

Living Bacterial Materials Integrated with Photocurable Matrices: Viability, Containment, Transport and Function

Engineering viability, confinement and mass-transfer windows for bacterial engineered living materials

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Abstract

Living bacterial materials combine a non-living matrix with metabolically active microorganisms. In photocurable systems the central engineering problem is not simply whether bacteria survive cure, but whether viability, containment, nutrient and analyte transport, mechanical integrity and programmed function remain compatible over the intended service interval. This review separates those requirements, treats cure exposure and matrix crosslinking as process variables that can alter cell state, and defines a qualification framework in which escape, overgrowth and functional drift are measured alongside viability. The discussion is bounded to bacteria integrated with photocurable or crosslinkable matrices and does not treat biosensing calibration in depth, which is owned by ER-436.

Keywords: engineered living materials; bacteria; photocurable hydrogel; viability; biocontainment; diffusion; mass transfer; functional stability

1. Significance and scope

Engineered living materials place viable cells inside a material architecture so that growth, metabolism or programmed gene expression becomes part of the material function. Bacterial systems are attractive because they are genetically tractable and can be maintained in hydrated matrices, but the matrix is not a passive container. Crosslink density, photoinitiator chemistry, oxygen availability, osmotic state and post-cure washing can all alter the cell population.

Bhusari and co-workers demonstrated that a bilayer Pluronic hydrogel could support engineered bacteria while restricting escape for more than two weeks, illustrating that viability and containment must be qualified together [1]. This review therefore treats the living material as a coupled biological-transport-mechanical system rather than as a resin with cells added. The exposure route also determines which biological subpopulation is being qualified: cells suspended before cure experience a different radical, osmotic and thermal history from cells seeded after a scaffold has been formed.

These routes should not be pooled under one viability claim. Where genetic circuits are used, strain identity, plasmid or genomic integration state and selective pressure belong in the material record because they can alter function even when colony counts remain stable.

2. Cure exposure defines a biological process window

A photocurable matrix imposes optical and chemical stresses during fabrication. Exposure wavelength, irradiance, radical flux and cure time should be reported together with the cell loading and the formulation state. A viability value obtained after cure is meaningful only when its assay time is stated, because delayed injury can appear after the initial exposure.

The process window must also include thermal history, mixing shear and any solvent or wash step. Hao and co-workers showed that engineered bacterial biomass can be integrated into printable living hydrogels with tunable physical properties, reinforcing the point that the biological state and matrix state must be co-designed rather than optimized sequentially [2]. Photoinitiator concentration and spectral overlap should be treated as coupled variables rather than independent recipe fields.

A wavelength that is mild for the organism can still be damaging if a high initiator concentration generates a large radical dose, while an apparently low dose can leave an under-cured matrix that subsequently releases reactive species. A useful process study therefore maps cell survival and conversion or gel state on the same exposure matrix instead of optimizing one endpoint first.

3. Containment is a transport problem, not only a barrier problem

A living matrix must admit water, nutrients, gases and often target analytes while preventing unacceptable organism release. Those objectives compete. Increasing crosslink density or adding a dense shell can suppress escape but also increase transport resistance and local starvation.

The containment metric should therefore include an escape assay, a permeability or uptake metric for relevant small molecules, and a spatial viability map. A pass/fail statement based only on colony recovery from the bulk gel can miss viable cells near the surface or rare release events. Containment testing also needs a stated detection limit. 'No colonies detected' is not equivalent to zero release unless the sampled volume, culture method, incubation time and limit of detection are reported.

For field-facing systems, challenge conditions should include swelling, repeated hydration and representative mechanical deformation because escape pathways can develop after the initial cure even when the pristine matrix is intact.

4. Geometry controls gradients and population structure

Thickness and surface-to-volume ratio determine diffusion path length, while bacterial consumption creates internal gradients that evolve with population growth. The relevant design variable is therefore not only initial cell density but the expected biomass distribution during service. Thick constructs can develop nutrient-depleted cores even when total viability remains high.

Qualification should compare multiple geometries or explicitly model the intended one, and should report whether the function scales with area, volume or accessible interface. Spatial heterogeneity should be treated quantitatively. Confocal imaging, sectioning or another validated spatial assay can distinguish a healthy peripheral shell from a starved core, while bulk ATP, fluorescence or colony counts cannot.

If the functional output consumes or produces a diffusible species, the response time should be evaluated as a function of thickness so that a transport-limited device is not mistaken for a biologically slow one.

5. Function must be separated from viability

A viable bacterium is not necessarily a functioning engineered bacterium. Reporter output, production rate, sensing response or catalytic activity can drift through plasmid loss, mutation, metabolic burden or nutrient limitation. Usai and co-workers showed that printed bacterial engineered living materials can retain genetically encoded sensing functions under challenging conditions, but their work also makes clear that longevity and functional stability are separate axes [3].

A qualification plan should therefore include viability, genotype or circuit stability where relevant, and a direct functional output.

Axis	Measurement	Failure mode
Biological state	strain, engineering state, inoculum history, viable fraction	survival without retained function
Cure exposure	wavelength, irradiance, time, initiator and temperature	delayed photochemical injury
Containment	escape assay, shell integrity, challenge after ageing	rare release or crack-assisted escape
Transport	nutrient/analyte uptake and geometry	starved core or diffusion-limited response
Function	output metric and stability interval	viable but functionally drifted population

Table 1. Domain-specific qualification matrix for living bacterial materials integrated with photocurable matrices.

6. Device-level verification

The device should be tested under the actual hydration, temperature, medium, mechanical load and replenishment conditions of use.

Batch-to-batch variation in cell state can dominate nominally identical matrix formulations, so biological inoculum history belongs in the process record. Containment must be challenged after ageing and deformation, because cracks, swelling and interfacial debonding can create release paths that were absent at day zero.

Sterility requirements for surrounding components and the intended disposal route should also be defined before field or biomedical use. ER-436 owns the calibration and signal-chain problem for living biosensors. The present review stops at whether bacterial function can be maintained and contained in a photocurable matrix; a sensing transfer function, detection limit and calibration drift belong to ER-436 even when the same engineered strain is used.

7. Minimum qualification framework

A minimum dataset should identify strain and engineering state, matrix chemistry, photoinitiator, cure conditions, cell loading, geometry, viability at multiple times, escape or leakage, transport of at least one relevant nutrient or analyte, mechanical state, functional output and an ageing interval. If genetic containment or kill switches are used, they should be tested as independent controls rather than credited by design intent. Claims of self-repair, biosynthesis or sensing should be tied to a measured output and not inferred from cell survival.

Replicate-to-replicate variability should be treated as a qualification output rather than hidden by a mean. Living systems can diverge through inoculum history, growth lag and stochastic circuit behaviour, so acceptance should include the fraction of constructs meeting both containment and function criteria. Where the material is intended for storage, qualification should repeat the complete viability-containment-function triad after the declared storage interval rather than testing storage survival alone.

The endpoint should also define what happens at end of service: continued growth, controlled inactivation or disposal. A kill step, if claimed, requires its own verification because loss of reporter output does not prove loss of viability.

8. Engineering conclusions

Living bacterial photopolymer systems are best qualified as coupled reactors with structural boundaries. The decisive question is whether the same network that protects and shapes the cells also supplies enough transport to preserve the required biological function without unacceptable escape. Designs that maximise viability at the expense of containment, or containment at the expense of transport, are not balanced systems.

The engineering objective is a bounded operating window in which biology, transport and structure remain simultaneously within specification.

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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.