

Agarose and Methacrylated Agarose in Photocurable Biofabrication: Algal Identity, Gelation Hysteresis, Derivatisation, Microgel Photoannealing, Support Baths, Network Attribution and Qualification

Juan Seguro

3Dresyns by Resyner Technologies S.L.

Registered tax address: General Etxague 15B, 20003 Donostia / San Sebastian, Gipuzkoa, Spain

VAT number: ESB24827511

Correspondence: info@3Dresyns.com · Website: www.3Dresyns.com

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Statement of Significance

Agarose can be the thermoreversible matrix, a methacrylated covalent participant, a jammed microgel ink, a removable support bath or a native additive inside another photocurable network. These roles are not interchangeable. Algal source, agarose purity, sulfate and agaropectin burden, molar mass, gelation-melting hysteresis, low-melting modification, derivatisation and article state must precede any claim of photocure or biofabrication performance.

Keywords: agarose; methacrylated agarose; thermogelation; microgel; photoannealing; GelMA; embedded printing; support bath; network attribution; qualification

Abstract

Agarose-enabled fabrication includes methacrylate-modified agarose hydrogels, photoannealable agarose microgels, native agarose used to pregel extrusion inks, agarose support baths for GelMA, and GelMA-agarose composites; the initiation chemistry of the methacrylate-modified route is not established in this corpus. De Paepe et al. established methacrylate-modified agarose. Mukundan et al. converted thermogelling agarose into a jammed microgel bioink with a photoannealing step. Gong et al., Yang et al. and Mokhtarzade et al. instead illustrate physical-matrix, support-bath and partner-owned photocure roles. This review makes polymer identity, thermal history, network owner and article state explicit.

1. Scope and boundaries

ER-136 owns material-specific identity and qualification of agarose and methacrylated agarose in photocurable biofabrication. It covers algal and recovery state, agaropectin and sulfate burden, molar mass, gelation and melting temperatures, derivatisation, microgels, extrusion and embedded printing, photoconversion, washing, hydration, mechanics and biological boundaries.

ER-06 retains biological-response methodology; ER-10 geometric metrology; ER-15 degradation and mechanical evolution; ER-27 conversion; ER-33 washing and drying; ER-67 network architecture; ER-86 extractables; ER-100 corpus architecture; ER-121 methacrylated silk fibroin; and ER-123 to ER-135 neighbouring photocurable polysaccharides. [C1,C2,C3,C4,C5,C6,C7,C8,C9,C10,C11,C12,C13,C14,C15,C16,C17,C18,C19,C20,C21,C22].

General AM characteristics and geometric test artefacts should follow the declared ISO/ASTM standards [S1,S3].

Agar, agarose, low-melting agarose and methacrylated agarose are not synonyms. Thermal gelation, mechanical jamming and support-bath recovery are not photoconversion. In a GelMA-agarose composite, GelMA owns the named radical cure unless agarose functionality and conversion are demonstrated. A removable agarose bath is process tooling, not necessarily part of the released article.

2. Controlled agarose-enabled photofabrication state chain

State	Minimum identity	Failure if omitted
Incoming agarose	Algal/recovery source, grade/lot, agaropectin/sulfate burden, molar mass, ash, moisture, microbial state and storage	Agarose name replaces feedstock identity
Thermogel, derivative or microgel	Role declaration; thermal history or reaction/purification, measured substitution, residuals, particle size/packing and storage	Physical gel, particle and covalent precursor are conflated

State	Minimum identity	Failure if omitted
Ink or support bath	Agarose role, partners/cells, solids, salts, initiator, temperatures, rheology, optical state, shear history and age	Network ownership and process tooling disappear
Article	Extrusion/light process, bath removal, post-cure, wash, conversion, soluble fraction, residuals, hydrated dimensions, mechanics and biology	Deposited, cured, washed and hydrated states are conflated

Table 1. Minimum state chain for agarose-enabled photocurable constructs.

This material and article chain is separate from process and production-site qualification under ISO/ASTM 52920:2023 [S2].

3. Primary evidence and controlled interpretation

3.1 Algal, purification and thermal-gel state precede formulation

Agarose is the less-sulfated gelling fraction isolated from red-algal agar. Species and harvest, extraction and purification, residual agarpectin and sulfate, pyruvate and ash, molar-mass distribution, moisture, microbial state and storage affect helix association and gel strength. Concentration, heating and dissolution, cooling rate, hold time and repeated thermal cycles govern gelation-melting hysteresis. Commercial grade or nominal concentration alone is not feedstock or thermal-history identity.

3.2 Methacrylate-modified agarose creates a distinct covalent precursor

De Paepe et al. reported hydrogels based on methacrylate-modified agarose [1]. The published title, authors and bibliography are established; the initiation chemistry is not, and this review therefore does not use [1] as evidence of photocure. The paper nevertheless establishes agarose derivatisation as a distinct literature route. Parent grade, reaction and purification, measured substitution, residual reagents, initiator, cure conditions and hydrated conditioning must accompany any future comparison with thermally gelled agarose.

3.3 Photoannealable agarose microgels add particle and interparticle states

Mukundan et al. described photoannealable agarose microgels for jammed-microgel printing [2]. Bulk agarose identity, microgel production and size distribution, packing fraction, interparticle chemistry, initiator and light treatment jointly define the printed material. Yield behaviour before irradiation and article cohesion after irradiation are separate measurements. Keyword indexing identifies agarose methacrylate and photo-annealing, but the accessible abstract does not report derivatisation degree, initiator or interparticle mechanism; no specific claim about those variables is made here.

3.4 Native agarose can pregel an ink without owning a photocure

Gong et al. used agarose physical crosslinking in gelatin-alginate-agarose inks to increase formation accuracy and stability at the tested temperatures [3]. Alginate was subsequently stabilised ionically with CaCl₂ and gelatin enzymatically with transglutaminase. The study contains no photochemical step of any kind; the evidence supports a thermal pregelation role, not methacrylate conversion. Agarose concentration, dissolution temperature, printing temperature, nozzle, alginate ionic treatment and gelatin crosslinking must remain attached to the observed print and cell results.

3.5 Agarose support baths and GelMA composites retain role ownership

Yang et al. printed low-viscosity GelMA inside an agarose-microgel suspension bath and irradiated the GelMA during deposition [4]. The bath supplied yield support and was rinsed from the article. Mokhtarzade et al. instead retained agarose within layered GelMA-agarose constructs [5]. In the first architecture agarose is process tooling; in the second it is a physical composite component, while GelMA owns the named photocure. Results cannot transfer between these architectures without rebuilding mass balance, conversion and article-state evidence.

4. Thermal, rheological and optical-process control

Agarose grade, concentration, dissolution and cooling history, microgel size and packing, partner macromers, cells and initiator define the precursor. Cartridge and bath temperatures, ageing, shear history, nozzle and pressure define extrusion. Spectrum, irradiance, exposure, oxygen, depth and geometry define photoconversion. Washing determines whether bath or soluble agarose remains in the article.

No-agarose, native-agarose, methacrylated-agarose, dark, initiator-light, no-photoactive-partner and complete-formulation controls are required. Matched thermal histories separate physical helix networks, microgel jamming, interparticle photoannealing and partner-macromer conversion. Without these controls, shape retention and mechanics cannot be assigned to a network owner.

5. Washing, hydration, degradation and biological boundaries

Evidence block	What it can show	What it cannot establish alone
Feedstock/derivative identity	Method-specific purity, sulfate, molar-mass, substitution, residual and particle state	Photoconversion or article performance
Thermal/rheology/printing	Grade-, concentration-, temperature-, shear-, nozzle-, bath- and geometry-specific deposition	Covalent network ownership or transferable settings
Conversion/article state	Agarose/partner conversion, bath removal, soluble fraction, hydration, dimensions and mechanics	Universal degradation or service life
Biological model	Response of the exact gel, microgel ink, bath-exposed print or composite in the stated model	General safety, clinical efficacy or regulatory approval

Table 2. Evidence blocks that must remain separate for agarose-enabled photofabrication.

ISO 10993-12:2021/Amd 1:2025 and ISO 10993-18:2020/Amd 1:2022 anchor sample preparation and chemical characterisation [S4,S5]. ISO 10993-1:2025 frames biological safety within risk management [S6]. Cell viability in one bulk gel, microgel ink, support-bath print or layered composite does not establish universal biocompatibility, therapeutic efficacy or regulatory approval.

6. Release matrix

Gate	Minimum evidence	Claim withheld
Parent identity	Algal/recovery source, lot, agarosectin/sulfate burden, molar mass, ash, moisture, microbial state and storage	Generic agarose equivalence
Derivative/particle state	Reaction/purification, measured substitution, residuals, microgel production, size/packing, stability and storage	Acronym or microgel name as chemical identity
Role attribution	No-agarose, native/modified agarose, no-partner, dark, initiator-light and complete controls plus conversion	Unverified thermal, support or covalent ownership
Process	Complete formulation, thermal/rheology/age state, hardware, bath, spectrum, irradiance, exposure and geometry	Transferable deposition or cure settings
Article state	Green, bath-removed, post-cured, washed, hydrated and dried x/y/z, mass, soluble fraction, residuals and mechanics	Unqualified composition, dimensions or stability
Biological boundary	Exact roles, thermal/print/light/bath stress, wash/extract, model, dose, endpoint, duration and statistics	Universal biocompatibility or clinical suitability

Table 3. Minimum release architecture for agarose-enabled photocurable constructs.

7. Failure-mode interpretation

- The nominally same agarose gels at a different temperature: verify source/grade, sulfate and agarosectin burden, molar mass, moisture, concentration, dissolution, cooling and thermal cycles before changing dose.
- A jammed microgel ink prints but lacks post-wash cohesion: separate particle packing and yield stress from interparticle conversion, soluble fraction and swollen-article mechanics.
- GelMA prints well in an agarose bath: quantify bath rheology, drag, recovery and removal independently from GelMA conversion and article fidelity.
- A GelMA-agarose composite is stiffer or degrades differently: use matched GelMA-only and agarose-only controls and retain formulation, cure, wash and hydration state before assigning causation.
- Agarose remains after bath removal: measure wash mass balance and chemical residuals; process tooling and article composition must not be inferred from the printing setup.
- A cell-laden construct remains viable: retain cell source, print and bath exposure, temperature history, light dose, wash, culture duration, controls and endpoint; withhold clinical claims.

8. Minimum workflow and research priorities

- Freeze algal source and recovery where available, grade and lot, residual agarosectin/sulfate, molar-mass distribution, ash, moisture, microbial state, storage, dissolution and thermal-cycle history.
- For methacrylated agarose or photoannealable microgels, report reaction and purification, measured substitution, residuals, particle production, size distribution, packing, annealing chemistry and storage.
- Map native or modified agarose, partner macromers, cells, initiator and salts, plus temperature, viscosity, yield stress, recovery, ageing, optical attenuation and conversion over the working interval.
- Record printer, nozzle, pressure or flow, speed, layer, cartridge and bath temperature, geometry, spectrum, irradiance, exposure, oxygen, post-cure, bath removal and wash.

- Measure agarose- and partner-specific conversion where applicable, insoluble and soluble fractions, residual initiator and derivatisation reagents, bath carryover and x/y/z dimensions through article states.
- Use no-agarose, native-agarose, modified-agarose, dark, initiator-light, no-partner and complete controls to assign thermogelation, jamming, deposition, interparticle annealing and covalent cure.
- Test swelling, dissolution, mass loss and mechanical retention in defined media over time; distinguish agarose release, partner degradation, interparticle separation and covalent-network cleavage.
- Separate temperature, extrusion and curing injury, bath exposure, extract cytotoxicity, proliferation and tissue models from safety, efficacy and regulatory conclusions.

9. Conclusions

Agarose-enabled photocurable materials are controlled algal-feedstock, purification, thermal, derivative, particle, formulation, support-bath, optical-process, washing and article states.

Primary evidence supports methacrylate-modified agarose, photoannealable agarose microgels, native agarose as a thermal pregel or support bath, and GelMA-agarose composites. Those roles are not interchangeable.

Release requires traceability through source, grade, thermal history, derivative, substitution, particle state, formulation role, process, conversion, bath removal, washing, soluble fraction, residuals, hydration, dimensions, mechanics and biological model.

Limitations

This paper is a qualitative engineering review, not a systematic meta-analysis. The cited studies use different agarose identities, roles, derivatives, partner networks, printing processes, thermal histories, article states and biological models. Numerical values are study-specific evidence, not universal design values.

Author contributions

Juan Seguro: conceptualisation, evidence synthesis, boundary definition, engineering interpretation, manuscript preparation and final author verification.

Competing interests

The author is founder and CEO of 3Dresyns by Resyner Technologies S.L. No comparative product recommendation is made.

Data availability

No new experimental dataset was generated. Scientific statements derive from the cited primary literature and official standards. The review package includes QA and source-boundary controls.

Company information

3Dresyns by Resyner Technologies S.L. | General Etxague 15B, 20003 Donostia / San Sebastian, Gipuzkoa, Spain | VAT ESB24827511 | info@3Dresyns.com | www.3Dresyns.com

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