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Systematic Review

Biocompatibility and Safety of Orthodontic Clear Aligners and Thermoplastic Retainers: A Systematic In Vitro Review (2015–2025)

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Abstract

Background: Clear aligners have become a common alternative to fixed appliances for tooth movement, and thermoplastic retainers hold the outcome. The prolonged intraoral contact of these devices has made the materials a focus of biocompatibility research. Objectives: This paper aims to summarize laboratory evidence on the biocompatibility of clear aligners and thermoplastic retainers. Materials included thermoformed polyethylene terephthalate glycol-modified (PETG), multilayer polyurethane, and directly printed resins. Primary outcomes were cytotoxicity, endocrine activity, and chemical or particle release. Methods: We systematically searched PubMed, the Cochrane Library, and Google Scholar through 31 May 2025, and we followed the PRISMA 2020 statement (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). We applied predefined eligibility criteria. Two reviewers screened records and extracted data in duplicate, including study design, extraction conditions, surface-area-to-volume ratio (SA/V), cell models, endpoints, and analytical sensitivity as the limit of detection (LOD) and limit of quantification (LOQ). We assessed the risk of bias across seven domains and graded certainty by outcome. We did not register a protocol prospectively. Results: Seventeen studies met the inclusion criteria. Materials spanned multilayer polyurethanes (SmartTrack, Clarity), PETG sheets (Essix ACE, Duran), and directly printed resins (Graphy TC-85DAC); a subset tested zinc-oxide (ZnO) nanoparticle coatings. Typical extractions immersed 0.1–1 g of material in cell-culture medium or artificial saliva at 37 °C for 24 h to 30 days. Cell viability usually remained ≥80%. Mild cytotoxicity (about 60–70% viability) appeared with harsher extractions, extended soaks, or an inadequate post-curing of printed parts. The estrogen-sensitive proliferation assay (E-Screen) returned negative results. In saliva-like media, bisphenol A (BPA) and related leachables were undetectable or in the low ng/mL range. In printed resins, urethane dimethacrylate (UDMA) sometimes appeared in water extracts, and amounts varied with curing quality. Evidence for chemical leaching and endocrine outcomes is sparse. We found no eligible in vitro study that quantified particle or microplastic release while also measuring a biological endpoint; we discuss particle findings from mechanical wear simulations only as the external context. Limitations: The evidence base is limited to in vitro studies. Many reports incompletely described extraction ratios and processing parameters. Risk of bias and certainty: Most studies used appropriate cell models and controls, but the reporting of surface-area-to-volume ratios, LOD/LOQ, and detailed post-processing parameters was often incomplete. Sample sizes were small, and dynamic wear or enzymatic conditions were uncommon. The overall risk of bias was moderate, and the certainty of evidence was low to moderate due to heterogeneity and in vitro indirectness. Conclusions: Under

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standard laboratory conditions, clear aligners and thermoplastic retainers show a favorable biocompatibility profile. For printed resins, outcomes depend mainly on processing quality, especially thorough washing and appropriate light-curing parameters. To improve comparability and support clinical translation, we recommend harmonized test protocols, transparent reporting, interlaboratory ring trials, and targeted clinical biomonitoring.

Keywords: biocompatibility; clear aligner; thermoplastic retainer; cytotoxicity; bisphenol A; PET-G; 3-D printing; chemical leaching; endocrine activity; in vitro

1. Introduction

The growing demand for orthodontic solutions that are both aesthetic and comfortable has fueled the widespread use of clear aligners made from thermoplastic polymers such as polyethylene terephthalate glycol (PET-G), polypropylene (PP), polycarbonate (PC), thermoplastic polyurethanes (TPU), and ethylene–vinyl acetate (EVA). Patients often prefer these removable, nearly invisible devices to fixed appliances [1–3]. Alongside thermoformed sheets, a fast-growing subset of appliances is produced directly from light-curable resins, which shortens fabrication, enables new design features, and shifts attention to process-dependent safety [4–6].

A 2021 bibliometric analysis of the 50 most-cited papers reflects this rapid growth [7]. Within that set, biocompatibility studies focus on cytotoxicity, endocrine activity, and chemical leaching, including work on retrieved Invisalign devices [7].

Three questions frame current safety debates: Do aligner materials remain cytocompatible under realistic extractions? Which chemicals are released, and in what amounts, in saliva-like media? Do devices shed measurable particulates under cyclic load? Recent scoping and umbrella reviews point to a broadly favorable profile, and they stress that study design and reporting determine how comparable results are across laboratories [8–11].

For printed aligners, post-processing matters more than resin label. Studies in polymerization and mechanics show that optimized curing—especially with oxygen control under nitrogen—increases the degree of conversion and mechanical stability and lowers residual monomer, while suboptimal protocols depress cell viability and performance [12,13]. For particulates, recent wear studies report low counts of micrometer-scale fragments and recommend standardized wear rigs, defined cycle numbers, and micro-FTIR (μFTIR) or Raman microspectroscopy to limit method-driven artifacts [14–16].

Regulatory developments also push toward transparent reporting. The European Food Safety Authority (EFSA) set a very low tolerable daily intake for bisphenol A (BPA) in 2023, and the European Union issued a 2024 restriction for BPA in food-contact uses [17,18]. Dental devices fall under different rules, so reporting should emphasize released amounts with medium, surface-area-to-volume ratio, and time rather than legacy intake ratios [11]. Clinical observations help separate polymer effects from confounders: during Invisalign therapy with composite attachments, salivary signals are transient and fall after attachment removal; fixed appliances show higher salivary BPA than removable devices, while plasma levels remain unchanged [19,20].

Given the growth of clear-aligner therapy and the gaps flagged by recent reviews, this article synthesizes 2015–2025 in vitro evidence on the biocompatibility of clear aligners and vacuum-formed thermoplastic retainers. We read biology alongside chemistry and link extraction severity and post-processing to multi-endpoint responses. [11–13,16,19–22]. Our aim is a reproducible framework—with transparent extraction ratios, media, and

analytical sensitivity—that makes results comparable and supports the safe evaluation of next-generation materials.

2. Materials and Methods

2.1. Protocol and Reporting

We followed PRISMA 2020 (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) [23]. Figure 1 shows the flow diagram. We did not register a protocol prospectively. The Supplementary File provides the full search strategies and the extraction workbook.

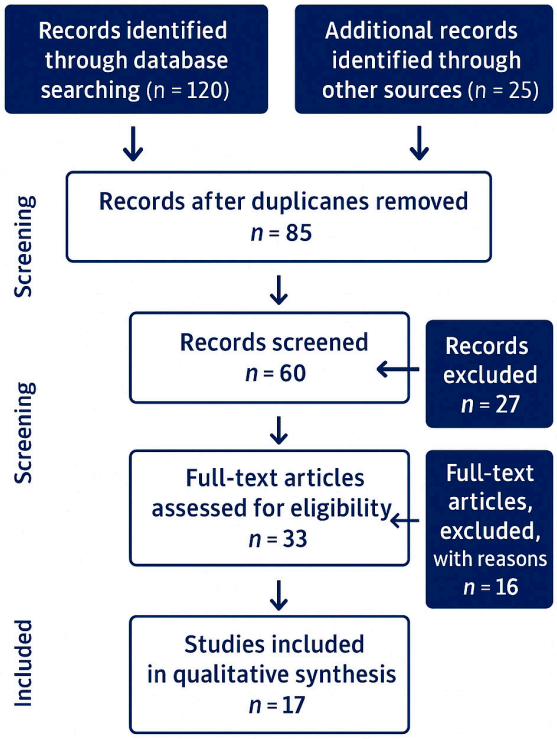

Figure 1. PRISMA flow.

2.2. Eligibility Criteria

We included in vitro studies of materials used for clear aligners or vacuum-formed thermoplastic retainers. Eligible materials were thermoformed sheets (for example, polyethylene terephthalate glycol-modified [PETG], thermoplastic polyurethane), multilayer films, directly printed light-curable resins, and coatings applied to these devices. Outcomes of interest were cytotoxicity, endocrine activity, chemical release, and particle release. We excluded animal and clinical studies, case reports, editorials, and studies focused on unrelated adhesives or composites or purely mechanical studies without biological endpoints. No language limits applied when an English version or translation was available.

2.3. Information Sources and Search Strategy

We searched PubMed, the Cochrane Library, and Google Scholar through 31 May 2025. We combined controlled vocabulary and keywords for clear aligners, thermoplastic retainers, materials, biocompatibility, cytotoxicity, chemical leaching, endocrine activity, and microplastics/particles. The timeframe spanned 1 January 2015 to 31 May 2025. Supplementary File S1 lists full search strings. We also screened reference lists of the included studies and recent reviews.

2.4. Study Selection

Two reviewers independently screened titles and abstracts, assessed full texts, and recorded exclusion reasons. A third reviewer resolved disagreements when needed. Supplementary Table S2 lists the full-text exclusions with reasons. The PRISMA diagram reports counts at each stage.

2.5. Data Extraction

We piloted a structured form and then extracted the following for each study:

- Material, device type, and post-processing steps (for example, washing, light source and energy, exposure time, atmosphere, and any thermal post-cure);
- Extraction medium, surface-area-to-volume ratio (SA/V), temperature, time, and any wear or agitation;
- Cell models and endpoints for cytotoxicity and endocrine activity, including readout normalization and acceptance thresholds;
- Analytical platform for chemistry (for example, high-performance liquid chromatography [HPLC], liquid chromatography–mass spectrometry [LC–MS or LC–MS/MS], gas chromatography–mass spectrometry [GC–MS]), target analytes, and analytical sensitivity (limit of detection [LOD], limit of quantification [LOQ]).
- For particle tests, wear rig, load, cycle count, dispersion method, size-measurement method (microscopy vs. micro-FTIR [μFTIR]/Raman), and environmental blanks.

Extraction tables and the data file appear in the Supplementary File (extraction workbook, per-study notes).

2.6. Quality Assessment and Risk of Bias

Because no single tool fits in vitro dental materials across biology, chemistry, and process reporting, we used a structured approach anchored in ISO 10993-5 items and ToxRTool-style domains, which were mapped to dentistry-specific instruments for comparability [24,25]. Two reviewers judged seven domains: (1) material identification and processing, (2) extraction reporting (medium, surface-area-to-volume ratio [SA/V], time, temperature, wear), (3) controls, (4) cell model and assay validity, (5) analytical methods and sensitivity, (6) replication and statistics, and (7) reporting completeness. We rated each domain as low risk, some concerns, or high risk, and resolved differences by consensus. Supplementary Tables S3a and S3b report the study-level judgments and standardized quality notes for each domain.

2.7. Synthesis Methods

We did not perform a meta-analysis because materials, media, extraction ratios, and endpoints were heterogeneous. We used narrative synthesis, grouped results by material class and processing quality, and standardized units when possible (for example, viability normalized to controls; leachables in ng/mL and μg/g).

2.8. Certainty of Evidence and Summary of Findings

We graded certainty by outcome using an adaptation of the GRADE approach for in vitro evidence. Supplementary File S4 describes decision rules; the Section 3 reports outcome-level ratings.

3. Results

3.1. Overall Results

Seventeen in vitro studies published between 2015 and 2025 met the criteria and cover the main clear-aligner materials in use today. These include multilayer polyurethanes used

in commercial trays (for example, SmartTrack and Clarity), polyethylene terephthalate glycol-modified (PETG) sheets for vacuum-formed retainers (such as Essix ACE and Duran), and directly printed light-curable resins (for example, Graphy TC-85DAC). A small subset tested antimicrobial surface modifications, including zinc oxide (ZnO) nanoparticle coatings on PETG. Table 1 summarizes study designs, extraction conditions, cell models, and the primary endpoints reported for cytotoxicity, endocrine activity, and chemical or particulate release. We counted Nemec et al. 2020 [26] and its 2021 extension as one study because both examined SmartTrack with direct-contact epithelial models; in Table 1, we list them as separate rows (Nemec 2020; Nemec 2021 [26,27]) to reflect different cell sources and endpoints, so the table shows 18 rows while the study count remains 17.

These papers report enough technical detail to compare outcomes across material classes and manufacturing steps, which sets up the stratified analyses in Sections 3.2–3.7.

No eligible in vitro study quantified particle or microplastic release and measured a biological endpoint in the same experiment. Mechanical wear and chewing-simulation papers exist, but they fall outside our inclusion set, and we discuss them only as external context in the Section 4 [14–16,21].

3.2. Cell Models

The cell models mirrored the tissues that aligners contact and used standard culture conditions. Human gingival fibroblasts appeared most often and align with International Organization for Standardization (ISO) 10993-5 guidance, which is clinically relevant because trays rest on the gingiva for much of the day [12,22,33,36]. Periodontal-ligament fibroblasts broadened soft-tissue coverage in one study that followed ISO-like mass-to-volume ratios [30]. For printed-resin work, HGF-1 (human gingival fibroblast-1) cells were paired with a direct-contact arm to probe surface effects [36].

Several teams added oral epithelium. Nemec and colleagues seeded Ca9-22 keratinocytes directly onto SmartTrack surfaces and tracked viability and interleukin-6/interleukin-8. Cells attached but proliferated more slowly than on tissue-culture plastic with a small interleukin-8 shift [26]. In a follow-up with primary human oral keratinocytes, proliferation again lagged tissue-culture plastic at two and seven days without an increase in dead cells. Barrier and adhesion genes shifted, including integrin-α6 (ITG-α6) and intercellular adhesion molecule-1 (ICAM-1) [27]. When the focus shifted to the post-curing of printed parts, groups tested supportive or bone-related cells. For example, MC3T3-E1 (mouse pre-osteoblast) showed that nitrogen-assisted curing restored non-cytotoxic behavior, while air curing could depress viability [12].

Endocrine activity screening used the estrogen-sensitive proliferation assay (E-Screen) with MCF-7 (estrogen-receptor positive) and MDA-MB-231 (estrogen-receptor negative) cells. Complementary readouts separated metabolic slowdown from true toxicity. Reactive oxygen species (ROS) assays were negative in one printed-resin extract study [32]. Real-time cell impedance (RTCA) confirmed recovery patterns under different post-processing protocols [33].

This panel-gingival and periodontal-ligament fibroblasts for soft tissues, epithelial models for surface contact, MC3T3-E1 for load-bearing context, and endocrine or functional readouts provides a reproducible, clinically anchored way to detect overt cytotoxicity and subtler bioactive effects across thermoformed and printed aligner materials [12,22,26–30,33,36,38].

Table 1. Process–outcome map by study.

Study (Ref)	Material/Device	Key Process Conditions	Extraction Medium/Temp/Time	SA/V Reported?	Cytotoxicity (Summary)	Endocrine Activity	Chemical Release
Campobasso 2023 [12]	Directly printed aligners (3D-printed resin)	Thermoforming: NA; Post-curing: Different post-curing procedures (duration/energy); Washing/Cleaning: NR; Atmosphere: NR	Culture medium; 37 °C; 24–72 h	NR	Short/insufficient post-curing reduced viability; optimized/longer post-curing restored non-cytotoxic range (≥70%)	Not assessed	Not assessed
Bleilöb 2025 [22]	Directly printed aligners (thickness series)	Thermoforming: NA; Post-curing: Nitrogen-assisted 20 min; Washing/Cleaning: NR; Atmosphere: Nitrogen	Saliva and other media; 37 °C; 24–72 h	Reported	Viability ≥70% across thicknesses with optimized curing	Not assessed	Not assessed
Martina 2019 [28]	Thermoformed thermoplastics (aligner sheets)	Thermoforming: Yes (vs non-thermoformed); Post-curing: NA; Washing/Cleaning: NR; Atmosphere: NA	Medium NR; ~37 °C; 24–96 h (typical)	NR	≥70–90% typical; dips ~65–70% under harsher extracts	Not assessed	Not assessed
Marsh 2022 [29]	Multilayer polyurethane films (3 brands)	Thermoforming: NR; Post-curing: NA; Washing/Cleaning: NR; Atmosphere: NA	Culture medium; 37 °C; up to 21 days	Partial	Non-cytotoxic to slight; viability often ≥80%	Not assessed	Not assessed
Lo 2024 [30]	PETG, PET, TPU (thermoformed vs. non-thermoformed)	Thermoforming: Yes (vs non-thermoformed); Post-curing: NA; Washing/Cleaning: NR; Atmosphere: NA	ISO-like; ~37 °C; 14-day extracts	Reported	Thermoformed PETG reduced viability; PET/TPU mostly ≥70%	Not assessed	Not assessed
Alhendi 2022 [31]	Commercial aligner systems (thermoformed)	Thermoforming: Yes; Post-curing: NA; Washing/Cleaning: NR; Atmosphere: NA	0.9% saline; 37 °C; 30 days; dilutions 5–20% *v*/*v*	NR	Concentration-dependent; ≥70% at lower concentrations	Not assessed	Not assessed
Pratsinis 2022 [32]	Prototype directly printed aligner resin	Thermoforming: NA; Post-curing: NR; Washing/Cleaning: NR; Atmosphere: NR	Water; 37 °C; 24–72 h	Reported	Non-cytotoxic in MTT; ROS negative	E-Screen negative	Not assessed
Bor 2025 [33]	Printed resins used for aligners	Thermoforming: NA; Post-curing: Multiple protocols; atmosphere NR; Washing/Cleaning: NR; Atmosphere: NR	ISO-like extracts; 24–72 h; includes 24 h undiluted test	Reported	24 h undiluted ~75–80% with recovery by 48–72 h	Not assessed	Not assessed
Nemec 2020 [26]	SmartTrack surface (direct contact)	Thermoforming: NR; Post-curing: NA; Washing/Cleaning: NR; Atmosphere: NA	Direct contact; 2–7 days	NA (surface model)	Attachment with slower proliferation; small IL-8 increase	Not assessed	Not assessed

Table 1. *Cont.*

Study (Ref)	Material/Device	Key Process Conditions	Extraction Medium/Temp/Time	SA/V Reported?	Cytotoxicity (Summary)	Endocrine Activity	Chemical Release
Nemec 2021 [27]	SmartTrack surface (primary oral keratinocytes)	Thermoforming: NR; Post-curing: NA; Washing/Cleaning: NR; Atmosphere: NA	Direct contact; 2 and 7 days	NA (surface model)	Proliferation lag vs. TCP; barrier/adhesion genes shifted	Not assessed	Not assessed
Ravi 2025 [34]	PETG with ZnO-nanoparticle coating	Thermoforming: NR; Post-curing: NA; Washing/Cleaning: NR; Atmosphere: NA	Direct contact and extract timepoints	NR	Slight cytotoxicity at 7–14 days vs. uncoated PETG	Not assessed	Not reported
Katras 2021 [35]	Thermoformed aligners (multi-media)	Thermoforming: Yes; Post-curing: NA; Washing/Cleaning: NR; Atmosphere: NA	Saliva-like, ethanol, gastric-like; plateau ~10 days	NR	Not assessed	Not assessed	BPA low ng/mL in saliva/gastric; higher in ethanol
Iodice 2024 [36]	Direct-printed aligners (post-cure time comparison)	Thermoforming: NA; Post-curing: Short vs. longer cure; Washing/Cleaning: NR; Atmosphere: NR	Saliva or culture medium; 37 °C; 24–72 h	Partial	Longer cure reduced cytotoxicity	Not assessed	Not assessed
Willi 2023 [37]	Direct-printed aligner resin	Thermoforming: NA; Post-curing: Post-cure studied; Washing/Cleaning: NR; Atmosphere: NR	Water; calibrated LC–MS/MS; timepoints NR	Reported (calibration + LOD/LOQ)	Not assessed	Not assessed	BPA ND; UDMA 29–96 µg/L (mean ~51 µg/L) with process dependence
Al Naqbi 2018 [38]	Vivera retainers (thermoformed)	Thermoforming: Yes; Post-curing: NA; Washing/Cleaning: NR; Atmosphere: NA	Physiologic media; standard timepoints	NR	Non-cytotoxic	E-Screen negative	Not assessed
El Idrissi 2020 [39]	Thermoformed aligners (brand NR)	Thermoforming: Yes; Post-curing: NA; Washing/Cleaning: NR; Atmosphere: NA	Artificial saliva; 24–168 h; LOQ stated	Partial	Not assessed	Not assessed	BPA trace; cumulative <1 µg per aligner
Özkan 2023 [40]	Multiple brands (polyurethane multilayers, etc.)	Thermoforming: Yes; Post-curing: NA; Washing/Cleaning: NR; Atmosphere: NA	0.9% saline; 37 °C; 8 weeks; 0.1 g/mL	Reported (calibration + LOD/LOQ)	Not assessed	Not assessed	Brand differences for BPA/BPS; BPF similar
Avan 2025 [41]	Thermoplastic appliances (3 brands)	Thermoforming: Yes; Post-curing: NA; Washing/Cleaning: NR; Atmosphere: NA	Beverages; 1 h; LOD 0.06 µg/L; LOQ 0.10 µg/L	Reported	Not assessed	Not assessed	BPA not detected for all three brands

3.3. Extraction Conditions

Extraction conditions were broadly similar. Most studies used about 0.1 g per mL in Dulbecco's Modified Eagle Medium (DMEM) or artificial saliva at 37 °C for 24–96 h [28–30]. Extended soaks included 21 days in culture medium [29] and 30 days in saline as a worst-case retainer scenario [31]. Several teams diluted extracts to 5–20 percent by volume before cell exposure to approximate salivary dilution or reduce assay artifacts [29,31,32]. Others assessed undiluted extracts, and some used direct-contact formats [26,27,33,34]. Media choice varied. Ethanol or gastric-like recipes served as harsher conditions and extracted more than saliva-like media [35]. Most extractions followed ISO 10993-5 guidance; several studies also included stress-test media outside physiologic conditions [42].

3.4. Biological Endpoints and Outcomes

Most cell-based papers assessed viability at 24–72 h in 96-well microplates using metabolic colorimetric assays. Common choices were MTT (tetrazolium reduction assay) [12,28–31,34], Cell Counting Kit-8 (CCK-8) or MTT in direct-contact formats [26,27], and resazurin (AlamarBlue) with live-cell confluence imaging [22]. Two groups added orthogonal readouts: ROS paired with MTT [32] and RTCA paired with XTT (tetrazolium reduction assay) to track recovery across 24–72 h [33]. Studies normalized readouts to untreated cells or to tissue-culture plastic or glass. A minority reported positive controls, for example natural rubber latex in Bor et al. 2025 [33] and a 1 percent anionic-detergent positive control in Marsh et al. 2022 [29].

Cytotoxicity outcomes: Under ISO-aligned extractions, no aligner or retainer material showed marked cytotoxicity [42]. Viability typically stayed at 70–90 percent across studies [22,28–30,32,33]. Mild dips around 60–70 percent appeared mainly with longer or harsher extractions [12,28,33].

Direct-contact findings. On SmartTrack surfaces, keratinocytes attached but proliferated more slowly than on tissue-culture plastic. Interleukin-8 rose slightly, while interleukin-6 was unchanged. Live/Dead staining did not show an increase in dead cells [26]. In the follow-up with primary oral keratinocytes, proliferation again lagged tissue-culture plastic, and barrier or adhesion genes shifted (integrin-α6, intercellular adhesion molecule-1, and E-cadherin) without overt cytotoxicity [27]. These findings point to a limited inflammatory or barrier cue at the surface rather than frank toxicity.

Endocrine and oxidative endpoints: The two E-Screen studies were negative under ISO-aligned extractions [32,38]. ROS remained unchanged under standard exposure in one printed-resin extract study [32]. These results match the metabolic findings.

Bottom line for biology: When studies confirmed viability with a second assay and paired it with inflammatory, oxidative, or endocrine checks, results were consistently non-cytotoxic across thermoformed and printed materials. Deviations were confined to severe extractions [12,22,26–34,36,38].

3.5. Post-Processing and Handling Effects

Post-processing is covered separately to highlight its central role in shaping viability and leaching outcomes across printed appliances. Across four studies of directly printed resins, air cures or short cures reduced cell viability, while optimized cures, especially nitrogen-assisted, restored non-cytotoxic values [12,22,33,36]. This pattern held across thicknesses and extraction designs. One study linked process quality to chemistry: better curing reduced residual monomer, and urethane dimethacrylate (UDMA) decreased as conversion increased [37]. Biology and chemistry moved together, which points to processing—not brand label—as the main driver.

Mechanism and practice align: Oxygen control during light exposure increases the degree of conversion at the surface. Adequate energy and time reduce unreacted species that can leach during extraction [12,22,33,36,37]. To make studies comparable, authors should report washing steps, light source and wavelength, irradiance or total energy, exposure time, cure atmosphere (air vs. nitrogen), and any thermal post-cure. When teams optimize and report these details, printed appliances meet the same non-cytotoxic range seen for thermoformed materials.

3.6. Chemical and Particle Leaching

Across solvents and time points, bisphenol A (BPA) was not detected or was present at low nanogram-per-milliliter levels under saliva-like extractions. In artificial saliva, El Idrissi 2020 reported trace BPA with cumulative release below about 1 microgram per aligner at 24–168 h and reported a limit of quantification (LOQ) near 0.023 micrograms per liter [39]. Katras 2021 [35] tracked BPA across media and time. Saliva-like and simulated gastric fluids stayed in the low-nanogram-per-milliliter range, while ethanol pulled higher values. Signals plateaued by about ten days [35]. An eight-week liquid chromatography–tandem mass spectrometry (LC–MS/MS) study across multiple brands quantified BPA, bisphenol F (BPF), and bisphenol S (BPS) under 0.9 percent saline at 37 °C with 0.1 g per mL. It used isotope-dilution calibration and reported between-brand differences for BPA and BPS with similar BPF across materials [40]. Beverage exposures for one hour showed no detectable BPA for Invisalign SmartTrack, ClearCorrect ClearQuartz, or Essix ACE with a limit of detection (LOD) and LOQ of 0.06 and 0.10 micrograms per liter [41].

For printed resins, Willi 2023 found no BPA in water but detected UDMA at about 29–96 micrograms per liter (mean near 51 micrograms per liter) and linked processing to conversion [37]. Across chemistry studies, platforms were high-performance liquid chromatography or LC–MS/MS with explicit calibration ranges. The stronger reports stated LOD and LOQ, which supports cross-study comparison [37,39–41]. These details help explain why saliva-like media return low signals while harsher recipes extract more. Saliva-like media approximate intraoral conditions; ethanol is a worst-case solvent that can over-extract hydrophobic additives and overestimate release.

Endocrine-activity checks. Where extractions were paired with the E-Screen, both studies reported negative results under ISO-aligned conditions [32,38]. This finding is consistent with the low measured releases in saliva-like media.

Particles: No eligible in vitro study measured particle shedding together with a biological response. Mechanical wear and chewing-simulation papers reported microplastic release with analytic identification, but they were mechanical-only and outside the included dataset [14–16,21]. We discuss those results as external context and a research gap in the Section 4.

Given the 2023 European Food Safety Authority opinion that set a tolerable daily intake for BPA at 0.2 nanograms per kilogram per day and the 2024 European Union decision to restrict BPA in food-contact materials, we report absolute release values and avoid legacy intake-ratio comparisons to older tolerable-daily-intake numbers [17,18]. Dental devices are not food-contact plastics. Reporting released amounts with medium, surface-area-to-volume ratio (SA/V), and timepoints gives readers a clearer basis for risk interpretation and replication.

3.7. Overall Findings, Risk of Bias, and Certainty

Across the 17 studies, aligner and retainer materials were consistently non-cytotoxic under ISO-aligned extractions with most values in the 80–90 percent range. Mild dips into about 60–70 percent appeared under long or harsh extractions or when post-curing was sub-

optimal [12,22,28–31,33]. Direct-contact models showed slower epithelial proliferation on SmartTrack than on tissue-culture plastic but no frank cytotoxicity and only small cytokine shifts. This points to surface cues rather than cell death [26,27]. For printed resins, processing determined biology. Nitrogen-assisted or longer cures restored non-cytotoxic behavior, while shorter or air cures sometimes depressed viability [12,22,30,33]. Bench chemistry linked this to conversion with UDMA detected at tens of micrograms per liter in water from a printed resin, while BPA was not detected [37]. Endocrine activity was negative in both E-Screen studies that used ISO-aligned extractions [32,38]. Bisphenol release in saliva-like or saline media was low and, when quantified with validated LC–MS/MS, varied by brand in one series [35,39,40]. One study reported no detectable BPA after one-hour beverage exposures across three brands [41].

Risk of bias: Study-level appraisal across seven domains was mostly "Some concerns." One study was "Low," and one was borderline "Low/Some concerns." Common issues included missing SA/V, incomplete post-processing details, the use of non-physiologic media in some extractions, and single-endpoint assays without a positive control. The reporting of LOD/LOQ was inconsistent. These patterns match Supplementary Table S3, which shows the weakest performance in exposure and extraction reporting. Domain definitions followed ISO 10993-5 reporting precedents and guidance from dental-materials appraisals [24,25,42]. Table 2 summarizes the risk-of-bias judgments.

Certainty of evidence: Using an adapted Grading of Recommendations, Assessment, Development and Evaluations (GRADE) profile summarized in Supplementary Table S4 as well as shown in the Table 3, outcome-level certainty was Moderate (adapted) for the cytotoxicity of thermoformed devices and for printed resins based on consistent process–response across laboratories with a coherent direction of effect. Endocrine activity and bisphenol release were Low because the evidence base is small and several studies used indirect media. Single-study topics, such as UDMA in water extracts or brief beverage exposures, were Very low. There was no eligible in vitro particulate evidence to grade.

Table 2. Risk of bias per study table.

No.	Study (Year)	Overall RoB	Exposure (Medium/Model)	SA/V Reported?	Positive Control (Type)	Endpoints (#)	Blinding Reported?	Calibration/ Validation	Why This Judgement	Outcome Area and Certainty (Adapted GRADE)
1	Al Naqbi 2018 [38]	Some concerns	Non-ISO extraction; endocrine + cytotoxic arms	No	Endocrine assay controls reported; no cytotoxic positive control	Multiple	Not reported	Not applicable	Extraction details incomplete and SA/V not stated. Endocrine arm well controlled.	Endocrine (Low); Cytotoxicity (Moderate—supportive single study)
2	Nemec 2020–2021 [26,27]	Some concerns	Direct-contact surface model	Not applicable	No positive control	Multiple	Not reported	Not applicable	Direct contact limits comparability; blinding not reported; gene-level signals lack protein confirmation.	Cytotoxicity/Surface response (Moderate—supportive direct-contact evidence)
3	Pratsinis 2022 [32]	Some concerns	Non-ISO water extract	No	Endocrine positive/negative controls; no cytotoxic positive control	Multiple	Not reported	Not applicable	Water extract and missing SA/V increase indirectness; endocrine panel well controlled.	Endocrine (Low); Cytotoxicity (Moderate—supportive)
4	Marsh 2022 [29]	Low	ISO-like DMEM extraction	Yes	Yes — 1% anionic detergent (cytotoxic positive control)	Single	Not reported	Not applicable	ISO-style extraction with clear controls and statistics; single assay keeps certainty modest but bias low.	Cytotoxicity (Moderate)
5	Alhendi 2022 (Angle) [31]	Some concerns	Non-ISO saline, month-long; dilution series	No	No positive control	Single	Not reported	Not applicable	Month-long saline extract with no SA/V; no positive control; reporting and replication clear.	Cytotoxicity (Moderate—supportive)
6	Katras 2021 [35]	Some concerns	Mixed media (saliva-like, ethanol, gastric-like)	No	Not applicable	Single	Stated	Yes	Calibrated LC–MS/MS but LOD/LOQ not explicit and SA/V missing; includes stress media.	Chemical release—BPA (Low)

Table 2. *Cont.*

No.	Study (Year)	Overall RoB	Exposure (Medium/Model)	SA/V Reported?	Positive Control (Type)	Endpoints (#)	Blinding Reported?	Calibration/ Validation	Why This Judgement	Outcome Area and Certainty (Adapted GRADE)
7	El Idrissi 2020 [39]	Some concerns	Non-ISO saliva-like; targeted BPA	No	Not applicable	Single	Not reported	Yes	Sensitive BPA quant with low totals; no SA/V and no biology in the same study.	Chemical release—BPA (Low)
8	Özkan 2023 [40]	Some concerns	Non-ISO saline; long extraction	No	Not applicable	Single	Stated	Yes	Full LC–MS/MS validation (IS, LOD/LOQ); saline medium and minor unit inconsistency.	Chemical release—BPA/BPF/BPS (Low)
9	Willi 2023 [37]	Some concerns	Non-ISO water; printed resin	No	Not applicable	Single	Not reported	Yes	Water extract and missing SA/V; calibrated UDMA quant, BPA screen negative; descriptive stats.	Chemical release—UDMA (Very low, single study)
10	Campobasso 2023 [12]	Some concerns	Non-ISO DMEM; post-curing protocols	No	No positive control	Single	Not reported	Not applicable	SA/V missing and 121 °C sterilization may alter surfaces; clear between-protocol differences.	Cytotoxicity (Moderate—supportive)
11	Lo 2024 [30]	Some concerns	ISO-like DMEM; thermoformed vs. flat	Yes	No positive control	Single	Not reported	Not applicable	ISO mass-to-volume and stats good; alcohol disinfection could confound; no positive control.	Cytotoxicity (Moderate—supportive)
12	Iodice 2024 [36]	Some concerns	Saliva extracts and direct-contact; post-cure time	No	Positive control not reported	Single	Not reported	Not applicable	Saliva composition and SA/V not reported; limited public statistics; clear direction of effect.	Cytotoxicity (Moderate—supportive)

Table 2. *Cont.*

No.	Study (Year)	Overall RoB	Exposure (Medium/Model)	SA/V Reported?	Positive Control (Type)	Endpoints (#)	Blinding Reported?	Calibration/ Validation	Why This Judgement	Outcome Area and Certainty (Adapted GRADE)
13	Bleilöb 2025 [22]	Some concerns	Saliva-like and culture medium; thickness series; nitrogen-assisted cure	No	No positive control	Multiple	Not reported	Not applicable	SA/V missing; saliva alone can suppress proliferation; otherwise well reported.	Cytotoxicity (Moderate—supportive)
14	Bor 2025 [33]	Low–Some concerns	ISO 6 cm^2/mL; multiple post-process conditions	Yes	Yes—natural rubber latex (cytotoxic positive control)	Multiple	Not reported	Not applicable	Strong ISO design with XTT and RTCA and latex positive control; blinding not stated.	Cytotoxicity (Moderate)
15	Ravi 2025 [34]	Some concerns	Direct-contact; ZnO-NP coating	Not applicable	No positive control	Single	Not reported	Not applicable	Direct contact and 200 °C sputtering reduce clinical comparability; replication and statistics adequate.	Cytotoxicity (Moderate—supportive)
16	Avan 2025 [41]	Some concerns	Beverages 1 h at room temperature	No (mass only)	Not applicable	Single	Not reported	Yes	Short, room-temperature beverage exposure; strong LOD/LOQ and blanks; scope limited.	Chemical release—BPA (Low)
17	Martina 2019 [28]	Some concerns	Non-ISO DMEM; static extract	No	Positive control not reported	Single	Not reported	Not applicable	SA/V not stated and low mass-to-volume in abstract; clear ANOVA and numeric outcomes.	Cytotoxicity (Moderate—supportive)

Table 3. Summary of findings and certainty (adapted GRADE).

Outcome	Evidence Base (Included In Vitro Studies)	Typical Test Conditions Behind the Finding	Main Finding (Direction/Magnitude)	Certainty (Adapted GRADE)	One-Sentence Rationale
Cytotoxicity	12 studies: [12,22,26–34,36]	Extractions aligned with ISO 10993-5 at ~0.1 g/mL in Dulbecco's Modified Eagle Medium (DMEM) or artificial saliva, 37 °C, 24 to 96 h; some extended soaks to 21 to 30 days; dose–response dilutions at 5 to 20% *v/v* in several studies.	Viability usually ≥80%; occasional dips to ~60 to 70% under harsher extractions or when post-curing of printed parts was insufficient.	Moderate	Consistent non-cytotoxic range under saliva-like conditions with a coherent process–response pattern; indirectness remains and several studies used single assays or incomplete process reporting.
Endocrine activity (E-Screen)	2 studies: [32,38]	Extractions aligned with ISO 10993-5; MCF-7 (estrogen-receptor positive) and MDA-MB-231 (estrogen-receptor negative) proliferation assays.	No estrogen-receptor-mediated proliferation detected.	Low	Small evidence base and few labs, but both studies agree under physiologic extractions.
Chemical release (bisphenols, monomers)	5 studies: [35,37,39–41]	Saliva-like or 0.9% saline media, ~0.1 g/mL, 37 °C, 24 h to 8 weeks; high-ethanol or gastric-like recipes used as stress tests.	In saliva-like media, BPA is low or not detected; brand-dependent differences for BPA/BPS at low levels; in a printed resin, UDMA appears in water at tens of µg/L with process-dependent variation.	Low	Consistent direction across chemistry studies but few studies, mixed media, and incomplete reporting of surface-area-to-volume ratio (SA/V) and limits of detection/quantification (LOD/LOQ) in some papers.
Particles/ microplastics	0 studies (eligible)	—	No eligible in vitro study paired particle measurements with a biological endpoint.	No eligible evidence	Wear and chewing simulations exist but sit outside the included dataset (mechanical only) and use diverse rigs without paired cell outcomes.

4. Discussion

Across seventeen in vitro studies, contemporary clear-aligner materials—polyethylene terephthalate glycol-modified (PETG), multilayer polyurethanes, and directly printed resins—show a favorable biocompatibility profile when extractions follow International Organization for Standardization (ISO) 10993-5 guidance [42]. What affected results was not the brand name but rather the way parts were processed and how extractions were designed. Washing steps, light energy and time, and cure atmosphere explained most differences in printed parts, and extraction severity explained most dips seen with thermoformed sheets [12,22,28,33]. With that lens, the dataset is consistent and practical: process quality preserves biocompatibility.

These conclusions line up with recent scoping and umbrella reviews, which also report mild effects in saliva-like media and call for a better reporting of extraction ratios and post-curing parameters [4,8–11]. Where this review adds value is emphasis. We read biology next to process, not just material labels, and we ask that the surface-area-to-volume (SA/V), media recipe, light source or energy, cure time, washing, and atmosphere be reported every time. That shift helps explain why laboratories sometimes disagree and makes results easier to compare and translate.

Moving from alignment to interpretation, the cytotoxicity signal is clearly process-driven. Thermoforming changed PETG mechanics in ways that can nudge responses, yet most values still met the ISO 10993-5 non-cytotoxic range [28,29,43]. For printed parts,

curing quality decided the outcome. Nitrogen assistance or longer cures restored high viability, while short or air cures sometimes depressed it [12,22,33,36]. Notably, thickness itself was not the cause once curing was adequate, even for multi-millimeter specimens [22]. This is the practical takeaway for laboratories and manufacturers: document and optimize post-processing before biological testing and then report it so others can reproduce the result.

Assay choice supports the same reading: Tetrazolium assays such as MTT are convenient, but they read metabolism, not survival, and leachates can skew signals [44]. Confidence improves when a metabolic assay is paired with a membrane-integrity or live/dead readout and at least one functional marker such as interleukin-6, interleukin-8 or reactive oxygen species (ROS). Where tested, the estrogen-sensitive proliferation assay (E-Screen) using MCF-7 and MDA-MB-231 was negative under ISO-aligned extractions, which fits the low-toxicity picture [26,32,38,45]. Findings from additional endocrine-screening paradigms corroborate this pattern [46].

Chemistry results fit that biology: In saliva-like or saline media, bisphenol A (BPA) is usually non-detectable or in the low nanogram-per-milliliter range with similar patterns for bisphenol F (BPF) and bisphenol S (BPS). Harsher recipes, like ethanol or simulated gastric fluid, extract more and should be read as stress tests rather than clinical surrogates [35,39–41,47]. A long liquid chromatography–tandem mass spectrometry (LC–MS/MS) series found brand-level differences for BPA and BPS, while BPF was similar across materials, again within low ranges [40]. Outside our dataset, Zhang et al. used a broader LC–MS/MS panel and again observed low BPA/BPF/BPS in saliva-like media, reinforcing the low-release pattern although the extraction was not physiologic and SA/V was not reported [48] A one-hour beverage exposure reported no detectable BPA for three brands with stated limits of detection and quantification (LOD and LOQ) [41]. Reporting absolute amounts together with medium, SA/V, and time is the most informative approach, especially because the 2023 European Food Safety Authority tolerable daily intake and the 2024 European Union restriction address food-contact plastics rather than dental devices [17,18]. For printed resins, chemistry echoed mechanics: urethane dimethacrylate (UDMA) appeared in water at tens of micrograms per liter when curing was weaker, while BPA was not detected, which points again to conversion and post-curing as the levers to watch [13,37,49–52].

Particles deserve a clear note: We planned to include in vitro particle-release studies that also measured a biological endpoint. None met that combined criterion, so no particulate study entered the synthesis. Mechanical wear and chewing-simulation papers exist and show low counts of micrometer-scale fragments with brand-specific size distributions, but they did not pair particle characterization with cells in the same experiment, and they used diverse rigs and loads [15,21]. This gap is understandable. Counts sit near analytical limits, contamination control is demanding, and it is not trivial to produce cell doses that truly mirror intraoral wear. Still, this is a valuable next step for the field [16].

Clinical data read cleanly against this laboratory picture: Adverse events are rare and usually isolated, including hypersensitivity case reports [53,54]. During Invisalign treatment with composite attachments, salivary BPA falls after attachment removal, which points to adhesive-related contributions rather than the trays themselves [19]. A non-randomized trial found nanogram-per-milliliter salivary values without within-group rises over time, and plasma values did not differ by appliance type. Fixed appliances produced higher salivary values than removable devices [20]. Real-world behaviors matter, too. Pre-soaking vacuum-formed retainers reduced the first-week salivary signal, and adhesive removal produced only a brief rise after debond [55,56]. Beverage exposures offer another check. A clinical beverage study did not show sustained rises under wear,

which aligns with the in vitro one-hour beverage test that reported non-detects with the stated LOD and LOQ [41,57]. Expansion appliances follow the same transient pattern with short-lived increases that returned to baseline within a week [58]. In short, modern aligner polymers contribute little under saliva-like conditions, while adhesives and fixed hardware are the main confounders. Earlier reviews flagged polymer degradation as a concern; the present methods quantify those risks more precisely and, importantly, keep them small in realistic use.

As a whole, these patterns give both researchers and clinicians a simple map: For researchers, the process choices you control—washing, light energy and time, and atmosphere—drive biology and chemistry. For clinicians, patient counseling can point to good processing, sensible cleaning, and awareness that attachments and adhesive steps, not the trays, often explain transient signals.

4.1. Future Directions

The field is close to a common language; only a few focused steps are still needed.

First, it is important for researchers to use saliva-like extractions paired with a defined mechanical-wear arm, and always report SA/V, media recipe and pH, timepoints, dilution series, and analytical sensitivity with LOD and LOQ [5,6,8,15].

Second, it is important for researchers in the field to raise biological realism where it adds value-co-cultures, organotypic gingiva, or oral-on-a-chip under flow while keeping endpoints practical and reproducible: one metabolic assay, one membrane integrity or live/dead readout, and an inflammatory or oxidative marker with an E-Screen when endocrine activity is plausible [6,8,10,24,45,46].

Third, it is important to treat post-processing as an exposure to document. Researchers need to log washing steps, light source and energy, exposure time, atmosphere, and any thermal post-cure; then, they need to track the degree of conversion and residual monomer. Optimizing curing, including nitrogen assistance, before biology is crucial [9,11,37].

Fourth, studies ought to anchor exposure to the clinic through mirroring tray changes and cleaning routines with sequential extractions as well as linking lab analytes to patient samples on the same scale in targeted biomonitoring studies [16,17,19,57]. Fifth, it is important to close the particle gap. Researchers need to run small inter-laboratory ring trials with shared lots and process logs, standardize loads and cycle numbers, and confirm identity by micro-FTIR or Raman. Where feasible, it is also ideal to pair wear-generated particle suspensions or wear-cell contactors with biological readouts in the same experiment [8,15]. Two additional gaps are worth flagging: the small number of endocrine-focused studies under physiologic media, and the under-reporting of SA/V and LOD/LOQ in several chemistry papers. Both limit certainty and are straightforward to fix.

4.2. Limitations

We separate the limitations of the included evidence from the limitations of our review process.

All included studies are in vitro, which means static extractions at 37 °C cannot fully reflect salivary flow, variable pH, enzymes, or continuous loading. Chewing simulations show surface change and small chips, which static soaks miss [14]. Many papers used single cell types and relied on MTT, which can misstate survival. The reporting of SA/V, LOD/LOQ, and process logs was uneven, especially for printed parts [8,24,37,44].

Our review did not register a protocol prospectively. We followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020), screened in duplicate, and used predefined criteria—all of which we include in our Supplementary File—yet post hoc decisions can still introduce bias [23]. We blended ISO 10993-5 reporting items

with dentistry-focused tools rather than a single instrument such as RoBDEMAT or QUIN, because the evidence spans cell biology, analytical chemistry, and process reporting, and no single tool covers all three. This choice may limit comparability with reviews that used one instrument [25,59]. Heterogeneity in materials, media, and endpoints precluded meta-analysis. We searched three major databases and English-accessible full texts, so some records may have been missed.

4.3. Limitations of the Included Studies

4.3.1. Methodological Issues

Most experiments used static extractions at 37 °C in closed vessels. In the mouth, aligners face salivary flow, fluctuating pH, enzymes, and continuous mechanical load. Saliva-borne esterases and proteases can alter additives over time, which static soaks do not capture [32]. Static tests also miss abrasion and fatigue. Chewing simulations with cyclic loading produced micro-cracks and small chips, indicating that wear can change the surface and release profile [15]. Because flow can dilute leachates or prevent saturation, a static beaker can under- or over-estimate exposure. Recent reviews recommend reporting or standardizing flow, load, cycle number, and surface-area-to-volume ratios in extraction and wear studies [5,6,8,15].

4.3.2. Biological Issues

Many papers relied on a single cell type. Monocultures miss crosstalk among epithelium, fibroblasts, and immune cells. An extract that nudges IL-8 in epithelial cells could recruit neutrophils in vivo, which a monoculture will not show. Reviews on printed-aligner cytotoxicity and materials testing call for co-cultures, organotypic epithelia, or "oral-on-a-chip" systems under flow to better reflect gingival biology [6,10].

4.3.3. Analytical Issues

MTT dominated viability testing, yet metabolic dye reduction is not identical to cell survival. Certain leachates can reduce tetrazolium or stimulate dehydrogenases and inflate readings, which is a point long noted by Wataha and Messer [44]. Only a minority paired MTT with a second viability readout or added morphology, IL-6/IL-8, ROS, or E-Screen. On the chemistry side, several studies did not report LOD/LOQ, surface-area-to-volume ratios, or full instrument settings, which limits cross-study comparison and weakens any dose–response analysis [8].

4.3.4. Reporting Issues

Process reporting was inconsistent, especially for directly printed parts. Post-curing time, light source and energy, washing steps, and atmosphere were often missing, despite evidence that these parameters drive cytocompatibility [9,11,37]. Some studies used small sample sizes and did not state whether positive and negative controls were run. Missing controls reduce confidence in "high viability" claims because assay failure can mask toxicity. Particle work is near analytical limits and is sensitive to rig design; standardized loads, cycle counts, μFTIR or Raman sizing, and environmental blanks are recommended [15]. While many papers aligned with ISO guidance, reporting remained uneven [42].

All evidence here is in vitro. Laboratory conditions approximate, but do not replicate, the intraoral environment. Clinical validation and post-market surveillance remain essential to confirm that these safety margins hold across patients with different wear habits and attachment use.

4.4. Limitations of the Review Process

Although we believe that our review adds a valuable insight into the biocompatibility of clear aligners, we must note one limitation. No prospective protocol registration was completed. This raises the risk of analytic flexibility and selective emphasis during screening, extraction, and synthesis. To limit this, we followed PRISMA 2020 [23], screened in duplicate, used predefined eligibility criteria, and now provide the full line-by-line search strings (Supplementary S1), a table of full-text exclusions with reasons (S2), study-level risk-of-bias judgments (S3), and outcome-level certainty (S4). Even with these safeguards, lack of registration can introduce bias through post hoc decisions.

We searched PubMed, the Cochrane Library, and Google Scholar, hand-searched reference lists, and conducted forward citation tracking. We did not search other bibliographic databases, and we required an English-accessible full text, so some eligible records may have been missed.

Our risk-of-bias tool blends ISO 10993-5 items with ToxRTool-style domains, which were aligned to dentistry-specific instruments. This improves transparency but is not a single validated instrument, which may affect comparability with reviews that used QUIN or RoBDEMAT exclusively [25,59]. We used an adapted GRADE approach for in-vitro evidence. Outcomes start very low due to indirectness with upgrades based on method quality and consistency. Given the heterogeneity in materials, media, and endpoints, we used narrative synthesis without meta-analysis.

Data extraction and judgment: Two reviewers extracted data and scored risk of bias independently, after which they reached consensus. Remaining judgment calls, such as outcome wording in the Summary-of-Findings, could still introduce subtle reviewer bias, which we mitigate by providing tables with the underlying decisions (S3, S4).

Our review adds standardized risk-of-bias and certainty tables to support that comparison while acknowledging the constraints above.

To sum up, we do not just report that aligners are broadly biocompatible in vitro; we show which process choices preserve that profile, and we publish the search strings, exclusion reasons, risk-of-bias calls, certainty ratings, and workbook needed for other groups to replicate and extend this evidence base.

4.5. Bottom Line for Practice and Research

Process quality, more than brand chemistry, explains the variation seen across laboratories. When curing is optimized and extractions reflect saliva-like conditions, aligner materials are broadly non-cytotoxic in vitro, endocrine-neutral in screening, and low-releasing in absolute terms. Clinicians can prioritize well-processed devices and focus on attachments and adhesive steps when counseling patients about transient exposures [19,20,53–58]. Researchers can accelerate translation by reporting SA/V, media, and process logs, by pairing simple viability with functional markers, and by closing the particle-with-biology gap.

5. Conclusions

Most in vitro evidence supports the cytocompatibility of clear aligners and vacuum-formed retainers when extractions follow International Organization for Standardization (ISO) 10993-5 thresholds. Cell viability typically remains at or above 70 percent.

Datasets for chemical leaching and endocrine outcomes are sparse. Within those limits, three signals are consistent. In saliva-like media, bisphenol A release is generally low or not detected. In directly printed resins, urethane dimethacrylate can be detected in water extracts, and amounts vary with curing quality and handling. In the available estrogen-sensitive proliferation assays, endocrine activity has been negative.

Certainty is limited. We rate confidence as moderate for cytotoxicity and low for chemical leaching and endocrine outcomes with very low certainty for single-study findings such as urethane dimethacrylate. Translation to clinical safety remains indirect because all included data are in vitro. No eligible study measured particle shedding and a biological response in the same experiment, so particulate evidence comes from mechanical studies only and remains a research gap.

The next steps are practical. These include using saliva-like extraction with a defined mechanical-wear arm; treating post-processing as a reportable exposure and documenting washing, light energy and time, and atmosphere, with basic quality control for conversion and residual monomers; sharing lots and methods across laboratories; and lastly, linking bench analytes to targeted biomonitoring and well-designed clinical studies. This approach will convert a consistent in vitro signal into durable, patient-facing guidance.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app152312494/s1, File S1: search strategies (S1), full-text exclusions with reasons (S2), study-level risk-of-bias judgments (S3), and summary-of-findings and certainty tables (S4), along with the structured extraction workbook.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

CA	Clear orthodontic aligners
VFR	Vacuum-formed thermoplastic retainers
PET-G	Polyethylene terephthalate glycol
BPA	Bisphenol A
HGF	Human gingival fibroblasts
Ca9-22	Human oral epithelial cell line Ca9-22
MCF-7	Estrogen-responsive human breast cancer cell line MCF-7
MDA-MB-231	Human breast cancer cell line MDA-MB-231
MTT	Methylthiazolyldiphenyl-tetrazolium bromide assay
XTT	2,3-bis-5-2H-tetrazolium assay
CCK-8	Cell Counting Kit-8
E-Screen	Estrogenicity screening assay
AlamarBlue	A resazurin-based fluorescent cell-viability assay
Live/Dead	Live/dead viability/cytotoxicity staining kit
NR	Not reported
NA	Not applicable

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