spatial control, and extrusion is the dominant modality because it is inexpensive and compatible with a broad range of hydrogels. Its limiting component remains the bioink, which must satisfy a set of partly contradictory requirements: it must flow through a fine nozzle under modest pressure, hold the deposited geometry until it is fixed, provide mechanical support to the construct, and remain hospitable to cells.^{1,2}

Gelatin methacryloyl has become the reference material for this purpose. Methacryloyl functionalization consumes only a small fraction of the amino acid residues, so the arginine–glycine–aspartate adhesion motifs and matrix metalloproteinase cleavage sites of gelatin survive the modification, and the resulting macromer photo crosslinks rapidly into a covalent network whose stiffness can be tuned through concentration and degree of substitution.^{1,3} These advantages are offset by two practical problems. First, GelMA at printable concentrations shows insufficient shear-thinning and yield stress to hold a deposited filament so pristine formulations spread on contact with the substrate.^{2,4} Second, and more restrictive in practice, the physical gelation of GelMA is governed by the gelatin coil–helix transition, which occurs close to room temperature; viscosity therefore changes by orders of magnitude over a few degrees, and reproducible extrusion demands tight print-head thermal control.^{3,5,6}

Strategies for overcoming this thermal sensitivity divide usefully into intrinsic approaches, which alter the macromer or its solvent environment so that the coil–helix transition itself is suppressed, and extrinsic approaches, which leave GelMA chemistry untouched and add a second component that carries the rheology. Recent work has advanced both, and the present study belongs to the second class. One intrinsic route exploits physical gelation itself, printing GelMA below its transition so that shear-thinning, self-healing physical gels retain shape before photocuring.²

Among intrinsic strategies, controlled hydrolysis of GelMA is the most direct. Kyeong et al. showed that enzymatically hydrolyzed GelMA loses thermal gelation below 30 °C altogether while remaining photo-crosslinkable, so network stiffness becomes a function of concentration rather than temperature.⁷ A second intrinsic route exploits Hofmeister ion effects on the gelatin helix: Chee et al. found that chaotropic ions suppress the sol–gel transition of gelatin methacrylate inks—above approximately 0.3 M CaCl₂ the transition disappears entirely, permitting room-temperature printing at very high polymer content, with the cations subsequently acting as crosslinkers.⁸ The complementary kosmotropic effect raises gel strength and crosslink density in gelatin-based networks.⁹ A third route replaces or supplements photochemistry with enzymatic or dual crosslinking: horseradish peroxidase-mediated phenolic coupling provides a cell-compatible secondary network for extrusion,¹⁰ and combined ionic and photo-crosslinking in GelMA–alginate systems raises modulus, although the ionic step can itself compromise cell viability.¹¹ Reformulating GelMA as a self-assembled colloidal gel likewise broadens the printable temperature range relative to the classical polymeric ink.¹²

Extrinsic additive strategies remain attractive because they require no new chemistry, no enzyme handling, and no change to established GelMA synthesis. Particulate and polysaccharide additives have both been used to this end: Laponite raises shear-thinning and thermal stability of GelMA and brings the printability index close to unity,¹³ hydroxyethyl cellulose increases bioink viscoelasticity by up to two orders of magnitude and converts non-printable inks into self-supporting ones,¹⁴ cellulose nanocrystals and methacrylated cellulose nanofibrils provide the shear-thinning needed for clog-free extrusion,^{15,16} and plant gums such as tragacanth raise the printability criterion of gelatin inks from about 0.3 to 1.¹⁷ More recent work has extended the polysaccharide repertoire: κ -carrageenan forms a synergistic network with GelMA that raises viscoelasticity while contributing glycosaminoglycan-mimetic cues,¹⁸ and a systematic

study of GelMA–alginate blends established that the molar mass of the polysaccharide, rather than the GelMA network, is the dominant variable setting the printable operating window.¹⁹ Nanoparticle and nanoclay fillers occupy the same functional niche and have been comprehensively reviewed.^{20,21}

Beyond polysaccharide and nanoclay additives, the wider additive-manufacturing literature indicates the direction in which reinforced photocurable inks are developing. Process-science treatments of GelMA-based biomaterial inks now extend to four-dimensional printing, in which a printed construct is designed to change shape or property with time or stimulus,³ and stimuli-responsive hydrogels have become a materials class in their own right for that purpose.²² Multi-component composite inks combine a hydrogel matrix with reactive inorganic fillers — silicates, nanoclays, calcium phosphates and bioactive glasses — to obtain in situ crosslinking, mechanical reinforcement and mineralisation capability from a single formulation,^{20,23} and bio-based photocurable resins extend the same logic to vat photopolymerisation.²⁴ Against that background the contribution sought here is deliberately narrow: chia mucilage is a non-engineered, food-grade additive whose effect is confined to rheology and stiffness, and its interest lies in delivering a processing benefit at negligible cost and without new chemistry rather than in multifunctionality.

These approaches trade against one another in ways worth stating plainly. Intrinsic modification gives the most complete decoupling — hydrolysed GelMA is genuinely temperature-independent⁷ — but requires an additional enzymatic processing step and reduces molecular weight, with consequences for network mechanics. Hofmeister modulation is elegant and requires only a salt, but changes ionic strength substantially, which constrains cell compatibility and downstream medium exchange.⁸ Dual-crosslinking systems achieve high stiffness at the cost of added reagents and, in the ionic case, measurable cytotoxicity.¹¹ Additive blending is the least invasive of the four, and its principal liability is that the additive must be well characterized and reproducibly sourced. Against that background, an inexpensive, food-grade, renewable polysaccharide that both widens the thermal window and reinforces the network is worth evaluating.

Chia (*Salvia hispanica* L.) is an attractive but largely unexplored source for this role. On hydration, the seed extrudes a mucilaginous capsule composed principally of xylose, glucose, arabinose, galactose, and uronic acid residues,^{25,26} recently identified as a glucoxylan,²⁷ which forms pseudoplastic dispersions whose viscosity and consistency index rise with concentration.²⁸ The mucilage is already used as a food hydrocolloid and has been formulated into wound-healing hydrofilms and pH-responsive drug-delivery hydrogels,^{27,29} establishing both its biocompatibility and its processability. It is inexpensive, renewable and obtainable without harsh chemistry. To our knowledge, however, chia mucilage has not previously been evaluated as a rheology modifier for a photocrosslinkable bioink.

A point of nomenclature should be settled before the study is described. Under the consensus definition of Groll et al.,³⁰ a bioink is a formulation that contains living cells at the moment of printing, whereas a cell-free formulation processed by the same route is a biomaterial ink. No cell-laden printing and no cytocompatibility assay were carried out in the present work. The GelMA–chia formulations characterised here are therefore biomaterial inks and are described as such; the study sits at the biomaterial-ink development stage and establishes the rheological and engineering basis on which a cell-laden bioink would subsequently be built. Where the term bioink appears below it refers to the general literature or to the intended downstream application.

We therefore prepared blends of microwave-synthesized GelMA with chia mucilage at 0.5–2% (w/v) and characterized them by nuclear magnetic resonance and infrared spectroscopy, electron microscopy, thermal and crystallographic analysis, compression testing and rheology, and finally by direct

measurement of deposited filament geometry across a pressure–speed matrix. Two questions organize the work: whether chia mucilage reinforces the photocrosslinked network, and whether it widens the thermal window within which GelMA can be extruded reproducibly. As will be shown, the answers differ in kind — reinforcement appears only above a concentration threshold and reverses sign below it, whereas the thermal benefit is progressive and is the more robust of the two effects.

2. Materials and methods

2.1. Materials

Gelatin (Type A, from porcine skin), methacrylic anhydride (MA), and lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphate-buffered saline (PBS, pH 7.4) tablets were obtained from BioShop Canada Inc. (Burlington, ON, Canada). Whole chia seeds (*Salvia hispanica* L.), a conventionally farmed commercial product supplied by Yayla (Türkiye), were obtained from a local market (Kayseri, Türkiye). The visible-light curing source (VALO Cordless) was supplied by Ultradent Products Inc. (South Jordan, UT, USA). Deuterium oxide (D₂O) was used for nuclear magnetic resonance (NMR). All other reagents were of analytical grade and used as received; distilled water was used throughout.

2.2. Preparation of chia mucilage

Chia mucilage was extracted from whole chia seeds through a sequential process of hydration, vacuum-assisted separation, purification, and freeze-drying. Whole chia seeds (13.3 g) were suspended in 250 mL of distilled water (seed-to-water ratio 1:18.8, w/v) and magnetically stirred at room temperature (~25 °C) for 2–3 h to allow complete hydration and release of the mucilage from the seed coat. The hydrated mixture was subjected to vacuum filtration using a Büchner funnel fitted with porous cheesecloth as the filter medium, connected to a glass filtration flask and a vacuum pump operating at 300 mbar; the viscous mucilage was drawn into the flask while the seeds were retained on the cheesecloth. The filtrate was transferred to Falcon tubes and centrifuged at 8000 rpm for 5 min to remove residual seed fragments and excess water. Floating debris and supernatant were carefully aspirated with a Pasteur pipette to yield purified mucilage. The isolated mucilage was freeze-dried (Teknosem, Türkiye), collected, sealed with parafilm in a glass beaker, and stored at –20 °C until use. This method provides a reproducible route to chia mucilage under ambient hydration without thermal treatment during extraction. The extraction was carried out in independent batches, each from 13.3 g of seed, and the material used in the experiments reported here derives from these batches rather than from a single extraction. The gravimetric yield of freeze-dried mucilage, defined as (mass of freeze-dried mucilage / mass of as-received seed) × 100, was 2.89%, 2.69% and 2.79% for three independent extractions (0.385, 0.358 and 0.372 g of freeze-dried mucilage from 13.3 g of seed), giving $2.79 \pm 0.10\%$ (mean ± standard deviation, SD; n = 3; coefficient of variation 3.6%). On a dry-seed basis, taking the 92.8% dry matter reported for seed from this supplier,³¹ this corresponds to approximately 3.0%. The closeness of the three yields indicates that the hydration and vacuum-filtration procedure is reproducible in the quantity of mucilage it recovers, which is the one compositional property of the extract that this study can report.

Because the composition and molecular weight of chia mucilage vary with seed provenance, hydration ratio, and extraction conditions,^{26,27} protein content, uronic acid content and molecular-weight distribution are the properties that determine batch-to-batch reproducibility and quantitative comparison with mucilage preparations from other laboratories. Comparison between laboratories should rest on protein content by Bradford or bicinchoninic acid assay, and uronic acid content by the carbazole or m-hydroxydiphenyl method, together with molecular-weight distribution by size-exclusion chromatography. The absolute values reported here are accordingly specific to the extract described above; this does not

affect the internal comparison among formulations, all of which were prepared by the same extraction procedure from the same commercial seed source. Provenance of the seed. The raw material was a conventionally farmed commercial chia seed supplied by Yayla (Türkiye), and whole seed from the same supplier has recently been characterised independently: Dirr et al. report a supplier-declared proximate composition of 23.2% protein, 31.4% fat, 31.9% fibre, 1.6% carbohydrate and 92.8% dry matter for conventionally farmed Yayla seed, together with an independently measured total fat content of $37.95 \pm 1.67\%$ by Soxhlet extraction with n-hexane.³¹ These values fix the identity and general composition of the raw material; they are not a characterisation of the mucilage used here, and two limits on their use must be stated. First, they describe the whole seed, whereas the material used in this study is the soluble mucilage of the seed coat, a polysaccharide fraction composed principally of xylose, glucose, arabinose, galactose and uronic acid residues^{25,26} and identified as a glucoxytan,²⁷ whose composition is not derivable from a whole-seed proximate analysis. Second, the 6.5 percentage-point difference between the supplier-declared and the independently measured fat content within that single study is itself a demonstration of lot-to-lot variation: figures quoted for a commercial agricultural product cannot be assumed to hold for a particular consignment. The consequence of compositional variation for the present result can be stated more specifically than as a general caution. The rigidity-percolation threshold located in this work between 0.5% and 1% (w/v) is a threshold in the number density and aspect ratio of load-bearing polysaccharide strands, not in mass concentration as such. A mucilage of higher weight-average molecular weight, or of higher uronic acid content and therefore greater chain extension and hydrodynamic volume at neutral pH, will reach network continuity at a lower mass loading; a depolymerised or lower-molecular-weight preparation will reach it at a higher loading, or not at all within the concentration range that remains extrudable. Seed provenance, hydration ratio and any thermal step during extraction all act on these two variables. A laboratory reproducing this work should therefore expect the threshold to move with its own mucilage and should locate it empirically over a concentration series rather than adopt the 1% (w/v) figure reported here. Reporting gravimetric yield, uronic acid content and molecular-weight distribution alongside the concentration series is what would make such thresholds comparable between laboratories, and we recommend that studies using chia mucilage state the supplier and cultivation category as well.

2.3. Microwave-assisted synthesis of GelMA

GelMA was synthesized by a microwave-assisted reaction. A 10% (w/v) gelatin solution was prepared in PBS (pH 7.4) at 50 °C in a glass vessel and stirred for 40 min to achieve homogeneous dissolution. The solution was placed in a temperature- and power-controlled microwave synthesis system (MicroSynth Milestone flexiWAVE, Italy). MA (4% v/v relative to the gelatin solution) was added at a flow rate of 0.4 mL/min under stirring. Microwave irradiation at 1000 W was applied for a total of 5 min in ten 30 s pulses (30 s $\times$ 10). The methacrylation reaction proceeded at 50–60 °C with periodic cooling of the solution between pulses to prevent overheating and gelatin degradation. After the reaction was complete, the mixture was dialysed against distilled water using 12–14 kDa molecular-weight cut-off dialysis tubing at 37 °C for 3 days to remove residual salts, unreacted methacrylic anhydride and the methacrylic acid by-product.³² The resulting GelMA was recovered, freeze-dried and stored at –20 °C for subsequent characterization and biomaterial ink preparation. Microwave heating has previously been shown to shorten methacrylation from hours to minutes while retaining light-curability.³³ The complete set of extraction, synthesis, and photo-crosslinking parameters is summarized in Table 1.

Table 1. Extraction, synthesis, and photo-crosslinking parameters.

Stage	Parameter	Value
Chia mucilage extraction	Seed-to-water ratio	1:18.8 (w/v)
	Hydration time and temperature	2–3 h, ~25 °C
	Vacuum filtration	300 mbar, cheesecloth
	Centrifugation	8000 rpm, 5 min
	Drying	Freeze-drying
Chia mucilage extraction	Extraction yield	2.79 ± 0.10% (w/w, as-received seed; n = 3 independent extractions)
GelMA synthesis	Gelatin concentration	10% (w/v) in PBS, pH 7.4
	Methacrylic anhydride	4% (v/v), 0.4 mL/min
	Microwave power and schedule	1000 W, 30 s × 10 (5 min net)
	Reaction temperature	50–60 °C
GelMA synthesis	Purification	Dialysis, 12–14 kDa MWCO, distilled water, 37 °C, 3 days
GelMA synthesis	Degree of methacrylation (DM)	81.92% (¹ H NMR; Figure A1)
Photo-crosslinking	Photoinitiator	LAP, 0.3% (w/v)
	Wavelength and irradiance	395–480 nm, 3200 mW/cm ²
	Exposure time	15 s

2.4. Preparation of GelMA–chia blend biomaterial inks and hydrogels

Photo-crosslinkable biomaterial inks and hydrogels were prepared by combining GelMA at a constant concentration of 10% (w/v) with different proportions of chia mucilage (0%, 0.5%, 1%, or 2% w/v), using LAP as the photoinitiator at a final concentration of 0.3% (w/v). LAP absorbs efficiently in the near-visible range and is well tolerated by cells at this concentration.^{34,35} LAP (0.3% w/v) was first completely dissolved in distilled water at ambient temperature under magnetic stirring for 20 min. Freeze-dried chia mucilage was then added to reach final concentrations of 0.5%, 1%, or 2% (w/v), and the solutions were stirred at ambient temperature for 1 h until uniform. GelMA (10% w/v) was subsequently incorporated into each chia solution and dissolved in an incubator at 37 °C for 1 h under moderate agitation.

The resulting pre-gel solutions were transferred into syringes and centrifuged at 4000 rpm for 5 min to remove air bubbles. Post-centrifugation, the syringes were maintained at 37 °C to ensure solution uniformity. Hydrogel specimens for compression testing and rheology were not cast but printed. Each pre-gel was loaded into the cartridge of the pneumatic extrusion bioprinter described in Section 2.6 and deposited layer by layer through the 22-gauge conical nozzle (410 µm) with the print head at 30 °C, as cylinders of 2.5 mm diameter and 6 mm height for compression testing and as discs of 16 mm diameter and 2 mm height for rheology, at the optimal pressure–speed setting identified for that formulation in Section 3.6: 3 psi / 20 V for 10% GelMA, and 5 psi / 15 V for GelMA-1% Chia and GelMA-2% Chia. GelMA-0.5% Chia, which was not included in the printability matrix, was printed at the pristine-GelMA setting. The completed construct was photo-crosslinked in a single exposure using a broadband light-emitting diode (LED) curing light (VALO Cordless) in Xtra Power mode (3200 mW/cm², 395–480 nm) for 15 s; this irradiance, a dental restorative regime delivering approximately 48 J/cm², is well above that used for cell-laden photocrosslinking and was chosen because the constructs contained no cells (Section

4.4). Specimens for scanning electron microscopy were prepared by extruding the pre-gel manually through the same nozzle into wells of a 24-well plate and photo-crosslinking identically (Section 2.5). A GelMA-only hydrogel was prepared analogously for comparison. A chia-only reference (1% w/v chia mucilage) was prepared without photoinitiator and was not photo-crosslinked, since the mucilage carries no photopolymerizable groups; it served as a reference in the spectroscopic, thermal and rheological analyses. Group compositions and crosslinking conditions are summarized in Table 2. A GelMA-5% chia formulation was prepared for morphological and pre-crosslinking viscosity measurements only and was excluded from the crosslinked-hydrogel comparison because of its excessive viscosity.

Table 2. Composition of the experimental groups.

Group	GelMA (% w/v)	Chia (% w/v)	LAP (% w/v)	Code
1% Chia	0	1.0	0	I
10% GelMA	10	0	0.3	II
GelMA-0.5% Chia	10	0.5	0.3	III
GelMA-1% Chia	10	1.0	0.3	IV
GelMA-2% Chia	10	2.0	0.3	V

A GelMA-5% chia formulation was additionally prepared for scanning electron microscopy and pre-crosslinking viscosity measurement only, and was excluded from the crosslinked-hydrogel comparison owing to excessive viscosity.

2.5. Hydrogel characterization

Characterization was performed using ^1H NMR, Fourier-transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), thermogravimetric analysis (TGA) with derivative thermogravimetry (DTG), X-ray diffraction (XRD), compression testing and rheology on five groups: (I) 1% chia; (II) 10% GelMA; (III) GelMA-0.5% Chia; (IV) GelMA-1% Chia; (V) GelMA-2% Chia. Unless stated otherwise, samples were lyophilized and analyses performed on the dried material.

^1H NMR spectroscopy. Proton NMR (Bruker, USA) was performed at 400 MHz. Dried samples were dissolved in 600 μL of D_2O and analyzed at room temperature. The degree of methacrylation (DM) was determined from the consumption of the lysine and hydroxylysine methylene resonances at $\delta \approx 2.9$ ppm, integrated relative to the aromatic protons of phenylalanine and tyrosine (δ 6.6–7.6 ppm), which are not affected by methacrylation and therefore serve as an internal reference. Unmodified gelatin was acquired in parallel under identical conditions as the reference material, and DM was calculated as

$$\text{DM (\%)} = [1 - (\text{lysine methylene peak area of GelMA} / \text{lysine methylene peak area of gelatin})] \times 100 \text{ (I)}$$

following the procedure established for microwave-synthesized GelMA.³² Determining DM from the disappearance of the lysine methylene signal rather than from the appearance of the methacryloyl vinyl protons avoids the region affected by the residual HOD resonance and is the more robust of the two ^1H NMR routes.³⁶ The spectra of gelatin and of the synthesized GelMA, together with the integration used, are given in Figure A1 (see Appendix).

FTIR spectroscopy. Infrared spectra (Bruker Lumos II, USA) of dried samples were collected over 4000–670 cm^{-1} .

SEM analysis. Specimens for microscopy were prepared by extruding the pre-gel manually from a syringe through the same 22-gauge conical nozzle (410 μm internal diameter) used for printing (Section 2.6), collecting the extrudate in the well and photo-crosslinking it as in Section 2.4. The imaged material had therefore passed through the printing nozzle, although at a hand-driven rather than pneumatically controlled flow rate and as a pooled deposit rather than as a single filament. Dried samples were mounted on stubs and sputter-coated with gold. Surface morphology was examined by scanning electron microscopy (Zeiss EVO LS10, Germany) at 25 kV, with images recorded at 500 $\times$, 2500 $\times$, and 10000 $\times$ magnification.

TGA. Thermogravimetric analysis (Perkin Elmer Diamond, USA) was performed on dried samples from 50 $^{\circ}\text{C}$ to 900 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}/\text{min}$ under nitrogen; DTG curves were computed from the mass-loss data.

XRD analysis. X-ray diffraction (Panalytical Empyrean, England) was used to assess crystallinity over $2\theta = 5\text{--}80^{\circ}$ at a scan rate of $2^{\circ}/\text{min}$.

Mechanical properties. Uniaxial compression testing was performed at room temperature on printed and photo-crosslinked cylinders (2.5 mm diameter, 6 mm height; Section 2.4), loaded along the cylinder axis, i.e. normal to the layer plane. Samples were equilibrated in PBS (pH 7.4) before measurement and compressed at a constant displacement rate to 80% strain ($n = 3$ per group). The compressive modulus was determined from the slope of the linear region of the stress–strain curve. The three specimens in each group were printed and tested independently from pre-gel solutions prepared with chia mucilage from independent extraction batches (Section 2.2), so $n = 3$ captures printing variability together with mucilage batch-to-batch variability. GelMA was synthesized in repeated microwave runs under the conditions of Section 2.3; this protocol reproducibly yields highly substituted GelMA in our hands ($\text{DM } 89.0 \pm 1.4\%$ in previous work³²), and the degree of methacrylation determined for the material used here (81.9%, Section 3.2) is consistent with that record.

Rheological properties. Rheology was measured on a rotational rheometer (Anton Paar, Austria) using a parallel-plate geometry. Printed, crosslinked discs (2 mm thickness, 16 mm diameter; Section 2.4) were placed between the plates and analyzed at 37 $^{\circ}\text{C}$. Dynamic frequency sweeps were performed over 2–60 rad/s, strain sweeps from 0 to 2% at 1 Hz, and temperature sweeps recorded shear stress from 15 to 45 $^{\circ}\text{C}$. The viscosities of pre-crosslinking biomaterial ink solutions were determined as a function of temperature over 15–45 $^{\circ}\text{C}$.

2.6. Printability assessment and bioprinting

Printability was assessed on a pneumatic extrusion bioprinter (Axolotl Biosystems, Türkiye) fitted with a temperature-controlled print head held at 30 $^{\circ}\text{C}$. Biomaterial inks were loaded into 3 mL cartridges and extruded through a 22-gauge conical plastic nozzle of 410 μm internal diameter. Three formulations were examined — 10% GelMA, GelMA-1% Chia and GelMA-2% Chia — across a 3×3 matrix of extrusion pressure (3, 4 and 5 psi) and print speed (10, 15 and 20 V in instrument units), giving nine settings per biomaterial ink. Single filaments were deposited on a flat substrate and imaged from above on an inverted microscope and from the side on a digital microscope; serpentine constructs (15.33×9.85 mm) were printed at the optimal setting of each biomaterial ink and imaged on the digital microscope. Print speed is commanded on this instrument as a drive voltage and is reported in the instrument's own units (V); the corresponding printhead travel speeds are 20, 30 and 40 mm/s for 10, 15 and 20 V, respectively.

At each of the nine pressure–speed settings per biomaterial ink, three filaments were deposited in three separate extrusion experiments, each a fresh deposition on the substrate, and each filament was imaged and its width W measured individually. The $n = 3$ reported for filament width therefore denotes three independently deposited filaments at that setting, and values are reported as mean $\pm$ standard deviation of these three independent depositions.

Top-view images carried a burned-in scale bar, which was measured independently in every image and applied on a per-image basis (172 px per 500 μm ; 176 px in one image). The filament boundary was taken as the specular ridge separating the in-focus deposit from the substrate. Side-view images contained the mirror reflection of the filament in the print bed, so the raw side measurement spans the filament plus its reflection and was halved to obtain the filament height H ; side views were calibrated against a stage micrometer imaged in the same session (24.476 μm per pixel). Construct images were calibrated from the same micrometer, giving a 15.7 mm field of view consistent with the 15.33 mm design width.

Two dimensionless descriptors were derived. The spreading ratio

$$\text{Sr} = W / D \text{ (II)}$$

where $D = 410 \mu\text{m}$ is the nozzle internal diameter, quantifies dimensional fidelity: a value of unity denotes a filament exactly as wide as the nozzle and values above unity denote lateral spreading after deposition.³⁷ The aspect ratio H / W quantifies shape retention, a filament preserving a circular cross-section giving unity. The optimal setting for each biomaterial ink was defined as that minimizing $|\text{Sr} - 1|$.

The printability index $\text{Pr} = L^2 / 16A$, where L and A are pore perimeter and area,³⁸ was not computed. That index is defined for a grid lattice with square pores, for which a geometrically perfect print returns unity. The construct printed here is a serpentine with elongated channels, for which even a perfect print returns a value governed by the design aspect ratio rather than by print quality; the index is therefore not interpretable for this geometry and a square-pore lattice would be required to obtain it.⁴

2.7. Statistical analysis

Compressive modulus was measured on three specimens per group, each printed from a pre-gel prepared with chia mucilage from an independent extraction batch, and filament width on three independently deposited filaments per setting, each from a separate extrusion experiment; both are expressed as mean $\pm$ standard deviation (SD). Biomaterial inks were compared across the nine pressure–speed settings using the Friedman test with settings as blocks and the Wilcoxon signed-rank test for pairwise contrasts, with effect size reported as the rank-biserial correlation. Significance was set at $\alpha = 0.05$. Analyses were performed in Python 3 (SciPy 1.15, statsmodels 0.14). Compressive moduli were compared across the four formulations by analysis of variance followed by a post hoc procedure corrected for multiple comparisons. Because the group variances differ by nearly three orders of magnitude (SD 5.8 kPa for pristine GelMA against 173.8 kPa for the 2% blend; variance ratio 898), the equal-variance assumption of Tukey's honestly significant difference (HSD) test is not met; Welch's heteroscedastic analysis of variance with the Games–Howell post hoc test, which assumes neither equal variances nor equal group sizes, was therefore used as the primary analysis, and Tukey HSD on the pooled error term is reported alongside it for reference. Pairwise Wilcoxon comparisons of filament width between biomaterial inks were corrected for multiplicity across the three contrasts by the Holm–Bonferroni procedure; both uncorrected and corrected P values are given. The dependence of filament width on extrusion pressure and commanded speed was examined within each biomaterial ink by Spearman rank correlation and by two-way analysis of variance on the 3×3 pressure–speed matrix ($n = 3$ independently deposited filaments per cell), with the variance partitioned into pressure, speed and interaction terms (η^2).

3. Results

3.1. Preparation and photo-crosslinking of the blend hydrogels

Microwave-assisted methacrylation reproducibly yielded GelMA within 5 min of net irradiation (1000 W, 30 s $\times$ 10 pulses), and blending with freeze-dried chia mucilage produced homogeneous, bubble-free pre-gel solutions after centrifugation. Upon 15 s exposure to the 395–480 nm curing light (3200 mW/cm²), all printed formulations formed self-supporting cylinders and discs that retained the printed geometry after crosslinking, and the manually extruded specimens likewise crosslinked to coherent gels. Pristine GelMA gels were transparent and colourless, whereas increasing chia content produced a progressively more opalescent, pale-brown appearance and a visibly more cohesive, less brittle texture. No macroscopic phase separation was observed at any chia concentration up to 2% (w/v), indicating good miscibility of the mucilage with the GelMA matrix under the mixing and crosslinking conditions used. The complete preparation route is summarized in Figure 1.

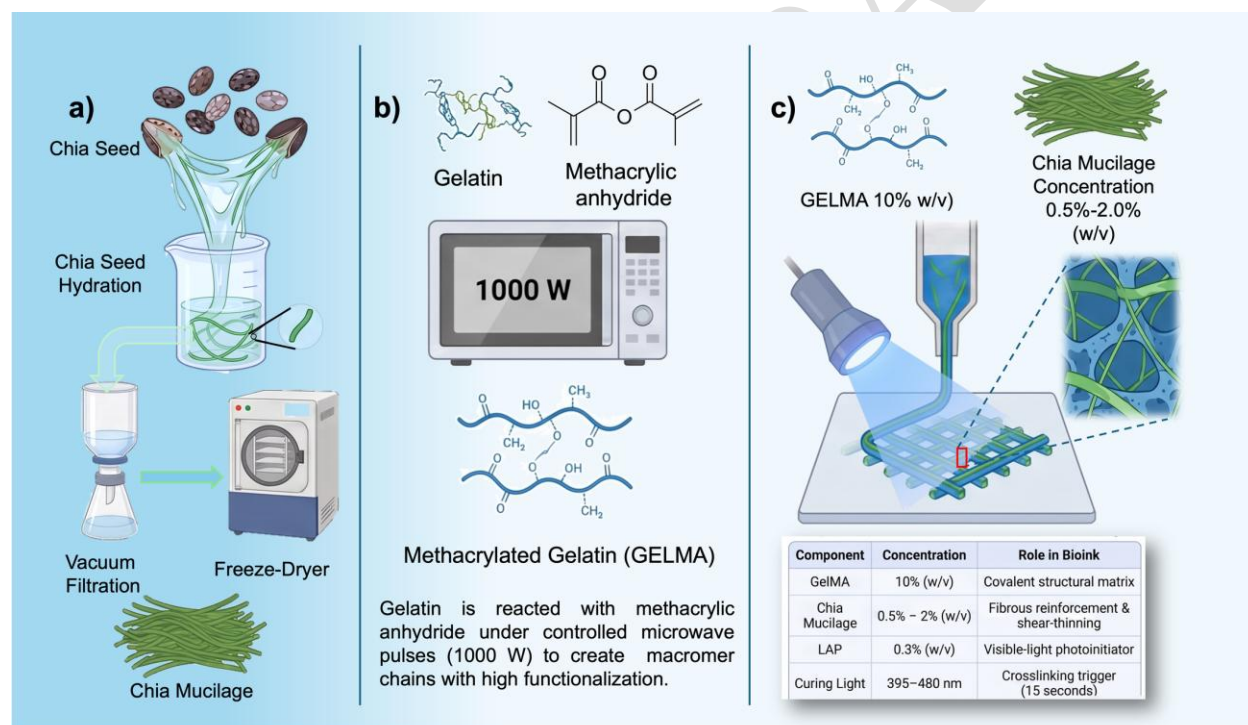


Figure 1. Preparation route for the GelMA–chia mucilage blend biomaterial ink. (a) Extraction of mucilage from *Salvia hispanica* L. seeds: seed hydration in distilled water, vacuum filtration through cheesecloth, centrifugation to remove residual seed fragments, and freeze-drying to yield the purified mucilage. (b) Microwave-assisted synthesis of GelMA: gelatin is reacted with methacrylic anhydride under power- and temperature-controlled microwave pulses (1000 W) to graft methacryloyl groups onto the gelatin backbone. (c) Blend biomaterial ink formulation and intended application: GelMA (10% w/v) is combined with chia mucilage (0.5–2.0% w/v) and LAP (0.3% w/v), extruded, and photo-crosslinked with a 395–480 nm curing light; the inset depicts the target architecture, in which chia fibers (green) interpenetrate and bridge the covalently crosslinked GelMA network (blue). The accompanying table summarizes the concentration and functional role of each component.

3.2. Chemical composition and intermolecular interactions

^1H NMR spectra of the five groups, acquired in D_2O at room temperature, are overlaid in Figure 2a. All GelMA-containing traces show the aliphatic envelope of the gelatin side chains between δ 0.8 and 2.1 ppm, the α -proton and side-chain methylene region between δ 2.8 and 4.2 ppm, and weak aromatic resonances of phenylalanine and tyrosine between δ 6.6 and 7.6 ppm. Signals attributable to the chia polysaccharide—anomeric and ring protons of its xylose, glucose and glucuronic acid residues—appear between δ 3.2 and 4.2 ppm and increase in relative intensity from GelMA-0.5% Chia to GelMA-2% Chia, confirming dose-dependent incorporation of the mucilage.

The degree of methacrylation was determined by ^1H NMR from the consumption of the lysine and hydroxylysine methylene protons. In the spectrum of unmodified gelatin a clear resonance is present at $\delta \approx 2.9$ ppm; in the microwave-synthesized GelMA this signal is almost entirely depleted, while two new sharp vinyl resonances appear at $\delta \approx 5.3$ and 5.5 ppm together with the methacryloyl methyl signal, confirming successful grafting (Figure A1, see Appendix). Integration of the lysine methylene region against the aromatic reference gave peak areas of 1461.31 for gelatin and 264.25 for GelMA, corresponding by Equation I to a degree of methacrylation of 81.92%.

This value is consistent with the behaviour expected of the synthesis route. Using the same microwave system and the same 1000 W / 4% (v/v) methacrylic anhydride condition, we previously obtained a DM of $89.0 \pm 1.4\%$ for microwave-assisted methacrylation against $23.1 \pm 5.6\%$ for conventional methacrylation under otherwise comparable conditions,³² and the modestly lower value obtained here falls within the batch-to-batch range expected for a different gelatin lot. A DM of approximately 82% places this material in the highly substituted regime, which is the relevant baseline for interpreting the absolute moduli reported in Section 3.4 and for comparison with literature GelMA systems.

Two qualifications should be noted. The five-group overlay of Figure 2a was acquired at room temperature without solvent suppression, so the methacryloyl vinyl region of those spectra sits on the shoulder of the residual HOD resonance at $\delta \approx 4.7$ ppm and is not suitable for quantification; the DM reported above derives from the dedicated gelatin/GelMA comparison of Figure A1, in which the lysine methylene region is well resolved. Signal overlap in the vinyl region is a documented and systematic source of bias in GelMA characterization, which is why the lysine-methylene route was used.³⁶

Because DM governs the covalent crosslink density of the photopolymerized network, quantifying it establishes the baseline against which the effect of chia mucilage is measured. All formulations compared in this study were prepared from GelMA synthesized by the same microwave protocol, which has repeatedly given highly substituted material in our laboratory,³² and whose degree of methacrylation was determined as described above; they were cured with identical photoinitiator concentration, irradiance and exposure time and differ only in mucilage content, so both the absolute moduli and the comparisons among formulations are referenced to a known and reported degree of substitution.

FTIR spectra (Figure 2b) resolve the composition of each group and provide evidence of interaction between the two networks. All gelatin-containing samples display the amide I band near 1640 cm^{-1} (C=O stretch) and the amide II band near 1535 cm^{-1} (N–H bend coupled to C–N stretch), together with C–H stretching near 2930 cm^{-1} . The chia-only control is dominated by a broad, intense O–H stretching envelope across $3700\text{--}3000\text{ cm}^{-1}$ and by a strong pyranose C–O–C absorption centered at 1038 cm^{-1} , with a carboxylate contribution near 1420 cm^{-1} consistent with the uronic acid residues of the mucilage; these assignments agree with published infrared data for chia mucilage.³⁹ In the blends, both the 1038 cm^{-1} pyranose band and the 1420 cm^{-1} carboxylate band grow systematically with chia loading, while the $3700\text{--}3000\text{ cm}^{-1}$ envelope broadens and deepens relative to pristine GelMA. This broadening, together

with the growth of the carboxylate feature, is consistent with intermolecular hydrogen bonding between the hydroxyl and carboxylate groups of chia and the amide groups of GelMA, and provides a molecular basis for the mechanical changes described below. We note that band broadening in this region is also sensitive to hydration state and to the extent of lyophilization, so the infrared evidence establishes that the two components interact rather than quantifying the strength of that interaction.

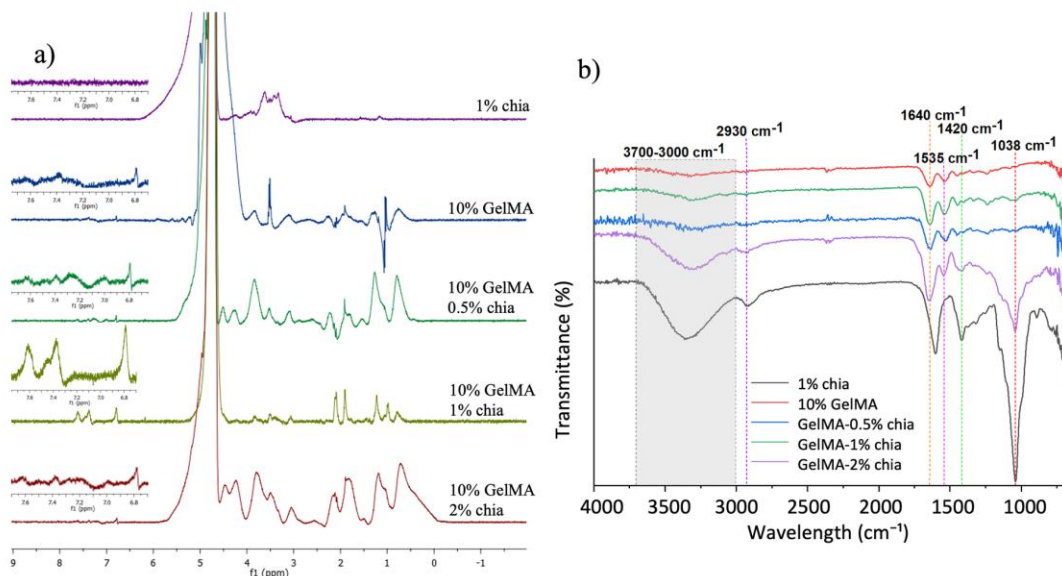


Figure 2. Spectroscopic characterization of the hydrogel groups. (a) Overlaid 400 MHz ¹H NMR spectra (D₂O, room temperature, δ 9 to -1 ppm) of 1% chia, 10% GelMA, GelMA-0.5% Chia, GelMA-1% Chia, and GelMA-2% Chia; carbohydrate ring-proton intensity in the δ 3.2–4.2 ppm region rises with chia content, while the methacryloyl vinyl region ($\delta \approx 5.3$ –5.7 ppm) is obscured by the residual HOD signal at $\delta \approx 4.7$ ppm, precluding determination of the degree of substitution from these overlay spectra (see Figure A1, in Appendix). (b) Overlaid FTIR transmittance spectra (4000–670 cm^{-1}) of the same five groups, with the O–H/N–H envelope (3700–3000 cm^{-1} , shaded) and the C–H (2930 cm^{-1}), amide I (1640 cm^{-1}), amide II (1535 cm^{-1}), carboxylate (1420 cm^{-1}), and pyranose C–O–C (1038 cm^{-1}) bands marked by dashed lines. The 1038 and 1420 cm^{-1} chia bands increase monotonically with mucilage content.

3.3. Microstructure

Scanning electron microscopy revealed a marked, concentration-dependent evolution of the internal architecture (Figure 3). Pristine 10% GelMA presented a smooth-walled, highly interconnected porous matrix typical of photocrosslinked gelatin methacryloyl (Figure 3a), whereas the chia-only control consisted of a comparatively dense, sheet-like matrix perforated by irregular pores and traversed by fine fibrils (Figure 3b). Introducing chia mucilage into GelMA superimposed a distinctive fibrous network on the porous GelMA walls. At 0.5% chia (Figure 3c), the fibres formed a fine, sparse mesh draped across the pore openings; at 1% (Figure 3d) and 2% (Figure 3e), the fibers became longer, thicker, and more interconnected; and at 5% (Figure 3f), they formed a coarse, heavily anastomosed network that coated and partially occluded the pore walls. High-magnification insets resolved individual mucilage fibrils,

typically 100–300 nm in apparent diameter, bridging adjacent pore walls and branching at nodal junctions.

The transition from a sparse, discontinuous mesh at 0.5% to a continuous, interconnected network at 1% (w/v) is the structural observation on which the mechanical interpretation in Section 4.1 rests, and its limits should be stated. The assessment of continuity is qualitative and two-dimensional: it is based on visual inspection of fracture surfaces of lyophilized specimens, not on quantitative image analysis of fibre length, connectivity or nodal density, and not on the hydrated state. Lyophilization is known to alter the apparent architecture of hydrogels, so the fibril dimensions and network geometry reported here should be regarded as the dried-state morphology rather than as a direct image of the hydrated network. What the images establish robustly is a monotonic increase in fibre density, length and apparent interconnectivity with mucilage content, with the largest qualitative step occurring between 0.5% and 1%. At 0.5–2% loading the network remains open and interconnected, which should permit nutrient and oxygen diffusion in thick constructs; at 5% the progressive occlusion of pores suggests that this benefit is lost at high loading, consistent with the exclusion of that formulation from the crosslinked-hydrogel comparison. Two consequences of the specimen preparation should be made explicit. Freeze-drying is the conventional preparation for hydrogel morphology, but ice nucleation and growth deform and compact the water-rich network and can generate cellular pores and aggregated fibrillar structures that are not present in the swollen state,⁴⁰ and comparative work on GelMA itself shows that the apparent architecture depends on the preparation route and that cryogenic or environmental electron microscopy is required to approach the native hydrated structure.⁴¹ The 100–300 nm fibrils and the network geometry reported here are therefore a dry-state morphology, and the transition from a sparse to a continuous network between 0.5% and 1% (w/v) is a qualitative observation on that morphology. It is used in Section 4.1 as the structural correlate of the mechanical threshold, not as a measurement of the hydrated network; the quantitative characterisation that would test the percolation reading directly is set out there. A second limit concerns the specimens themselves. The material imaged had been extruded manually through the printing nozzle before crosslinking (Section 2.5), so it had experienced nozzle shear, but it was collected as a pooled deposit in a well rather than as a deposited filament, and printed filaments were not sectioned or imaged. Shear and extensional flow through the nozzle can align anisotropic building blocks along the filament axis, as shown for cellulose nanocrystal inks^{42,43} and for collagen,⁴⁴ and such alignment would confer a mechanical anisotropy that the pooled specimens imaged here cannot show. The compression and rheology specimens were themselves printed layer by layer (Section 2.4), so the moduli reported in Section 3.4 are those of printed constructs loaded normal to the layer plane; in-plane anisotropy along the filament direction was not measured. Whether it occurs in deposited filaments of these blends is not established by the present data and is identified as a limitation of the microstructural evidence.

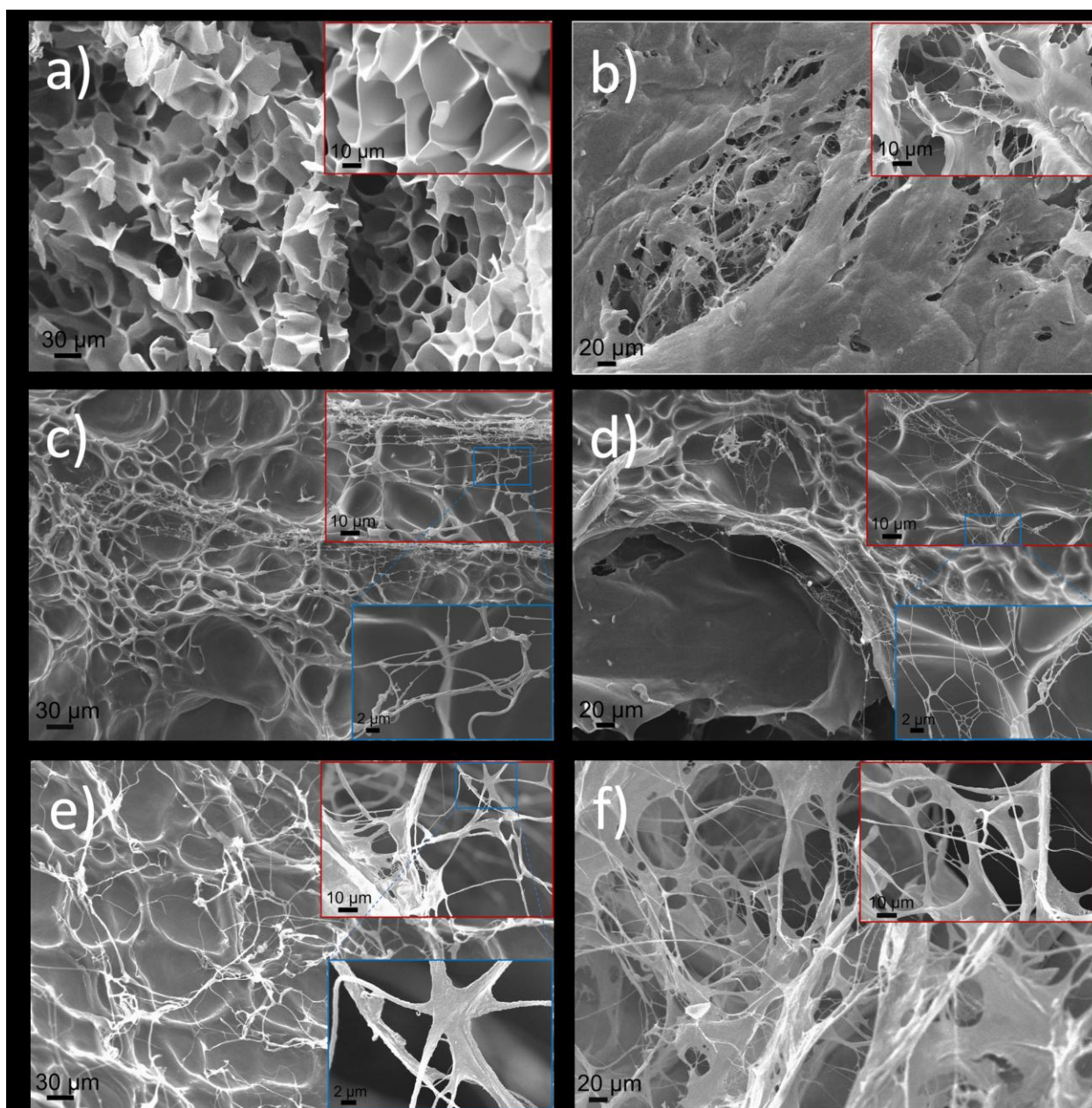


Figure 3. Morphology of chia mucilage and GelMA–chia hydrogels by scanning electron microscopy. (a) 10% GelMA (scale bar 30 μm); (b) chia mucilage (20 μm); (c) GelMA-0.5% Chia (30 μm); (d) GelMA-1% Chia (20 μm); (e) GelMA-2% Chia (30 μm); (f) GelMA-5% chia (20 μm); all main panels 500 $\times$. Red-bordered insets, 2500 $\times$ (scale bar 10 μm); blue-bordered insets, 10000 $\times$ (scale bar 2 μm). Chia introduces a fibrous network whose density, length, and interconnectivity increase with concentration, decorating and bridging the porous GelMA walls; the network becomes continuous between 0.5% and 1% (w/v). Assessment of continuity is qualitative and based on fracture surfaces of lyophilized specimens; the dried-state morphology may differ from the hydrated network. Specimens were freeze-dried before imaging; the fibril dimensions and network geometry shown are a dry-state morphology that may be templated in part by ice formation during freezing, and are not a direct image of the hydrated network (Section 3.3).

3.4. Thermal, crystallographic and mechanical properties

Thermogravimetric analysis (Figure 4a) showed the two-stage mass loss characteristic of hydrated biopolymer networks. The first stage, below approximately 150 °C, corresponds to release of bound and loosely associated water and was largest for pristine 10% GelMA, which lost approximately 8% of its mass by 150 °C, whereas all chia-containing blends and the chia-only control lost only 4–5%. The principal degradation step began near 270 °C and was essentially complete by 400 °C for all GelMA-containing samples. Residual mass at 900 °C was higher for every chia-containing blend than for pristine GelMA, but did not scale monotonically with mucilage content: 10% GelMA retained approximately 21%, GelMA-0.5% Chia approximately 26%, GelMA-1% Chia approximately 33% and GelMA-2% Chia approximately 30%, while the chia-only control retained approximately 55% (Table 3). Given the reproducibility typical of residual-mass determination, the 1% and 2% values are close, and we draw no interpretation from their ordering; the reliable observation is that mucilage incorporation raises the carbonaceous residue relative to pristine GelMA.

The derivative curves (Figure 4b) locate the maximum rate of mass loss at approximately 315–320 °C for all four GelMA-containing formulations, with no systematic shift attributable to chia content; the chia-only control degraded earlier, peaking near 300 °C with a much lower peak amplitude. Chia mucilage therefore increases the carbonaceous residue of the network and reduces the amount of weakly bound water it retains, but does not measurably raise the onset or peak temperature of backbone degradation.

X-ray diffraction (Figure 4c) confirmed that all samples are predominantly amorphous, each pattern being dominated by a broad halo centered near $2\theta \approx 20^\circ$. Increasing chia content broadened the principal halo modestly without generating new polysaccharide reflections, indicating that the mucilage is incorporated in a well-dispersed, non-crystalline state. Two weak but sharp reflections were nevertheless resolved at $2\theta \approx 31.8^\circ$ and 45.5° in the 10% GelMA and GelMA-2% Chia patterns; their positions correspond to the (200) and (220) reflections of sodium chloride. Because the GelMA used here was dialysed against distilled water after synthesis (Section 2.3), these reflections are unlikely to represent salt carried over from the PBS reaction medium. They appear in only two of the five patterns, whereas a systematic synthesis residue would be expected in every GelMA-containing sample; the most plausible origin is therefore PBS carry-over during specimen handling and equilibration before lyophilization. We note this as an inference from the distribution of the reflections rather than a determination and recommend that specimens for diffraction be rinsed in distilled water before drying in future work.

Uniaxial compression revealed the most striking effect of chia loading, and one that is distinctly non-monotonic. The stress–strain responses (Figure 4d) were J-shaped and typical of hydrated networks, with stress at 80% strain increasing in the order GelMA-0.5% Chia (≈ 53 kPa) < 10% GelMA (≈ 72 kPa) < GelMA-1% Chia (≈ 1250 kPa) < GelMA-2% Chia (≈ 2150 kPa). The corresponding compressive moduli (Figure 4e) were 55.7 ± 5.8 kPa for 10% GelMA, 42.6 ± 6.2 kPa for GelMA-0.5% Chia, 974.8 ± 101.3 kPa for GelMA-1% Chia and 1671.0 ± 173.8 kPa for GelMA-2% Chia. Adding 0.5% chia lowered the compressive modulus by 24% relative to GelMA alone, a difference that does not reach statistical significance (Welch $t = 2.67$, $df = 4$, $P = 0.056$; 95% confidence interval of the difference -0.5 to 26.7 kPa), whereas 1% and 2% chia increased it 17.5-fold and 30.0-fold ($P = 0.004$ and $P = 0.004$, respectively). The step between 0.5% and 1% (w/v) is therefore the reliable feature of the series. The reinforced values fall in the same range as GelMA systems reinforced with a second interpenetrating network,⁴⁵ although such cross-study comparison is weakened by differences in degree of substitution between studies (Section 3.2). Across the four formulations the compressive modulus differed overall (Welch's analysis of variance $F(3, 4.00) = 86.2$, $P = 0.0004$; classical one-way analysis of variance (ANOVA) $F(3, 8) = 184.0$, $P = 1.0 \times 10^{-7}$, $\eta^2 = 0.986$). Games–Howell post hoc comparisons, corrected

across all six pairwise contrasts, reproduce the threshold pattern: the 1% and 2% blends each exceed both pristine GelMA and the 0.5% blend ($P = 0.010, 0.010, 0.010$ and 0.009) and differ from one another ($P = 0.023$), whereas pristine GelMA and the 0.5% blend do not differ ($P = 0.169$; 95% confidence interval of the difference -6.9 to 33.1 kPa). Correction for multiplicity therefore moves the one contrast that was already non-significant further from significance and leaves every contrast involving 1% or 2% chia significant. Tukey HSD on the pooled error term gives the same ordering ($P = 0.998$ for pristine GelMA versus the 0.5% blend; $P \leq 1.3 \times 10^{-4}$ for every contrast involving 1% or 2% chia) but is reported for reference only, because the variance ratio of 898 across groups violates its equal-variance assumption.

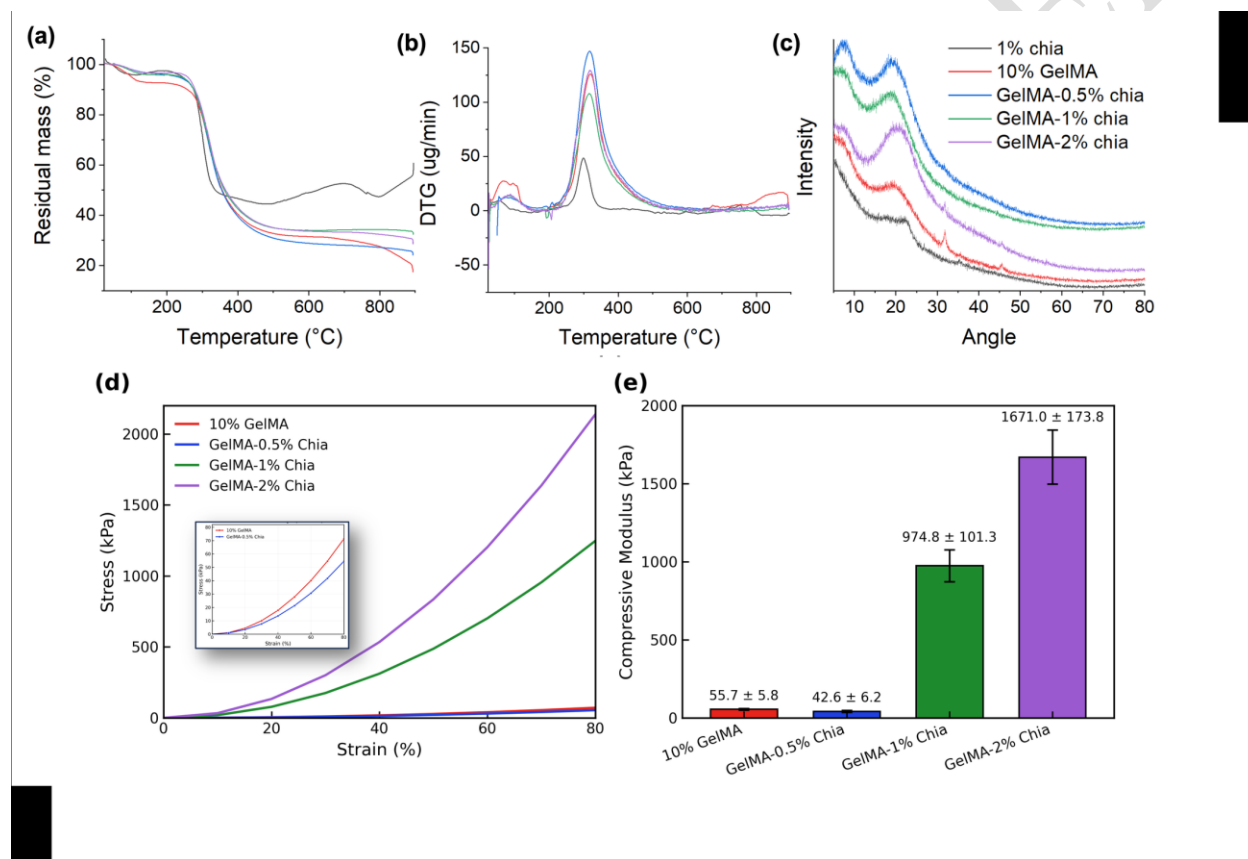


Figure 4. Thermal, crystallographic, and mechanical characterization. (a) TGA residual-mass curves and (b) derivative thermogravimetric (DTG) curves recorded from 50 to 900 °C at 10 °C/min under nitrogen, and (c) XRD patterns recorded over $2\theta = 5-80^\circ$, for 1% chia, 10% GelMA, GelMA-0.5% Chia, GelMA-1% Chia, and GelMA-2% Chia. Weak sharp reflections at $2\theta \approx 31.8^\circ$ and 45.5° in the 10% GelMA and GelMA-2% Chia patterns are indexed as the (200) and (220) reflections of face-centred-cubic NaCl; their most plausible origin is PBS carry-over during specimen handling and equilibration before lyophilisation (Section 3.4). (d) Uniaxial compressive stress-strain curves to 80% strain and (e) compressive moduli (mean $\pm$ SD, $n = 3$ specimens prepared with chia mucilage from independent extraction batches). Reinforcement is non-monotonic: 0.5% chia lowers the modulus below that of pristine GelMA, whereas 1% and 2% chia raise it 17.5-fold and 30.0-fold.

Table 3. Summary of mechanical, rheological, and thermal characteristics.

Property	1% Chia	10% GelMA	GelMA-1% Chia	GelMA-2% Chia
Compressive modulus (kPa)	n.d.	55.7 ± 5.8	974.8 ± 101.3	1671.0 ± 173.8
G', LVE plateau (Pa)	n.d.	196 ± 7	2550 ± 153	2972 ± 541
G'', LVE plateau (Pa)	n.d.	39	241	414
tan δ	n.d.	0.201	0.094	0.139
Shear stress at 30 °C (Pa)	n.d.	0.010	0.319	1.764
Viscosity at 30 °C (mPa·s)	209	63	397	1047
Viscosity at 15 °C (mPa·s)	258	19 630	4664	15 729
Thermal collapse, 15→45 °C	1.4×	3838×	49×	41×
Viscosity loss per °C at 30 °C	1%	63%	32%	15%
TGA residue at 900 °C (%)	≈55	≈21	≈33	≈30
DTG peak (°C)	≈300	≈318	≈317	≈316

Compressive modulus is mean ± SD (n = 3 specimens, each printed from a pre-gel prepared with chia mucilage from an independent extraction batch) from Figure 4e. The linear viscoelastic (LVE) plateau is the mean over 0.1–1% strain at 1 Hz and 37 °C; $\tan \delta = G''/G'$ at the plateau. Shear stress and viscosity at 30 °C are interpolated from the temperature ramps of Figure 5; viscosity refers to the pre-crosslinking biomaterial ink. Thermal collapse is the ratio of viscosity at 15 °C to that at 45 °C. Viscosity loss per °C is the fractional decrease across 29.5–30.5 °C, i.e. the local sensitivity at the print-head temperature. Values prefixed with ≈ were read from the corresponding figure panels. The shear stress value for 10% GelMA lies close to the low-torque resolution limit of the rheometer; ratios formed with it as denominator are correspondingly uncertain (Section 3.5). GelMA-0.5% Chia is omitted for legibility; its values lie below those of 10% GelMA for every mechanical and rheological property listed (compressive modulus 42.6 ± 6.2 kPa). n.d., not determined.

3.5. Rheological behavior

Oscillatory frequency sweeps confirmed solid-like behaviour for all crosslinked gels, with the storage modulus exceeding the loss modulus across the accessible range (Figure 5a,b). Strain sweeps at 1 Hz (Figure 5c,d) reproduced the concentration threshold identified by compression testing: within the linear viscoelastic (LVE) plateau over 0.1–1% strain, the storage modulus was 196 ± 7 Pa for 10% GelMA, 2550 ± 153 Pa for GelMA-1% Chia and 2972 ± 541 Pa for GelMA-2% Chia — increases of 13.0-fold and 15.2-fold respectively — while GelMA-0.5% Chia fell below pristine GelMA. The corresponding loss tangents were 0.201, 0.094 and 0.139, all well below unity, confirming that every formulation is solid-like in the linear region; the 1% blend is the most gel-like of the series.

Temperature sweeps of shear stress from 15 to 45 °C (Figure 5e) gave the same ordering. At the 30 °C print-head temperature the shear stress was 0.010 Pa for 10% GelMA, 0.319 Pa for GelMA-1% Chia and 1.764 Pa for GelMA-2% Chia. Computed from the tabulated values this is a 176-fold increase for the 2%

blend. Because the GelMA and GelMA-0.5% Chia traces lie close to the low-torque resolution limit of the instrument, the denominator of this ratio is poorly determined, the separation between those two formulations should be regarded as indicative, and the fold-change is best stated as exceeding two orders of magnitude rather than as a precise multiple.

The viscosity of the pre-crosslinking biomaterial inks (Figure 5f) is the most consequential result for extrusion. Pristine 10% GelMA behaves as expected of a physically gelling gelatin derivative: interpolated from the measured ramp, its viscosity falls from 19 630 mPa·s at 15 °C to 63 mPa·s at the 30 °C print-head temperature and 5.1 mPa·s at 45 °C, a collapse of 3838-fold driven by the coil–helix transition and concentrated between 28 and 32 °C. Chia progressively suppresses this collapse: viscosity at 30 °C rose to 397 mPa·s for GelMA-1% Chia and 1047 mPa·s for GelMA-2% Chia, increases of 6.3-fold and 17-fold, while the overall thermal collapse fell to 49-fold and 41-fold, respectively. Expressed as local sensitivity at the print head, a 1 °C rise across 29.5–30.5 °C removed 63% of the remaining viscosity of pristine GelMA but only 32% and 15% for the 1% and 2% blends. The chia-only control was almost athermal, declining only from 258 to 186 mPa·s over the full range (a factor of 1.4), which identifies the mucilage as the component responsible for decoupling ink viscosity from temperature. Below the coil–helix transition, which for GelMA lies at approximately 20–30 °C,⁵ the GelMA-containing inks are physically gelled, so the values recorded at the low-temperature end of the ramp are apparent viscosities of a yielding soft solid rather than true flow viscosities, and the collapse ratios computed from the 15 °C endpoint should be read as operational descriptors of resistance to flow. The per-degree sensitivity at the 30 °C print head, which carries the thermal-decoupling conclusion, is determined above the transition, where the inks flow and the rotational measurement is well posed.

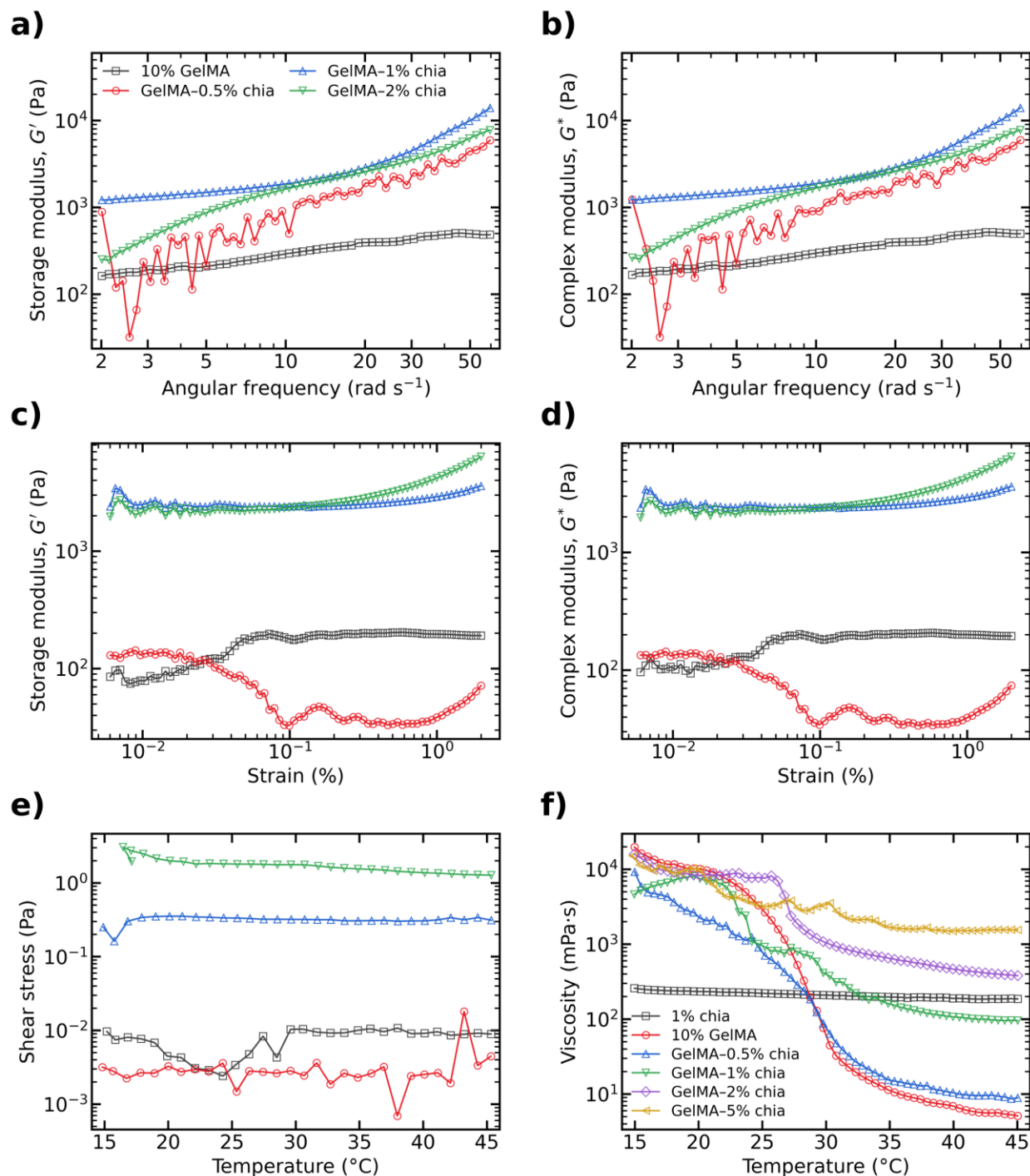


Figure 5. Rheological characterization of the hydrogels and pre-crosslinking biomaterial inks. (a) Storage modulus G' and (b) complex modulus G^* from frequency sweeps (2–60 rad/s, 37 °C); (c) storage modulus G' and (d) complex modulus G^* from strain sweeps (0–2% strain, 1 Hz, 37 °C) for 10% GelMA, GelMA-0.5% Chia, GelMA-1% Chia, and GelMA-2% Chia. (e) Shear stress as a function of temperature (15–45 °C) for the same four crosslinked gels; the 10% GelMA and GelMA-0.5% Chia traces lie close to the instrument's low-torque resolution limit. (f) Viscosity of the pre-crosslinking biomaterial inks (1% chia, 10% GelMA, GelMA-0.5%, -1%, -2%, and -5% chia) as a function of temperature (15–45 °C); below the coil-helix transition the GelMA-containing inks are physically gelled and the plotted values are apparent viscosities (Section 3.5). Chia mucilage raises the plateau

moduli above a threshold between 0.5% and 1% (w/v) and progressively decouples ink viscosity from temperature, suppressing the sol–gel collapse that pristine GelMA undergoes between 28 and 32 °C. The scatter in those two traces at the higher temperatures reflects approach to the instrument's baseline torque detection limit rather than material behaviour, and those values are indicative only.

3.6. Filament geometry and printing conditions

Filament geometry across the nine pressure–speed settings is given in Table 4 and Figure 6. Every biomaterial ink deposited filaments wider than the nozzle at every setting, with S_r ranging from 1.016 to 3.174, confirming that lateral spreading occurs in all three inks before photo-crosslinking arrests the geometry.

The optimal setting was 3 psi / 20 V for pristine GelMA and 5 psi / 15 V for both chia blends. At their respective optima, S_r was 1.158 for GelMA, 1.164 for the 1% blend and 1.016 for the 2% blend, so only the 2% blend deposited filaments essentially matching the nozzle dimension, at 1.6% above it.

Across the nine settings the three inks differed overall (Friedman $\chi^2 = 6.89$, $P = 0.032$), with GelMA producing wider filaments than the 1% blend at seven of nine settings (Wilcoxon $W = 6.0$, $P = 0.055$, $r = 0.73$). Aspect ratios spanned 0.085 to 0.540, with medians of 0.153, 0.244 and 0.254 for GelMA, the 1% blend and the 2% blend, so all filaments flattened substantially on contact and none preserved a near-circular cross-section. After Holm–Bonferroni correction across the three pairwise contrasts, none of the individual between-ink comparisons of filament width reaches significance (GelMA versus the 1% blend, $W = 6.0$, $P = 0.055$ uncorrected, 0.164 corrected, rank-biserial $r = 0.73$; GelMA versus the 2% blend, $W = 7.0$, $P = 0.074$ and 0.164, $r = 0.69$; 1% versus 2% blend, $W = 17.0$, $P = 0.570$ and 0.570, $r = 0.24$), although the omnibus Friedman test remains significant. The between-ink difference in deposited width is therefore an overall effect with a consistent direction — pristine GelMA depositing the widest filaments at most settings — rather than an established pairwise difference, and it is reported here as such. Multi-layer square-pore lattices (four layers) printed with each biomaterial ink at its optimal setting are shown in Figure A2 (see Appendix) as a qualitative demonstration of layer stacking, pore retention and interlayer adhesion; those images were not acquired for quantitative analysis and no fidelity metrics were derived from them. Neither extrusion pressure nor commanded speed showed a monotonic relationship with filament width within the 3×3 matrix: Spearman rank correlations of width with pressure were $\rho = +0.08$, $+0.16$ and -0.16 for pristine GelMA, the 1% blend and the 2% blend ($P = 0.84$, 0.69 and 0.69), and with speed $\rho = -0.29$, -0.11 and $+0.16$ ($P = 0.45$, 0.79 and 0.69). Two-way analysis of variance on each biomaterial ink located the reason: the pressure $\times$ speed interaction accounted for the largest share of the variance in width ($\eta^2 = 0.77$, 0.71 and 0.43 for pristine GelMA, the 1% blend and the 2% blend), exceeding the pressure ($\eta^2 = 0.11$, 0.08 , 0.25) and speed ($\eta^2 = 0.12$, 0.15 , 0.32) main effects in every case. The two parameters therefore do not act additively on the deposited width, and a regression of geometry on either parameter alone is not supportable from this design; the optimum is an operating point located by search over the matrix rather than a value read from a monotonic trend, and resolving a quantitative parameter–geometry relationship would require a finer grid with independent replicate prints in every cell.

Table 4. Filament geometry across the pressure–speed matrix.

Biomaterial ink	Pressure (psi)	Speed (V)	Width (μm , mean $\pm$ SD)	Sr	H/W
10% GelMA	3	10	1247.1 $\pm$ 5.0	3.042	0.085
	3	15	1106.6 $\pm$ 14.9	2.699	0.142
	3	20	474.8 $\pm$ 3.4	1.158	0.425
	4	10	726.1 $\pm$ 1.7	1.771	0.279
	4	15	1090.1 $\pm$ 5.8	2.659	0.153
	4	20	608.5 $\pm$ 1.7	1.484	0.276
	5	10	1090.1 $\pm$ 5.8	2.659	0.118
	5	15	716.1 $\pm$ 1.7	1.747	0.158
	5	20	1301.4 $\pm$ 1.7	3.174	0.132
GelMA-1% Chia	3	10	545.9 $\pm$ 31.8	1.331	n.d.
	3	15	732.0 $\pm$ 42.4	1.785	0.141
	3	20	672.7 $\pm$ 36.3	1.641	0.188
	4	10	1056.3 $\pm$ 88.2	2.576	0.239
	4	15	657.2 $\pm$ 39.9	1.603	0.246
	4	20	595.5 $\pm$ 3.8	1.452	0.415
	5	10	751.8 $\pm$ 27.4	1.834	0.242
	5	15	477.4 $\pm$ 42.9	1.164	0.257
	5	20	864.1 $\pm$ 60.3	2.108	0.332
GelMA-2% Chia	3	10	611.4 $\pm$ 1.7	1.491	0.207
	3	15	712.2 $\pm$ 0.0	1.737	0.286
	3	20	938.0 $\pm$ 8.9	2.288	0.124
	4	10	611.7 $\pm$ 1.6	1.492	0.540
	4	15	539.7 $\pm$ 1.7	1.316	0.355
	4	20	533.9 $\pm$ 4.4	1.302	0.254
	5	10	781.0 $\pm$ 12.1	1.905	0.227
	5	15	416.7 $\pm$ 4.4	1.016	0.312
	5	20	861.4 $\pm$ 3.4	2.101	0.223

Width is the mean $\pm$ SD of three independently deposited filaments at each setting, each from a separate extrusion experiment. Sr = W / D with D = 410 μm (22-gauge conical nozzle). H is the filament height obtained from side-view images after correcting for the mirror reflection in the print bed. Bold values identify the optimal setting of each biomaterial ink, defined as that minimizing $|\text{Sr} - 1|$: 3 psi / 20 V for 10% GelMA and 5 psi / 15 V for both blends. n.d., not determined.

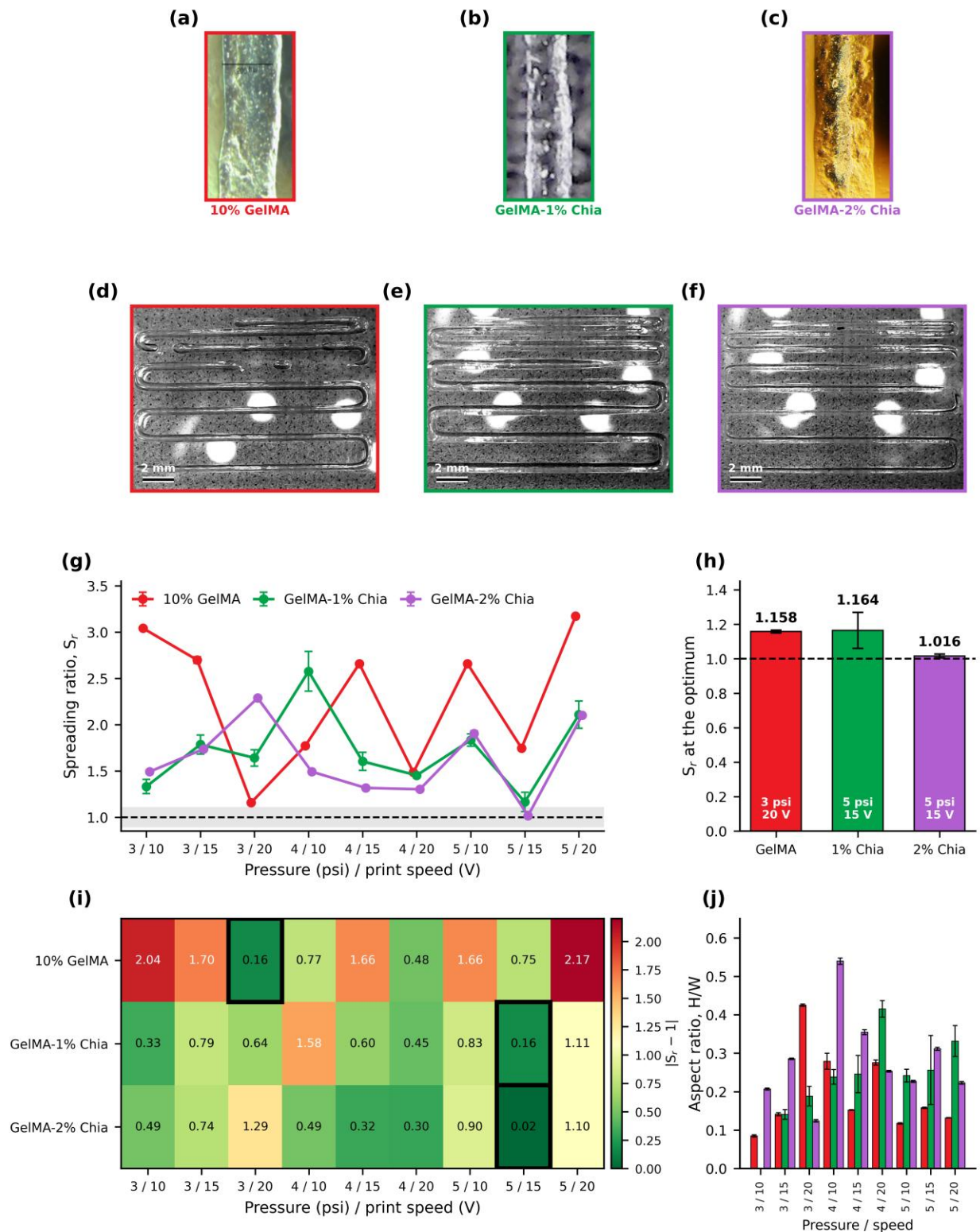


Figure 6. Printability of the GelMA–chia biomaterial inks. (a–c) Representative filaments, one image selected from each formulation for illustration only, deposited through a 22-gauge conical nozzle (410 μm internal diameter) with the print head at 30 $^{\circ}\text{C}$: (a) 10% GelMA, (b) GelMA-1% Chia, (c) GelMA-2% Chia; scale bar, 500 μm . (d–f) Serpentine constructs printed with each biomaterial ink at its optimal pressure–speed setting — (d) 10% GelMA at 3

psi / 20 V, (e) GelMA-1% Chia and (f) GelMA-2% Chia at 5 psi / 15 V; scale bars, 2 mm. (g) Spreading ratio $S_r = W / D$, where W is the deposited filament width and D the nozzle diameter, across the nine combinations of extrusion pressure (3, 4 and 5 psi) and print speed (10, 15 and 20 V); the dashed line marks $S_r = 1$ and the shaded band a $\pm 10\%$ window around it. (h) Spreading ratio at each biomaterial ink's optimal setting, defined as the setting minimizing $|S_r - 1|$; the corresponding pressure and speed are given within each bar. (i) Deviation from the nozzle diameter, $|S_r - 1|$, for every biomaterial ink and setting; the boxed cell in each row marks that biomaterial ink's optimum. (j) Filament aspect ratio H / W , where H is the filament height measured from side-view images; lower values denote greater flattening after deposition. In (g), (h), (i) and (j) each value is the mean of three independently deposited filaments, each from a separate extrusion experiment, and error bars in (h) show the corresponding standard deviation. The aspect ratio is unavailable for GelMA-1% Chia at 3 psi / 10 V, the only condition without a side-view measurement. The quantitative data in (g–j) derive from all independently deposited filaments at every setting (Section 2.6), not from the single illustrative images shown in (a–c). Error bars in (g) and (j) likewise show the standard deviation of the three independently deposited filaments at each setting; standard deviations of the aspect ratio were computed from the three paired height and width measurements.

4. Discussion

Two questions organized this study, and the data answer them differently. Chia mucilage reinforces the photocrosslinked GelMA network, but only above a concentration threshold; below it, the network is statistically unchanged. Chia mucilage also decouples ink viscosity from temperature, and this second effect is progressive, monotonic and — for extrusion bioprinting — the more valuable and the better supported of the two.

4.1. Reinforcement is a threshold phenomenon

The compressive modulus did not increase smoothly with mucilage content. Relative to pristine GelMA (55.7 ± 5.8 kPa), 0.5% (w/v) chia gave a modestly lower value (42.6 ± 6.2 kPa) that does not reach statistical significance (Welch $t = 2.67$, $df = 4$, $P = 0.056$; 95% confidence interval of the difference -0.5 to 26.7 kPa), whereas 1% and 2% raised the modulus 17.5- and 30.0-fold ($P = 0.004$ in both cases; Games–Howell $P \leq 0.010$ after correction for all six pairwise contrasts, against $P = 0.169$ for the GelMA–0.5% contrast; Section 3.4). We therefore do not interpret the low-loading value as evidence of weakening. The reliable feature of the series is a steep, discontinuous rise between 0.5% and 1% (w/v), and the same step recurs in the strain-sweep plateau modulus and in the temperature-dependent shear stress, so it is not an artefact of a single mechanical test.

Scanning electron microscopy supplies the structural correlate: the mucilage network is sparse and discontinuous at 0.5% and becomes long, dense and interconnected between 0.5% and 1%. This is the behaviour expected of rigidity percolation, and we offer that reading as the most economical interpretation of the data rather than as a demonstrated mechanism. Discontinuous fibrils dispersed in a compliant matrix cannot transfer load between them, so a fibrous filler contributes little to stiffness until its network becomes continuous; disordered fibre networks possess a rigidity-percolation threshold in connectivity, below which the network is mechanically floppy and above which the shear modulus rises with critical scaling.^{46–48} The classic experimental demonstration in a soft matrix is that of cellulose whiskers in latex, where the shear modulus rose by more than two orders of magnitude at a few percent loading through formation of a hydrogen-bonded percolating network.²¹ That reading — a percolating, hydrogen-bonded filler network inferred from the discontinuity in modulus with loading together with the microstructure, rather than from a fitted critical exponent — has since become the standard framework for threshold reinforcement by rod-like cellulose nanocrystals and nanofibrils,^{49,50} and it accounts for modulus changes of more than an order of magnitude that are switched on and off by forming and

disrupting the whisker network.⁵¹ Comparable threshold stiffening has since been shown in composite hydrogels, where filler particles remain mechanically inert until polymer bridging renders them interconnected,⁵² and percolation models fit the modulus of cellulose-nanofibril-reinforced networks quantitatively.⁵³ The hydrogen bonding detected by FTIR provides the interfacial coupling that load transfer through such a network requires.

Two qualifications apply. The assessment of network continuity is qualitative, two-dimensional and performed on lyophilized, pooled rather than printed, specimens (Section 3.3); we have not quantified fibre connectivity or determined a percolation threshold volume fraction, and with three non-zero loadings we cannot fit the power-law scaling that would test the mechanism directly. A finer concentration series between 0.25% and 1.5% (w/v), together with equilibrium swelling and sol-fraction measurements to quantify effective crosslink density, hydrated-state imaging of the network, and sectioning of printed filaments with compression parallel and perpendicular to the print direction, would locate the threshold, test that scaling and resolve any flow-induced anisotropy. The practical implication is nevertheless specific: a GelMA–polysaccharide blend cannot be optimized by extrapolating from a single low concentration, because the modulus is essentially unchanged below the threshold and rises by more than an order of magnitude above it.

The sub-percolation behaviour merits a brief mechanistic comment even though the difference does not reach significance, because its sign recurs in the compression, strain-sweep and temperature-sweep data. Two non-exclusive mechanisms would each produce a modest softening at 0.5% (w/v). The first is steric: GelMA at 10% (w/v) retains a physical contribution from triple-helix junction zones formed on cooling before and during photocuring, and a dispersed but still discontinuous polysaccharide phase increases the mean separation between gelatin chains, suppressing helix nucleation while contributing no load-bearing network of its own; above the percolation threshold the mucilage network carries load and this cost is repaid. The second is optical: the blends are already opalescent at 0.5% (w/v), and scattering by the polysaccharide phase attenuates the 395–480 nm curing light within the specimen, lowering the rate of LAP photolysis and hence the covalent crosslink density. The two are experimentally separable — equilibrium swelling with sol-fraction determination would isolate the photochemical contribution, and circular dichroism or temperature-resolved rheology of the uncured pre-gel the steric one — but neither measurement was performed here, and the account is offered as a testable hypothesis.

The degree of methacrylation of the GelMA used throughout was 81.9% (Section 3.2), so the covalent crosslink density against which the mucilage effect is measured is defined rather than assumed. This matters for the interpretation above: at high substitution the GelMA network is densely crosslinked, and the softening produced by 0.5% mucilage therefore cannot be attributed to an intrinsically weak or under-substituted matrix. Because all formulations derive from GelMA synthesized by the same microwave protocol, which reproducibly yields highly substituted material,³² and were cured under identical conditions, both the absolute moduli and the comparisons among them are referenced to this value.

4.2. Thermal decoupling is the principal processing benefit

Pristine GelMA is difficult to extrude reproducibly not because its viscosity is too low in absolute terms but because its viscosity is a steep function of temperature near the working point. The measurements here quantify that steepness: a 1 °C rise at the 30 °C print head removed 63% of the remaining viscosity of 10% GelMA, so a thermal excursion of ± 0.5 °C — well within the drift of an uninsulated cartridge — changes ink viscosity by roughly a factor of two. Chia mucilage, whose own viscosity varies only from 258 to 186 mPa·s over the entire 15–45 °C range, flattens this dependence: the per-degree loss fell to 32% at 1% loading and 15% at 2%, a two- to four-fold reduction in thermal sensitivity at the working point.

This matters more than the accompanying rise in absolute viscosity. Bioink rheology is conventionally optimized for shear-thinning and yield stress, both of which govern whether a filament forms and holds,^{3,4,54,55} but a formulation can satisfy both criteria and still print irreproducibly if those properties are strongly temperature-dependent. By reducing the thermal sensitivity of the ink, the chia blends relax the requirement for tight print-head thermostating that constrains pristine GelMA. The mechanism is straightforward and does not require the mucilage to interact specifically with gelatin: an athermal, pseudoplastic polysaccharide contributes a temperature-insensitive component to the total viscosity, so the fractional contribution of the strongly temperature-dependent gelatin coil–helix term is diluted. The chia-only control, which varies by a factor of only 1.4 across the full range, confirms that the mucilage supplies this athermal contribution.

4.3. Comparison with current strategies for stabilizing GelMA

Placing this system among recent alternatives requires acknowledging what those alternatives do better. Intrinsic modification achieves the most complete decoupling available: hydrolysed GelMA does not thermally gel below 30 °C at all, making stiffness a function of concentration alone,⁷ which is a cleaner solution than the partial suppression reported here — our 2% blend still loses 15% of its viscosity per degree and collapses 41-fold across 15–45 °C. Hofmeister modulation abolishes the sol–gel transition with nothing more than a salt and simultaneously supplies ionic crosslinking,⁸ and dual-crosslinked and colloidal GelMA systems reach stiffnesses and fidelities that physical blending does not.^{10–12} Within the additive class, Laponite,¹³ hydroxyethyl cellulose,¹⁴ cellulose nanomaterials,^{15,16} tragacanth,¹⁷ and κ -carrageenan¹⁸ all deliver comparable rheological benefits, and the GelMA–alginate literature has shown that polysaccharide molar mass is a powerful and tunable control variable in its own right.¹⁹

The advantages of the present system are correspondingly specific rather than general. First, it requires no additional chemistry, enzyme handling, or change in ionic strength: the mucilage is dissolved into the photoinitiator solution before GelMA is added, and the crosslinking chemistry is unaltered — an advantage over enzymatic and Hofmeister routes for laboratories with established GelMA workflows. Second, the additive is a food-grade material from a renewable, inexpensive and widely available agricultural source, obtained without organic solvents or harsh chemistry, which compares favourably with synthetic nanoclays and engineered nanocelluloses on cost and supply. Third, and least common among the alternatives, a single additive delivers both effects simultaneously: thermal decoupling that is progressive with concentration, and mechanical reinforcement that is available above a threshold, so the two can be traded against one another by formulation alone. We note, however, that this combination is a convenience rather than a unique capability, and that the natural-extract origin which makes chia mucilage cheap is also the source of its principal liability — compositional variability (Section 2.2), which engineered additives do not share.

4.4. Filament geometry

The rheological differences translated only partly into filament geometry. Every ink spread beyond the 410 μm nozzle at every setting (Sr 1.016–3.174), and only the 2% blend approached the nozzle dimension at its optimum. Aspect ratios of 0.085–0.540 show that all filaments flattened on contact, so height retention would require post-deposition support or in-line photo-crosslinking rather than crosslinking after deposition. Lateral spreading of this kind is commonly reported for GelMA printed without support.⁵⁶

The printability index $\text{Pr} = L^2/16A$ is defined for square-pore lattices and is not interpretable for the serpentine geometry printed here, and was therefore not computed.³⁸

The platform combines rapid microwave GelMA synthesis,³³ visible-light crosslinking with LAP that is compatible with cell encapsulation,^{34,35} and simple physical blending that requires no additional reactive chemistry, with stiffness and thermal sensitivity adjustable through a single formulation variable. Cytocompatibility and cell-laden printing remain to be established and are the subject of ongoing work. Two further limitations should be stated. Equilibrium swelling, equilibrium water content and mass retention in PBS at 37 °C were not measured. Because the mucilage carries no photopolymerisable groups and is retained in the network only by entanglement, hydrogen bonding and physical entrapment, progressive leaching during prolonged incubation cannot be excluded on present evidence, and the persistence of the reinforcement reported here under tissue-culture conditions remains to be established by gravimetric swelling and mass-retention measurement at 24–72 h with carbohydrate assay of the supernatant. The photocuring regime also requires qualification. The 3200 mW/cm² for 15 s used here (approximately 48 J/cm²) was chosen for rapid and complete conversion in cell-free specimens; cell-laden photocrosslinking with LAP is conventionally performed at far lower irradiance at 405 nm,⁵⁷ and both irradiance and dose govern cytotoxicity.⁵⁸ Whether the blends reach comparable gel fraction and modulus under such a regime has not been tested, and the opalescence that increases with mucilage loading will shorten the optical attenuation length and make the cure more surface-weighted in thick constructs; photorheology at matched dose and depth-resolved gel-fraction measurement are the appropriate tests and are planned with the cell-laden work.

5. Conclusion

Blending microwave-synthesized GelMA with chia seed mucilage yields a photocrosslinkable biomaterial ink governed by two distinct mechanisms operating on different concentration scales. Mechanical reinforcement is a threshold phenomenon: the compressive modulus is statistically unchanged at 0.5% (w/v) mucilage but rises 17.5- and 30.0-fold at 1% and 2%, in step with the transition of the fibre network from sparse to continuous observed by electron microscopy. We interpret this as rigidity percolation of the mucilage network, an interpretation that a finer concentration series and independent crosslink-density measurement would be needed to establish. Thermal decoupling, by contrast, is progressive and is the better-supported effect: the mucilage suppresses the coil–helix viscosity collapse of GelMA from 3838-fold to 49- and 41-fold across 15–45 °C, and cuts the per-degree sensitivity at the 30 °C print head from 63% to 32% and 15%. Because the mucilage is itself nearly athermal, it supplies a temperature-insensitive viscosity contribution that dilutes the steep gelatin term. Direct measurement of deposited filaments showed that the 2% blend reached a spreading ratio of 1.016, the closest approach to the nozzle dimension recorded in this study. The practical contribution is therefore a widened and more forgiving thermal processing window obtained from an inexpensive, renewable, food-grade plant polysaccharide, at the cost of a compositional variability that engineered additives do not carry. Cytocompatibility and cell-laden printing are the necessary next steps.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

Not applicable. This study did not involve human participants, human tissue, or animals.

Consent for publication

Not applicable.

Availability of data

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Further disclosure

During the preparation of this manuscript the authors used Jenni AI to assist with language editing and to check the reference list. The tool was not used to generate scientific content, to analyse or interpret data, or to produce figures. The authors reviewed and edited all resulting text and take full responsibility for the content of the published article.

References

1. Kim YH, Dawson JI, Oreffo ROC, et al. Gelatin methacryloyl hydrogels for musculoskeletal tissue regeneration. *Bioengineering (Basel)*. 2022;9(7):332. doi:10.3390/bioengineering9070332
2. Liu W, Heinrich MA, Zhou Y, et al. Extrusion bioprinting of shear-thinning gelatin methacryloyl bioinks. *Adv Healthc Mater*. 2017;6(12):1601451. doi:10.1002/adhm.201601451
3. Das S, Jegadeesan JT, Basu B. Gelatin methacryloyl (GelMA)-based biomaterial inks: process science for 3D/4D printing and current status. *Biomacromolecules*. 2024;25(4):2156-2221. doi:10.1021/acs.biomac.3c01271
4. Schwab A, Levato R, D'Este M, et al. Printability and shape fidelity of bioinks in 3D bioprinting. *Chem Rev*. 2020;120(19):11028-11055. doi:10.1021/acs.chemrev.0c00084
5. Suntornnond R, An J, Chua CK. Bioprinting of thermoresponsive hydrogels for next generation tissue engineering: a review. *Macromol Mater Eng*. 2017;302(1):1600266. doi:10.1002/mame.201600266

6. Rebers L, Granse T, Tovar GEM, et al. Physical interactions strengthen chemical gelatin methacryloyl gels. *Gels*. 2019;5(1):4. doi:10.3390/gels5010004
7. Kyeong J, Jeong D, Bae H, et al. Temperature-independent and photocrosslinkable hydrolyzed GelMA with tunable mechanics for biofabrication. *Mater Today Bio*. 2025;35:102402. doi:10.1016/j.mtbio.2025.102402
8. Chee HL, Koo JW, Sim EEI, et al. Hofmeister ions-induced thinning of gelatin to enhance 3D printing precision. *Adv Mater Technol*. 2024;9(23):2302230. doi:10.1002/admt.202302230
9. Das S, Majumdar S. Enhancing the properties of self-healing gelatin alginate hydrogels by Hofmeister mediated electrostatic effect. *ChemPhysChem*. 2023;25(1):e202300660. doi:10.1002/cphc.202300660
10. Bracchi M, Nicotra F, Russo L. 3D bioprintable hydrogels via enzymatic crosslinking of hyaluronic acid and phenol-functionalized gelatin to mimic extracellular matrix (ECM) network. *Carbohydr Polym Technol Appl*. 2025;11:100928. doi:10.1016/j.carpta.2025.100928
11. Jahani Kadousaraei M, Yamada S, Aydin MS, et al. Bioprinting of mesenchymal stem cells in low concentration gelatin methacryloyl/alginate blends without ionic crosslinking of alginate. *Sci Rep*. 2025;15(1):6609. doi:10.1038/s41598-025-90389-2
12. Wang Q, Chen K, Zhang Y, et al. Nanostructured GelMA colloidal gels as bioinks for freeform multi-mode 3D printing: better replacement for the classical GelMA polymeric inks. *Bioact Mater*. 2025;53:188-204. doi:10.1016/j.bioactmat.2025.07.010
13. Davern JW, Hipwood L, Bray LJ, et al. Addition of Laponite to gelatin methacryloyl bioinks improves the rheological properties and printability to create mechanically tailorable cell culture matrices. *APL Bioeng*. 2024;8(1):016101. doi:10.1063/5.0166206
14. Li X, Deng Q, Wang S, et al. Hydroxyethyl cellulose as a rheological additive for tuning the extrusion printability and scaffold properties. *3D Print Addit Manuf*. 2021;8(2):87-98. doi:10.1089/3dp.2020.0167
15. Wu Y, Lin ZY, Wenger A, et al. 3D bioprinting of liver-mimetic construct with alginate/cellulose nanocrystal hybrid bioink. *Bioprinting*. 2018;9:1-6. doi:10.1016/j.bprint.2017.12.001
16. Brusentsev Y, Yang P, King AWT, et al. Photocross-linkable and shape-memory biomaterial hydrogel based on methacrylated cellulose nanofibres. *Biomacromolecules*. 2023;24(8):3835-3845. doi:10.1021/acs.biomac.3c00476
17. Minooei HZ, Kaffashi B. Viscoelastic properties of thermosensitive gelatin/tragacanth hydrogel as novel ink suitable for additive manufacturing. *J Appl Polym Sci*. 2023;140(34):e54328. doi:10.1002/app.54328
18. Chaves Filho GP, Santos P, Richardson SM, et al. Glycosaminoglycan-mimetic gelatin-methacrylate/ κ -carrageenan bioink: a synergistic approach for enhanced printability, osteogenesis, and apatite mineralization in bone constructs. *Carbohydr Polym*. 2026;380:125092. doi:10.1016/j.carbpol.2026.125092
19. Hájovská P, Kroneková Z, Cseriová Z, et al. Tuning rheology and printability of gelatin methacryloyl/sodium alginate bioinks: the role of sodium alginate molar mass and sterilization. *ACS Omega*. 2025;10(42):49776-49793. doi:10.1021/acsomega.5c05476
20. Elkhoury K, Patel D, Gupta N, et al. Nanocomposite GelMA bioinks: toward next-generation multifunctional 3D-bioprinted platforms. *Small*. 2025;21(45):2505968. doi:10.1002/smll.202505968
21. Favier V, Canova GR, Cavaillé JY, et al. Nanocomposite materials from latex and cellulose whiskers. *Polym Adv Technol*. 1995;6(5):351-355. doi:10.1002/pat.1995.220060514

22. Champeau M, Heinze DA, Viana TN, de Souza ER, Chinellato AC, Titotto S. 4D printing of hydrogels: a review. *Adv Funct Mater.* 2020;30(31):1910606. doi:10.1002/adfm.201910606
23. Heid S, Boccaccini AR. Advancing bioinks for 3D bioprinting using reactive fillers: a review. *Acta Biomater.* 2020;113:1-22. doi:10.1016/j.actbio.2020.06.040
24. Voet VSD, Guit J, Loos K. Sustainable photopolymers in 3D printing: a review on biobased, biodegradable, and recyclable alternatives. *Macromol Rapid Commun.* 2021;42(3):2000475. doi:10.1002/marc.202000475
25. Motyka S, Koc K, Ekiert H, et al. The current state of knowledge on *Salvia hispanica* and *Salviae hispanicae* semen (chia seeds). *Molecules.* 2022;27(4):1207. doi:10.3390/molecules27041207
26. Alejo-Jacuinde G, Nájera-González HR, Chávez Montes RA, et al. Multi-omic analyses reveal the unique properties of chia (*Salvia hispanica*) seed metabolism. *Commun Biol.* 2023;6(1):820. doi:10.1038/s42003-023-05192-4
27. Khatoon M, Ali A, Haseeb MT, et al. A chia (*Salvia hispanica* L.) seed mucilage-based glucoxyllan-grafted-acrylic acid hydrogel: a smart material for pH-responsive drug delivery systems. *Mater Adv.* 2025;6(8):2636-2647. doi:10.1039/d5ma00046g
28. Silva LLG, Silva CAS, Santana RC, et al. Rheology of dispersions and emulsions composed of chia mucilage and the application of chia in food. *J Sci Food Agric.* 2022;102(13):5585-5592. doi:10.1002/jsfa.11921
29. Qiao Z, Zhang K, Liu H, et al. CSMP: a self-assembled plant polysaccharide-based hydrofilm for enhanced wound healing. *Adv Healthc Mater.* 2024;13(6):2303244. doi:10.1002/adhm.202303244
30. Groll J, Burdick JA, Cho DW, et al. A definition of bioinks and their distinction from biomaterial inks. *Biofabrication.* 2019;11(1):013001. doi:10.1088/1758-5090/aaec52
31. Dirr S, Ozmutlu Karslioglu O, Ates EG, Oztup MH. Defatting strategies for chia protein production: effects on physicochemical properties. *Future Foods.* 2025;12:100729. doi:10.1016/j.fufo.2025.100729
32. Irmak G, Demirtaş TT, Gümüşderelioğlu M. Highly methacrylated gelatin bioink for bone tissue engineering. *ACS Biomater Sci Eng.* 2019;5(2):831-845. doi:10.1021/acsbomaterials.8b00778
33. Baghban SA, Ebrahimi M, Bagheri-Khoulenjani S, et al. A highly efficient microwave-assisted synthesis of an LED-curable methacrylated gelatin for bio applications. *RSC Adv.* 2021;11(25):14996-15009. doi:10.1039/d1ra01269j
34. Nguyen AK, Goering PL, Reipa V, et al. Toxicity and photosensitizing assessment of gelatin methacryloyl-based hydrogels photoinitiated with lithium phenyl-2,4,6-trimethylbenzoylphosphinate in human primary renal proximal tubule epithelial cells. *Biointerphases.* 2019;14(2):021007. doi:10.1116/1.5095886
35. Monteiro N, Thirivikraman G, Athirasala A, et al. Photopolymerization of cell-laden gelatin methacryloyl hydrogels using a dental curing light for regenerative dentistry. *Dent Mater.* 2018;34(3):389-399. doi:10.1016/j.dental.2017.11.020
36. Claaßen C, Claaßen MH, Truffault V, et al. Quantification of substitution of gelatin methacryloyl: best practice and current pitfalls. *Biomacromolecules.* 2018;19(1):42-52. doi:10.1021/acs.biomac.7b01221
37. Cristea DD, Liciu E, Trifan A, et al. Volumetric control vs. pneumatic pressure: a comparative analysis of extrusion in 3D bioprinting. *Micromachines (Basel).* 2026;17(5):521. doi:10.3390/mi17050521

38. Ouyang L, Yao R, Zhao Y, et al. Effect of bioink properties on printability and cell viability for 3D bioplotting of embryonic stem cells. *Biofabrication*. 2016;8(3):035020. doi:10.1088/1758-5090/8/3/035020
39. Darwish AMG, Khalifa RE, El-Sohaimy SA. Functional properties of chia seed mucilage supplemented in low fat yoghurt. *Alex Sci Exch J*. 2018;39(3):450-459. doi:10.21608/asejaiqjsae.2018.13882
40. Kaberova Z, Karpushkin E, Nevoralová M, et al. Microscopic structure of swollen hydrogels by scanning electron and light microscopies: artifacts and reality. *Polymers (Basel)*. 2020;12(3):578. doi:10.3390/polym12030578
41. Aigoin J, Payré B, Minvielle Moncla J, et al. Comparative analysis of electron microscopy techniques for hydrogel microarchitecture characterization: SEM, cryo-SEM, ESEM, and TEM. *ACS Omega*. 2025;10(15):14687-14698. doi:10.1021/acsomega.4c08096
42. Siqueira G, Kokkinis D, Libanori R, et al. Cellulose nanocrystal inks for 3D printing of textured cellular architectures. *Adv Funct Mater*. 2017;27(12):1604619. doi:10.1002/adfm.201604619
43. Hausmann MK, Rühls PA, Siqueira G, et al. Dynamics of cellulose nanocrystal alignment during 3D printing. *ACS Nano*. 2018;12(7):6926-6937. doi:10.1021/acsnano.8b02366
44. Nerger BA, Brun PT, Nelson CM. Microextrusion printing cell-laden networks of type I collagen with patterned fiber alignment and geometry. *Soft Matter*. 2019;15(28):5728-5738. doi:10.1039/c8sm02605j
45. Gao F, Xu Z, Liang Q, et al. Osteochondral regeneration with 3D-printed biodegradable high-strength supramolecular polymer reinforced-gelatin hydrogel scaffolds. *Adv Sci (Weinh)*. 2019;6(15):1900867. doi:10.1002/advs.201900867
46. Broedersz CP, Mao X, Lubensky TC, MacKintosh FC. Criticality and isostaticity in fibre networks. *Nat Phys*. 2011;7(12):983-988. doi:10.1038/nphys2127
47. Broedersz CP, MacKintosh FC. Modeling semiflexible polymer networks. *Rev Mod Phys*. 2014;86(3):995-1036. doi:10.1103/RevModPhys.86.995
48. Sharma A, Licup AJ, Jansen KA, et al. Strain-controlled criticality governs the nonlinear mechanics of fibre networks. *Nat Phys*. 2016;12(6):584-587. doi:10.1038/nphys3628
49. Azizi Samir MAS, Alloin F, Dufresne A. Review of recent research into cellulosic whiskers, their properties and their application in nanocomposite field. *Biomacromolecules*. 2005;6(2):612-626. doi:10.1021/bm0493685
50. Habibi Y, Lucia LA, Rojas OJ. Cellulose nanocrystals: chemistry, self-assembly, and applications. *Chem Rev*. 2010;110(6):3479-3500. doi:10.1021/cr900339w
51. Capadona JR, Shanmuganathan K, Tyler DJ, Rowan SJ, Weder C. Stimuli-responsive polymer nanocomposites inspired by the sea cucumber dermis. *Science*. 2008;319(5868):1370-1374. doi:10.1126/science.1153307
52. Duan Y, Zhao Y, Ai S, et al. Programmable processing toward stiff composite hydrogels. *Macromolecules*. 2022;55(16):7071-7079. doi:10.1021/acs.macromol.2c00852
53. Li K, Wei P, Huang J, et al. Mechanically strong shape-memory and solvent-resistant double-network polyurethane/nanoporous cellulose gel nanocomposites. *ACS Sustain Chem Eng*. 2019;7(19):15974-15982. doi:10.1021/acssuschemeng.9b02341

54. Mouser VHM, Melchels FPW, Visser J, et al. Yield stress determines bioprintability of hydrogels based on gelatin-methacryloyl and gellan gum for cartilage bioprinting. *Biofabrication*. 2016;8(3):035003. doi:10.1088/1758-5090/8/3/035003
55. Cooke ME, Rosenzweig DH. The rheology of direct and suspended extrusion bioprinting. *APL Bioeng*. 2021;5(1):011502. doi:10.1063/5.0031475
56. Pasanaphong K, Pukasamsombut D, Boonyagul S, et al. Fabrication of fish scale-based gelatin methacryloyl for 3D bioprinting application. *Polymers (Basel)*. 2024;16(3):418. doi:10.3390/polym16030418
57. Fairbanks BD, Schwartz MP, Bowman CN, Anseth KS. Photoinitiated polymerization of PEG-diacrylate with lithium phenyl-2,4,6-trimethylbenzoylphosphinate: polymerization rate and cytocompatibility. *Biomaterials*. 2009;30(35):6702-6707. doi:10.1016/j.biomaterials.2009.08.055
58. Nguyen AK, Goering PL, Elespuru RK, Sarkar Das S, Narayan RJ. The photoinitiator lithium phenyl (2,4,6-trimethylbenzoyl) phosphinate with exposure to 405 nm light is cytotoxic to mammalian cells but not mutagenic in bacterial reverse mutation assays. *Polymers (Basel)*. 2020;12(7):1489. doi:10.3390/polym12071489

Appendix

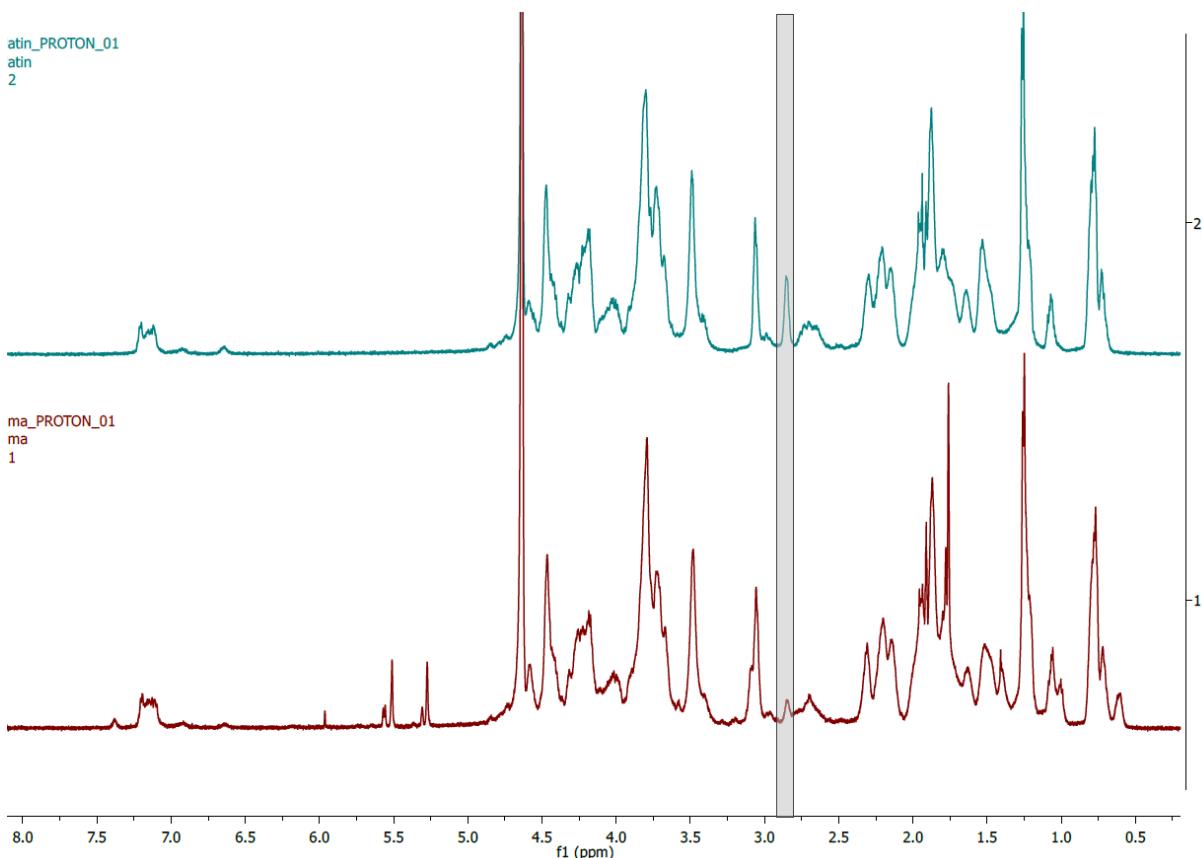


Figure A1. Determination of the degree of methacrylation of microwave-synthesized GelMA by ^1H NMR (400 MHz, D_2O , room temperature). Overlaid spectra of unmodified gelatin (upper trace) and microwave-synthesized GelMA (lower trace). The shaded band at $\delta \approx 2.9$ ppm marks the lysine and hydroxylysine methylene resonance, which is strongly depleted in GelMA relative to gelatin; integration of this region against the aromatic protons of phenylalanine and tyrosine (δ 6.6–7.6 ppm) as internal reference gave peak areas of 1461.31 and 264.25 respectively, corresponding to DM = 81.92% by Equation I. The new sharp resonances at $\delta \approx 5.3$ and 5.5 ppm in the GelMA trace are the methacryloyl vinyl protons, confirming grafting; these signals lie close to the residual HOD resonance at $\delta \approx 4.7$ ppm and were not used for quantification.

$$\text{DM (\%)} = [1 - (\text{lysine methylene peak area of GelMA} / \text{lysine methylene peak area of gelatin})] \times 100$$

$$\text{DM (\%)} = [1 - (264.25 / 1461.31)] \times 100 = 81.92\%$$

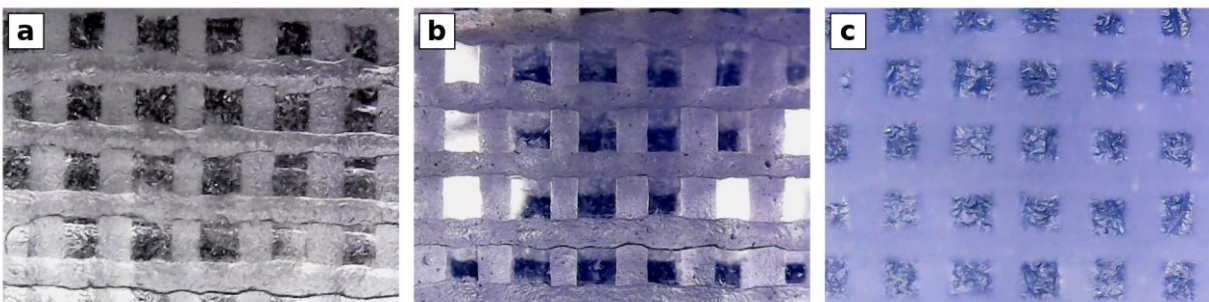


Figure A2. Multi-layer lattice constructs printed with the biomaterial inks. Four-layer square-pore lattices printed with (a) 10% GelMA, (b) GelMA-1% Chia and (c) GelMA-2% Chia at the optimal pressure–speed setting of each formulation identified in Figure 6 (3 psi / 20 V for 10% GelMA; 5 psi / 15 V for the 1% and 2% blends), through the 22-gauge conical nozzle (410 μm internal diameter) with the print head at 30 $^{\circ}\text{C}$, and photo-crosslinked after printing. Images were recorded on a USB digital microscope; no scale bar is available, and the strand width at each setting is given in Table 4. The images are presented as a qualitative demonstration that the formulations stack in successive layers with retained pore geometry and interlayer adhesion; they were not acquired for quantitative analysis, and no pore-area, line-merging, or printability-index metrics were derived from them.