

Cytotoxicity-Test Artefacts in Photopolymer Evaluation: Extraction Conditions, Dose Metrics, Controls and Result Interpretation

A failure-resistant framework for distinguishing material response from test-system bias

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Abstract

In vitro cytotoxicity is often the first biological screen applied to a printed photopolymer, yet the measured response can be dominated by the test configuration. Extraction surface-area-to-volume ratio, specimen thickness, medium composition, serum, temperature, time, agitation, dilution, storage and evaporation determine which substances reach the cells and at what dose. Cell lineage, density, exposure duration and endpoint then shape sensitivity. Pigments, particles, photoinitiator-related species, pH or solvent carryover can also alter optical or metabolic readouts without matching cell death. This review treats cytotoxicity testing as a measurement system requiring mass-balance logic, controls and orthogonal endpoints. It distinguishes device extraction from cell-exposure dose, blank and negative controls from assay-interference controls, and a thresholded screening outcome from a mechanistic or clinical conclusion. A result becomes interpretable only when the final material state, extraction loading, extract handling, cell model, signal normalisation and uncertainty are traceable. The proposed gates prevent favourable dilution, invalid control response, optical interference or an unrepresentative coupon from being hidden inside one viability percentage. No universal extraction condition or assay is claimed for all photopolymers or devices. Keywords: cytotoxicity; photopolymer; extraction; dose metric; assay interference; controls; cell viability; ISO 10993-5

1. Significance and scope

ISO 10993-5:2009 describes direct-contact, diffusion and extract approaches for in vitro cytotoxicity [1], while ISO 10993-12:2021, together with Amendment 1:2025, addresses sample preparation, extraction ratios and reference materials [2,8]. The public ISO record confirms that the amendment applies to the 2021 standard; no additional amendment-specific technical claim is inferred here. These standards provide a framework, but test choices still require device-specific scientific justification.

1.1 Corpus boundary

ER-252 owns artefacts and interpretation in cytotoxicity testing of photopolymer devices. ER-175 retains washing engineering; ER-181 retains post-cure engineering; ER-206 retains micro-computed-tomography metrology; ER-251 owns the overall biological-evaluation plan; ER-253 owns analytical evaluation thresholds; ER-254 owns toxicological assessment; ER-255 to ER-259 own application-specific biological questions; and ER-260 owns device traceability. ER-252 evaluates whether the cytotoxicity measurement validly represents the declared material state and exposure.

2. Define the test article

Record resin lot, printer, orientation, layer schedule, wash, drying, post-cure, support removal, finishing, sterilisation, ageing and storage. Include the clinically relevant surface and difficult-to-clean geometry. A laboratory coupon made smoother, thinner or better washed than the device can underestimate accessible residues. If specimens are sectioned, document newly exposed bulk surfaces. If they are sterilised, separate sterilisation effects from aseptic handling and include suitable controls.

3. Distinguish extraction loading from cell dose

A basic extraction loading is $\text{Dext} = \text{Acontact} / \text{Vmedium}$ where Dext is the declared extraction loading, Acontact is the exposed specimen area relevant to extraction and Vmedium is extraction-medium volume. For irregular, porous or internal-channel parts, geometric area can be uncertain; mass-to-volume or complete-device extraction may be justified, but the selected basis and uncertainty must be explicit. Cell dose further depends on extract dilution, exposure volume, well area, cell number and exposure duration. Two studies using the same extraction ratio can impose different cellular doses.

4. Control extraction conditions

Declare medium, serum, temperature, time, agitation, headspace, light, container, specimen orientation and extract storage. Medium polarity and protein content influence solubilisation and binding. Extended or elevated-temperature extraction may increase recovery but can also create degradation not representative of use. Use sealed or controlled vessels where evaporation changes concentration. Record pH, osmolality, visible colour, turbidity and precipitation before cell exposure.

5. Preserve mass-balance logic

Follow sample area, medium volume, transfer volume, dilution and discarded fractions. Report nominal concentration and, where available, measured chemical concentration. Avoid calling a percentage extract a dose unless the original loading is known. An apparently weak response after repeated dilution may reflect low delivered dose rather than low hazard. Conversely, an exaggerated extraction should be labelled as a challenge condition, not silently equated with clinical exposure.

6. Choose the cell model deliberately

L929 fibroblasts support standardised screening, but tissue-specific cells can reveal different sensitivity or mechanisms. Cell passage, confluence, seeding density, growth phase, medium and exposure duration affect response. The model must be justified against the intended contact and the question being asked. Brooks and Yadavalli compared fibroblast and myoblast responses while changing post-print treatment [4]. Their results illustrate both process and cell-model dependence, not a universal ranking of materials.

7. Separate assay dimensions

Metabolic assays measure enzyme-dependent signal, membrane assays measure leakage or permeability, live/dead imaging estimates cell integrity, morphology captures structural response and cell counting measures attachment or proliferation. These endpoints are related but not interchangeable. Use at least one orthogonal endpoint when extract colour, particles, redox activity, metabolic suppression or a borderline result could bias a single assay. Turkalj, Pully, Li, Sun, Ghosh, Čokić, Vanoirbeek, Van Meerbeek and Van Landuyt combined XTT, LDH and cell-cycle analysis with conversion and monomer measurements [5].

8. Detect optical and chemical interference

Run cell-free extract-plus-reagent controls at the measurement wavelength and appropriate spectral blanks. Check whether colour, fluorescence, turbidity, particles or reducing species add to or quench the assay signal. Confirm that pH and solvent carryover remain compatible with the assay. An interference correction is valid only within the linear range and with the same extract matrix. If correction is large or unstable, use a different endpoint rather than relying on subtraction.

9. Use a complete control architecture

Include untreated cells, medium blank, extraction blank, negative material control and positive control. Add process controls for sterilisation, container, wash residue or coating when they can influence the result. Each control answers a different question. Positive-control failure shows inadequate assay sensitivity; negative-control failure shows excessive background or handling problems. Neither can be repaired by normalising the sample result.

10. Normalise transparently

A common relative signal expression is $V_{rel} = 100 (S_{sample} - S_{blank}) / (S_{negative} - S_{blank})$ where V_{rel} is relative signal percentage, S_{sample} is sample-well signal, S_{blank} is the relevant cell-free background and $S_{negative}$ is the valid negative-control signal. This calculation is meaningful only if response is linear, denominators are stable and interference is negligible. Report raw and normalised values, replicate structure, variability and exclusions. Do not treat a rounded threshold as measurement precision.

11. Challenge interlaboratory variability

Gruber and Nickel conducted an interlaboratory comparison involving 52 laboratories and found substantial variation despite a common ISO 10993-5 framework [3]. Serum supplementation and exposure duration influenced sensitivity. This primary comparison demonstrates that nominal standard compliance does not guarantee comparable execution. For transfer, predefine critical conditions, reference materials, acceptance ranges and raw-data reporting. A method-transfer study is stronger than reliance on a shared standard citation.

12. Resolve direct-contact and extract disagreement

Direct contact includes local concentration, surface adhesion and mechanical interaction; extract testing distributes leachables through a medium and may miss contact-localised effects. A disagreement can therefore be mechanistically plausible. Investigate surface topography, sedimentation, local pH, extractability, contact pressure and cell displacement before declaring one method correct. The intended clinical exposure determines relevance.

13. Treat post-processing as part of the specimen

Rengarajan, Clyde, Pontsler, Valiente, Peel and Huang observed leachable cytotoxicity in several stereolithography polymers and compared further curing, passivation and solvent extraction [7]. Brooks and Yadavalli found strong effects of wash-and-cure sequence [4]. Turkalj, Pully, Li, Sun, Ghosh, Čokić, Vanoirbeek, Van Meerbeek and Van Landuyt found post-processing altered conversion and elution [5]. These outcomes cannot be transferred to an undeclared resin or process. Test both the qualified state and justified adverse states when processing variability is a known risk. Record elapsed time between manufacture, extraction and assay.

14. Interpret concentration response

Use a dilution series when it can distinguish saturation, threshold-like response, interference or non-monotonic behaviour. A single concentration cannot show whether the assay is operating in a responsive range. Tincea, Cârstea, Vasilescu, Cucuruz, Banciu, Nicoară, Mateiaş, Tâncu, Pițuru, Imre and Ciocan reported concentration-dependent morphological responses for three printed resins [6]. Their qualitative screen explicitly requires quantitative confirmation for stronger conclusions.

15. Use non-compensatory validity gates

Gate	Required evidence	Blocking failure
Specimen	Final relevant material and surface state	Testing an idealised coupon
Extraction	Loading, medium, time, temperature and handling	Reporting only percent extract
Controls	Valid blank, negative and positive responses	Normalising an invalid run
Interference	Cell-free matrix checks or orthogonal assay	Mistaking colour or redox effects for viability
Dose	Extract and cell-exposure dose traceability	Comparing unlike concentrations
Replication	Independent specimens and assay replicates	Treating technical wells as independent builds
Interpretation	Endpoint-specific and uncertainty-bounded conclusion	Converting a screen into clinical safety

No favourable gate offsets failure of another.

16. Design replication correctly

Distinguish independent print batches, specimens, extracts and replicate wells. Technical wells estimate assay repeatability; they do not replace independent manufacturing or extraction replicates. Randomise plate position and include plate-level controls. Report missing data and predefined outlier rules. Pooling extracts can mask specimen variability and should be justified.

17. Handle borderline results

For a result near a decision threshold, inspect uncertainty, control performance, dose-response shape, raw morphology and interference. Repeat with independently manufactured specimens and a predefined method. Do not average an invalid run with a valid one. Borderline outcomes can motivate process tightening, chemical analysis or a second endpoint; they are not proof of either safety or harm.

18. Connect cytotoxicity to chemistry

Where response occurs, analyse extracts at matched conditions if feasible. Candidate substances, total organic load, pH, ions or particles can guide root-cause testing. Absence of an identified compound does not erase a reproducible biological response when analytical coverage is incomplete. ER-253 owns threshold and uncertainty treatment for extractables, while ER-254 owns toxicological interpretation. ER-252 supplies the valid biological observation and its exposure conditions.

19. Minimum reporting set

Report specimen and process state; geometry, mass and exposed area; extraction basis; medium and serum; container, time, temperature, agitation and storage; dilution and delivered volume; cell identity, passage, density and exposure; assay chemistry; raw and normalised results; control acceptance; interference checks; replicate hierarchy; statistics; morphology; exclusions; uncertainty; deviations; and endpoint-limited conclusion.

20. Knowledge boundary

Standards define the main in vitro and preparation framework [1,2]. The interlaboratory study demonstrates execution-dependent variability [3]. Photopolymer studies show process, cell-model, endpoint and concentration dependence [4-7]. None establishes one universal extraction ratio, cell line, viability threshold or post-process for every device.

21. Limitations

References [1] and [2] were verified from official primary records. References [3-7] were examined at full-text or detailed primary-record depth. Studies use different resins, geometries, cells and processes. This review does not validate a particular product, substitute for the current controlled standard text or disclose proprietary formulations, process windows or acceptance limits.

22. Conclusions

Cytotoxicity testing of printed photopolymers is reliable only when specimen state, extraction loading, cell dose, controls, interference and endpoint are all traceable. A percentage viability without this measurement chain is not portable evidence. Valid interpretation is endpoint-specific, uncertainty-bounded and integrated with chemistry and intended exposure.

References

1. International Organization for Standardization. ISO 10993-5:2009 Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity. Geneva: ISO; 2009, reviewed and confirmed 2022. <https://www.iso.org/standard/36406.html>
2. International Organization for Standardization. ISO 10993-12:2021 Biological evaluation of medical devices - Part 12: Sample preparation and reference materials. Geneva: ISO; 2021. <https://www.iso.org/standard/75769.html>
3. Gruber S, Nickel A. Toxic or not toxic? The specifications of the standard ISO 10993-5 are not explicit enough to yield comparable results in the cytotoxicity assessment of an identical medical device. *Frontiers in Medical Technology*. 2023;5:1195529. <https://doi.org/10.3389/fmedt.2023.1195529>
4. Brooks AK, Yadavalli VK. Post-print processing to minimize cytotoxicity of 3D-printed photopolymer resins for biomedical applications. *Journal of Applied Polymer Science*. 2025;142:e56545. <https://doi.org/10.1002/app.56545>
5. Turkalj M, Pully D, Li Q, Sun W, Ghosh M, Čokić SM, Vanoirbeek J, Van Meerbeek B, Van Landuyt KL. Monomer elution and cytotoxicity of 3D-printed resin-based composites. *Dental Materials*. 2025;41(9):1140-1150. <https://doi.org/10.1016/j.dental.2025.06.016>
6. Tincea TA, Cârstea AE, Vasilescu VG, Cucuruz A, Banciu A, Nicoară AI, Mateiaş CG, Tâncu AMC, Pişuru SM, Imre M, Ciocan LT. In vitro cytotoxicity of three dimethacrylate-based photopolymer resins for 3D-printed dental restorations: a qualitative morphological screening on human fibroblasts. *Journal of Functional Biomaterials*. 2026;17(8):421. <https://doi.org/10.3390/jfb17080421>
7. Rengarajan V, Clyde A, Pontsler J, Valiente J, Peel A, Huang Y. Assessing leachable cytotoxicity of 3D-printed polymers and facile detoxification methods. *3D Printing and Additive Manufacturing*. 2023;10(5):1110-1121. <https://doi.org/10.1089/3dp.2021.0216>
8. International Organization for Standardization. ISO 10993-12:2021/Amd 1:2025 Biological evaluation of medical devices - Part 12 - Amendment 1. Geneva: ISO; 2025. <https://www.iso.org/standard/88205.html>

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