

Gelatin–Norbornene Thiol–Ene Bioinks: Macromer Identity, Stoichiometry, Oxygen Response and Cell-Compatible Cure

Step-growth photocrosslinking as an alternative network architecture for gelatin biofabrication

Juan Seguro

3Dresyns by Resyner Technologies S.L.

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

ER-326 · Engineering Review · Version 0.4 · 28 September 2026

Abstract

Gelatin-norbornene (GelNB) bioinks use thiol-ene step-growth photochemistry to separate the gelatin backbone from the choice of thiolated crosslinker. This architecture offers rapid, controllable gelation and is less sensitive to oxygen than conventional chain-growth methacrylate curing, but it introduces a different set of state variables: degree of norbornene substitution, thiol functionality, thiol-to-ene stoichiometry, crosslinker molecular size and photoinitiating conditions. This review treats GelNB as a family of stoichiometrically designed networks rather than a direct substitute for GelMA. It examines dithiothreitol and macromolecular thiol crosslinkers such as thiolated heparin, oxygen response, radical burden, network homogeneity and cell compatibility. It also emphasises that oxygen tolerance does not eliminate the need to characterise conversion or cytocompatibility. A qualification framework is proposed that links functional-group balance and light dose to mechanics, swelling, residual groups, cell response and printed fidelity.

Keywords: gelatin norbornene; GelNB; thiol-ene; stoichiometry; oxygen tolerance; bioink; photocrosslinking

1. Significance and scope

GelNB combines gelatin-derived biological motifs with norbornene functionality that reacts with thiols through light-triggered thiol-ene chemistry. GelNB systems have been used for high-definition and extrusion bioprinting, with reports of rapid crosslinking and reduced reactive oxygen burden relative to GelMA under studied conditions [1-4].

1.1 Corpus boundary

ER-326 owns gelatin-norbornene thiol-ene bioinks: GelNB identity, thiol source and stoichiometry, oxygen response, dose and cell-compatible cure. ER-323 retains PEG-based network architecture, including PEG-norbornene when the question is PEG mesh design rather than GelNB chemistry. ER-322 retains GelMA, ER-325 retains elastin- and tropoelastin-derived photocurable materials, and ER-327 retains sequence-defined recombinant proteins.

2. Macromer identity and substitution

Degree of norbornene functionalisation determines ene density. Gelatin source still matters, so GelNB identity requires both the gelatin precursor and its substitution state. A high nominal polymer concentration with low substitution can have fewer reactive groups than a lower concentration with higher substitution. [1,2]

3. Crosslinker identity

Small-molecule dithiols and macromolecular thiols produce different network structures and biological environments. Thiolated heparin has been used as a multifunctional crosslinker that also provides growth-factor-binding capability [1]. The crosslinker is therefore part of both the mechanical and biological specification.

4. Stoichiometry and residual functionality

Thiol-ene step growth is sensitive to the balance of reactive groups. Deviations from equivalence can leave residual thiol or norbornene groups and reduce effective connectivity. Stoichiometric ratio should be reported in functional-group equivalents rather than only as mass concentration. [1-3]

5. Oxygen response and photochemical burden

Thiol-ene systems are generally less inhibited by oxygen than acrylate chain-growth curing because chain-transfer and propagation mechanisms differ. Recent GelNB work explicitly describes rapid oxygen-insensitive crosslinking [3]. This advantage should be verified under the actual geometry, because oxygen transport, dose and photoinitiator still influence radicals and cell exposure.

6. Cell-compatible cure

Cell compatibility depends on the full system, including initiator, thiol chemistry, dose, osmolarity and residual reagents. A comparison between GelNB and GelMA is meaningful only when conversion and final mechanics are matched or explicitly reported. Higher viability at lower conversion is not an equivalent-network comparison. [2,5]

7. Qualification framework

Minimum reporting includes gelatin source, norbornene substitution, thiol crosslinker identity and substitution, thiol:ene equivalents, photoinitiator, spectrum, dose, conversion or gel fraction, swelling, modulus and cell assay. These parameters define the GelNB network more directly than polymer percentage alone. [1-5]

7.1 Evidence hierarchy and transfer

GelNB evidence must specify norbornene substitution, thiol identity and functionality, thiol:norbornene stoichiometry, initiator/light conditions and oxygen state. These variables jointly determine gelation and network defects, so a result cannot be transferred from 'GelNB concentration' alone [1-5].

7.2 Research priorities

Priority experiments should measure residual functional groups and network properties across stoichiometric imbalance, compare macromolecular and small-molecule thiols, and quantify whether oxygen tolerance persists at the relevant cell density and geometry. Recent interfacial-thiol strategies should be evaluated for spatial uniformity as well as viability [1-3].

7.3 Coupled state variables

Thiol-ene step-growth chemistry changes the usual relation between oxygen and cure, but network quality still depends on functional-group balance and transport. A formulation can gel rapidly while retaining unreacted groups or spatially heterogeneous crosslink density if thiol delivery is nonuniform [1,3,4].

7.4 Decision logic for qualification

Qualification should therefore combine stoichiometric accounting, a conversion or residual-group measurement, mechanical/transport testing and the intended cell endpoint. Fast gelation by itself is not evidence of a uniform or cell-compatible network [1-5].

Table 1. Minimum qualification matrix for gelatin-norbornene thiol-ene bioinks.

Variable	Engineering role	Minimum report
GelNB substitution	ene density	analytical substitution level
Crosslinker functionality	network connectivity	thiol identity and functionality
Thiol:ene ratio	stoichiometric balance	functional equivalents
Oxygen condition	surface/depth cure sensitivity	air/inert state and geometry
Light field	radical generation and conversion	spectrum, irradiance, dose
Cell assay	cytocompatibility	cell type, timing and endpoint

8. Interpretation matrix

GelNB troubleshooting should first test thiol:norbornene balance and thiol delivery before attributing a defect to oxygen or irradiance. The same weak region can reflect local thiol depletion, optical underdose or an off-stoichiometric precursor state [1-4].

Table 2. Diagnostic interpretation matrix for gelatin-norbornene thiol-ene bioinks.

Observation	Interpretation	Control
Fast gel but low modulus	low connectivity or off-stoichiometry	functional-group balance and gel fraction
Surface and bulk cure similarly in air	oxygen inhibition is limited under test	repeat at controlled oxygen and depth
Macromolecular thiol improves biology	specific biochemical effect or altered mechanics	mechanics-matched crosslinker control
Good viability but residual thiol high	under-conversion/off-stoichiometry	chemical conversion assay
Different print fidelity at same wt%	substitution or crosslinker differs	reactive-equivalent normalisation

9. Limitations and evidence gaps

Published GelNB studies use different norbornene substitution levels, thiol crosslinkers, photoinitiators and printing modalities. Direct comparisons of oxygen sensitivity and dose are therefore conditional. Quantitative residual-group maps and long-term network homogeneity data remain limited [1-5].

10. Conclusions

Gelatin-norbornene bioinks should be qualified as stoichiometrically defined thiol-ene networks. Their attraction is rapid, comparatively oxygen-tolerant gelation, but reproducibility still depends on macromer identity, thiol delivery, functional-group balance and spatially adequate dose [1-5].

References

1. Zhao C, Wu Z, Chu H, Wang T, Qiu S, Zhou J, Zhu Q, Liu X, Quan D, Bai Y. Thiol-Rich Multifunctional Macromolecular Crosslinker for Gelatin-Norbornene-Based Bioprinting. *Biomacromolecules*. 2021;22(6):2729-2739. doi:10.1021/acs.biomac.1c00421.
2. Dobos A, Van Hoorick J, Steiger W, Gruber P, Markovic M, Andriotis OG, Rohatschek A, Dubruel P, Thurner PJ, Van Vlierberghe S, Baudis S, Ovsianikov A. Thiol-Gelatin-Norbornene Bioink for Laser-Based High-Definition Bioprinting. *Advanced Healthcare Materials*. 2020;9(15):e1900752. doi:10.1002/adhm.201900752.
3. Prasetyaningrum PW, Sakai S. Cell-Friendly 3D Bioprinting of Gelatin-Norbornene Bioink via Thiol-Ene Crosslinking Enabled by Interfacial DTT Delivery from Alternating Support Layers. *ACS Biomaterials Science & Engineering*. 2026; online ahead of print. doi:10.1021/acsbiomaterials.6c00677.
4. Lim KS, Galarraga JH, Cui X, Lindberg GCJ, Burdick JA, Woodfield TBF. Fundamentals and Applications of Photo-Cross-Linking in Bioprinting. *Chemical Reviews*. 2020;120(19):10637-10669. doi:10.1021/acs.chemrev.9b00812.
5. Lai Y, Xiao X, Huang Z, Duan H, Yang L, Yang Y, Li C, Feng L. Photocrosslinkable Biomaterials for 3D Bioprinting: Mechanisms, Recent Advances, and Future Prospects. *International Journal of Molecular Sciences*. 2024;25(23):12567. doi:10.3390/ijms252312567.

Competing interests

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

Data availability

No new experimental dataset was generated for this review. The analysis is based on the cited literature and the stated engineering scope.