

Living Biosensing Materials Made by Photopolymerisation: Recognition, Signal Coupling, Containment and Calibration

Qualification of genetically encoded recognition and measurable output in living printed biosensors

Juan Seguro

3Dresyns by Resyner Technologies S.L.

© 2026 Juan Seguro. All Rights Reserved.

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

ER-436 · Engineering Review · Version 0.1 · 8 October 2026

Abstract

Living biosensing materials combine viable engineered cells with a shaped matrix that transports analyte, confines the organism and couples biological recognition to an observable signal. A useful sensor therefore requires more than viability or reporter expression: analyte access, response function, selectivity, containment, calibration, storage and drift must all be demonstrated. This review distinguishes biosensor transfer functions from general living-material viability and defines minimum qualification for response time, dynamic range, matrix effects and biological stability.

Keywords: living biosensor; engineered bacteria; photopolymer; hydrogel; calibration; containment; response time; drift

1. Significance and scope

A living biosensor is a material-device system in which cells perform recognition and signal generation while the matrix provides geometry, transport and containment. Usai and co-workers demonstrated bioprinted bacterial materials with genetically encoded sensing circuits, multiplexed configurations and field-relevant testing [1]. Their results illustrate that sensing performance must be measured as an input-output function, not inferred from reporter capability in planktonic culture.

The sensor should be decomposed into recognition, biological processing, reporter generation, transport through the matrix and external readout. A change in any one of those stages can alter the apparent sensitivity. The matrix is therefore part of the signal chain, not merely a containment material, and its thickness, porosity and optical or electrochemical background should be included in calibration.

2. Recognition and matrix transport are coupled

The analyte must reach the cells before the genetic or metabolic circuit can respond. Crosslink density, thickness and binding to the matrix can delay or attenuate the effective input. Calibration should therefore be performed on the final material geometry.

A circuit calibrated in liquid culture cannot simply transfer its detection limit or response time to an encapsulated format. Selectivity testing should use plausible interferents at declared concentrations rather than only a clean buffer. For whole-cell sensors, nutrient state and growth phase can change baseline expression and gain.

If calibration is performed immediately after fabrication but service occurs after storage or repeated exposure, the transfer function should be re-measured at those later states. A quantitative sensor should also state its operating decision rule. Threshold classification, continuous concentration estimation and qualitative presence/absence sensing require different validation.

For threshold devices, false-positive and false-negative rates near the decision boundary matter more than the brightest reporter output. For concentration measurements, calibration residuals and uncertainty across the useful range should be reported rather than only a coefficient of determination.

3. Signal coupling sets the observable metric

Fluorescence, colour, luminescence, electrical output or secreted products each impose different transport and instrumentation constraints. Optical readout can be affected by matrix absorbance, scattering and growth. The baseline, dynamic range and saturation response should be recorded under the same illumination and imaging configuration used for service.

Signal coupling can be limited by analyte uptake before the genetic circuit is limiting. Response time should therefore be reported against geometry and analyte transport, and a thin-reference or planktonic control can help separate matrix delay from cellular circuit delay. Optical reporters additionally require correction for matrix absorbance, scattering and cell density when quantitative intensity is used.

4. Containment and function must coexist

Bhusari and co-workers demonstrated a bilayer hydrogel architecture that retained bacteria while preserving lactate sensing, showing that physical confinement can be compatible with function when the transport window is designed appropriately [2]. Containment should be challenged over the full sensing interval and after storage or deformation. Containment qualification should be coupled to sensor performance over the same interval. Tang and co-workers independently demonstrated hydrogel-based physical biocontainment that preserved bacterial sensing and communication while challenging environmental escape, reinforcing that containment should be measured as a device function rather than assumed from encapsulation [3].

A shell or tighter network that reduces escape can also slow analyte transport and change calibration. Bhusari and co-workers provide a useful demonstration of this coupled requirement: confinement and sensing were evaluated together rather than as independent claims [2]. Genetic containment and physical containment should be reported separately.

An auxotrophy or kill switch can reduce the consequence of escape but does not demonstrate that escape is physically prevented; conversely, a strong physical barrier does not prove genetic stability. If both mechanisms are credited, each should have its own challenge test and failure criterion.

5. Calibration includes biological drift

Living sensors can change through growth, mutation, plasmid loss and metabolic adaptation. A calibration curve is therefore time-stamped. Repeated calibration or internal controls may be needed for long-term devices.

The fraction of non-responding or escaped cells should not be hidden by averaging a strong population-level reporter signal. Calibration drift can arise from cell proliferation, mutation, metabolic adaptation, reporter accumulation and matrix ageing. Repeated standards or reference channels are therefore valuable.

A detection limit reported from the initial day cannot be assumed to hold after prolonged storage or repeated sensing cycles unless the calibration is shown to remain stable.

Axis	Measurement	Failure mode
Recognition	input range and interferents	reporter activation mistaken for specificity
Transport	geometry and analyte uptake/lag	liquid-culture calibration transferred unchanged
Readout	baseline, dynamic range, instrument settings	matrix optics alter apparent signal
Containment	escape after storage/use	sensor works but releases organism
Drift	repeat calibration over time	single-day curve treated as lifetime calibration

Table 1. Domain-specific qualification matrix for living biosensing materials made by photopolymerisation.

6. Device-level verification

Test the final construct against a concentration series, interferents, blanks and recovery cycles where applicable. Report lag time, time to stable output, dynamic range, limit of detection only if the method supports it, and hysteresis or memory. Storage conditions and reactivation protocol are part of the sensor specification.

ER-431 owns the general viability-containment-transport problem for bacterial living materials. ER-436 begins where a quantitative sensing claim is made: recognition specificity, transfer function, detection limit, response time and calibration stability are its defining outputs. Device-level testing should include a calibration blank containing the same matrix and readout components but no sensing circuit, plus a biological control that lacks the recognition element where feasible.

These controls separate matrix background, constitutive reporter drift and true analyte-dependent response. For field use, temperature and ionic-strength variation should be included because both can change transport and gene-expression kinetics.

7. Minimum qualification framework

Report organism and circuit, matrix and cure history, geometry, analyte transport condition, calibration curve, selectivity controls, response time, signal normalization, containment, viability, storage stability and drift. A qualitative colour change can support a threshold sensor claim but not a quantitative concentration claim without a calibrated transfer function. The minimum dataset should report calibration model, dynamic range, detection and quantification limits where appropriate, response and recovery time, selectivity panel, storage time, number of sensing cycles, containment result and failure rate across replicate devices.

A single successful trace should not define sensitivity. Batch-to-batch variation in cell state and printing geometry should be propagated into confidence intervals or acceptance bands for the sensor output.

8. Engineering conclusions

Living biosensing materials should be qualified as sensors with a biological recognition layer, not as living hydrogels that happen to change colour. The engineering challenge is to preserve a stable relationship between external analyte concentration and measured output while the cells remain contained and biologically variable. Calibration, containment and drift are therefore co-equal with viability.

References

- [1] F. Usai, G. Loi, F. Scocozza, M. Bellato, I. Castagliuolo, M. Conti, L. Pasotti, “Design and biofabrication of bacterial living materials with robust and multiplexed biosensing capabilities,” *Materials Today Bio* 18 (2023) 100526. <https://doi.org/10.1016/j.mtbio.2022.100526>
- [2] S. Bhusari, J. Kim, K. Polizzi, S. Sankaran, A. del Campo, “Encapsulation of bacteria in bilayer Pluronic thin film hydrogels: A safe format for engineered living materials,” *Biomaterials Advances* 145 (2023) 213240. <https://doi.org/10.1016/j.bioadv.2022.213240>
- [3] T.-C. Tang, E. Tham, X. Liu, K. Yehl, A. J. Rovner, H. Yuk, C. de la Fuente-Nunez, F. J. Isaacs, X. Zhao, T. K. Lu, “Hydrogel-based biocontainment of bacteria for continuous sensing and computation,” *Nature Chemical Biology* 17 (2021) 724-731. <https://doi.org/10.1038/s41589-021-00779-6>

Competing interest

Juan Segurola is founder, CEO and CTO of 3Dresyns by Resyner Technologies S.L., which develops and commercialises photopolymer materials and related additive-manufacturing technologies. This review is an engineering analysis and does not constitute a product-performance claim or a device qualification.