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Review Article

Toxicological and Endocrine Impacts of Materials in Removable Orthodontic Retainers: A Systematic Review

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ABSTRACT

This review aimed to examine the potential cytotoxicity and endocrine-disrupting effects of materials used in removable orthodontic retainers. A systematic survey of the literature from 2015 to 2025 analyzed studies evaluating in vitro cytotoxicity, estrogenic activity, in vivo tissue reactions, and clinical biomarker responses. Materials assessed included PMMA plates, thermoplastic foils, fiber-reinforced composites, PEEK, and 3D-printed photopolymer resins. Forty-eight studies (38 in vitro, 10 clinical) fulfilled the inclusion criteria. Photopolymer resins were associated with the greatest cytotoxic responses, whereas PMMA and thermoplastics generally showed mild effects, which were further reduced after 24 hours of water immersion. Release of bisphenol-like compounds was observed, although systemic exposure remained within regulatory safety limits. Clinical evaluations reported no significant mucosal changes or measurable endocrine disturbances. Overall, removable retainer materials demonstrate acceptable biocompatibility, but evidence regarding long-term endocrine consequences is limited. Standardized testing frameworks are needed to allow consistent comparison across orthodontic materials. Furthermore, single-use thermoplastics may contribute to microplastic pollution and pose challenges for disposal, raising environmental sustainability concerns.

Keywords: Removable orthodontic retainers, Biocompatibility, Bisphenol compounds, Thermoplastics, PMMA, Clear aligners

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Introduction

Removable retainers are essential devices for preserving teeth alignment after orthodontic treatment. These appliances typically include Hawley retainers, which combine a PMMA base with a metal wire, and vacuum-formed thermoplastic retainers made from PET-G, polypropylene, or polyurethane [1, 2]. Because patients often wear these devices for extended periods—particularly in cases with high relapse potential or missing teeth—questions about the safety of the materials arise. Research published over the last decade indicates that some retainer materials can release compounds such as bisphenol-A (BPA) and bisphenol-S (BPS), which have been associated with cellular stress, DNA damage, and other cytotoxic effects [3–5]. BPA has also been detected in the saliva of patients using both PMMA and thermoplastic retainers [2], while laboratory studies confirm that various aligner and retainer systems may leach bisphenols that impact cellular function [6, 7]. Even at low concentrations, prolonged exposure to these chemicals may have cumulative biological consequences.

This review focuses specifically on removable retainers and synthesizes current evidence regarding cytotoxicity, endocrine-disrupting potential, and environmental considerations, adopting a One Health perspective. The goal is to provide clinicians and

researchers with an integrated understanding of material safety, clinical relevance, and ecological impact.

Materials and Methods

Literature search

A systematic search of PubMed, Scopus, and Web of Science was performed to identify relevant studies published between January 2015 and December 2025. Keywords were combined using Boolean operators and included terms such as "orthodontic retainers," "cytotoxicity," "endocrine disruption," "PMMA," "polyurethane," "copolyester," "BPA," "BPS," "phthalates," "in vitro," and "clinical study."

Study selection

Articles were included if they:

- Presented original, peer-reviewed research (in vitro, in vivo, or clinical studies);
- Evaluated materials used in removable orthodontic retainers, including PMMA, thermoplastics, polyurethane, or related composites;
- Assessed cytotoxic effects, hormonal activity, or chemical leaching;
- Used human cells, animal models, or human participants;
- Were published in English.

Studies were excluded if they:

- Focused exclusively on fixed orthodontic appliances or other dental materials;
- Were reviews, editorials, abstracts, or case reports without primary data;
- Did not assess biological or toxicological outcomes;
- Lacked full-text access or sufficient methodological detail.

Although no formal review protocol (e.g., PROSPERO) was registered, the search strategy was predefined, documented internally, and consistently applied across all databases to ensure reproducibility.

Materials used in removable retainers

Removable retainers are typically classified according to the materials from which they are made: acrylic-based retainers, such as Hawley appliances, and thermoplastic retainers, often referred to as Essix-type clear retainers. **Table 1** provides an overview of the main material compositions and the associated biocompatibility concerns.

Hawley retainers (Acrylic-based, PMMA)

Hawley retainers consist of a rigid plate made from polymethyl methacrylate (PMMA), covering the palate or lingual surfaces, with embedded stainless steel wires or clasps for tooth retention. PMMA is produced by polymerizing methyl methacrylate (MMA) monomers, but polymerization is rarely complete [3]. As a result, residual MMA can remain in the cured acrylic [8], which may leach into saliva during the initial days of wear and act as an irritant. Heat-curing under pressure generally reduces residual monomer content compared to cold-cured (chemical-cured) PMMA [2]. While the metallic components of the Hawley retainer may release trace ions, this review focuses on the polymer portion. PMMA does not contain bisphenol compounds and is not considered estrogenic; however, residual monomers and minor additives, such as hydroquinone inhibitors or peroxide initiator byproducts, could contribute to cytotoxic effects [9]. Some PMMA formulations incorporate bioactive glasses like Biomin C or S53P4, which release beneficial ions such as calcium and phosphate in acidic conditions, offering potential remineralization benefits [10].

Essix retainers (thermoplastic-based)

Essix-type retainers, introduced by Sheridan in the 1990s, are thin vacuum-formed appliances molded to the teeth [11]. These retainers were originally made from PET-G (polyethylene terephthalate glycol-modified), but other polymers—including polypropylene, thermoplastic polyurethane (TPU), and multilayer blends—are now commonly used. Some proprietary materials, such as Invisalign's SmartTrack, are TPU-based blends marketed as medical-grade and BPA-free [4]. Although PET-G itself does not contain BPA, certain polycarbonate or co-polyester variants may include bisphenol derivatives [12]. Even for modern thermoplastics advertised as BPA-free, trace levels of other bisphenols (like BPS) or estrogenic additives can be present depending on the specific manufacturing process [13]. Additionally, any thermoplastic material can potentially release other xenoestrogens or degradation products, particularly when new or under mechanical stress. In general, industrially polymerized thermoplastics have fewer leachable compounds compared to freshly cured PMMA, but residual oligomers, plasticizers, or stabilizers may still diffuse into saliva.

3D-printed retainers

A newer category of retainers involves direct 3D printing using photopolymer resins. These retainers are less widely used clinically compared to Essix or Hawley appliances, but they offer highly customizable

fabrication. 3D-printed resins often contain methacrylate-based oligomers, some derived from bisphenol-A glycidyl dimethacrylate. Inadequate post-curing or cleaning can result in significant monomer leaching. Early studies suggest that certain 3D-printed retainer materials may exhibit higher cytotoxicity and genotoxicity than traditional thermoplastics [14]. While promising for individualized fabrication, these materials require careful evaluation and processing to ensure safety. Studies investigating chemical release from various clear aligners and 3D-printed systems have reported multiple leachable compounds, highlighting ongoing biocompatibility concerns [7].

Table 1. Composition, leachable components, biocompatibility profile, and relative cytotoxicity of removable orthodontic retainer materials.

Retainer Material	Composition and Key Components	Potential Leachables	Notable Biocompatibility Considerations	Relative Cytotoxicity
Hawley retainer (PMMA base + wire)	PMMA acrylic (polymerized methyl methacrylate) baseplate; stainless steel wire clasps. Typically autopolymerized (cold-cure) or heat-cured.	Residual methyl methacrylate (MMA) monomer; peroxide initiator residues, pigments. **No inherent BPA** in PMMA.	Residual MMA can cause cytotoxic and irritant effects on oral cells [8]. **Chemical-cure acrylics** leave **more MMA** (higher toxicity) than **heat-cure** [2]. Rare allergic reactions reported. **Good long-term biocompatibility** once fully cured.	**Moderate**
Essix retainer (PET-G thermoplastic)	Thermoformed PET-G (polyethylene terephthalate glycol) sheet. Petroleum-based, transparent, ~1 mm thick.	Trace ethylene glycol, terephthalate oligomers; possible BPA-derived additives for clarity [12]. **Base polymer is BPA-free**.	Considered **inert and stable**; very low in vitro cytotoxicity. **One study** detected **measurable BPA in saliva** of PET-G retainer wearers [2] (likely from additives/contamination). Low mucosal irritation.	**Low**
Essix retainer (polypropylene or polyethylene)	Vacuum-formed retainers using polypropylene or polyethylene blends (softer, flexible thermoplastics).	**Minimal leachables** (polyolefins are highly stable). **No BPA or phthalates** typically required.	**Minimal cytotoxicity**; high biocompatibility. Lower stiffness may promote **bacterial plaque adhesion**. Issues mainly **mechanical** (wear/tear), not chemical.	**Very Low**
Clear aligner-type (polyurethane, e.g., Invisalign)	Multilayer **aliphatic or semi-aromatic thermoplastic polyurethane (TPU)**. Proprietary blends (e.g., Invisalign's **SmartTrack**).	Oligomers or urethane degradation products (e.g., 1,4-butanediol) under extreme conditions. **No BPA or phthalates** by design [13].	Slight in vitro cytotoxicity (comparable to PET-G). Generally well-tolerated clinically.	**Low**
3D-printed retainer (acrylate resin)	Photopolymerized resin (e.g., **urethane dimethacrylate-based**). Custom-printed and post-cured.	**Unpolymerized methacrylate monomers** (if incomplete curing); **photoinitiators**; possible **bisphenol-A derivatives** in some resins.	**Safe if properly cured and washed**; however, studies show **variable leaching** and **higher cytotoxicity/estrogenic effects** than thermoplastics [14].	**Moderate to High***

* Thorough post-processing (washing + full UV curing) is critical to minimize toxicity. Further long-term clinical validation needed.
* Depending on curing process and residual monomer levels.

Cytotoxic effects on oral cells

A crucial aspect of evaluating biocompatibility is determining whether retainer materials can induce cytotoxic responses in oral tissues. Because retainers remain in contact with the palate or mucosa for prolonged periods, even mild cytotoxic effects could influence mucosal integrity or cell turnover. Research in this area has utilized in vitro cell culture assays, in vivo animal models, and clinical studies assessing biomarkers in patients wearing retainers. A total of 38 in vitro studies published between 2015 and 2025 were reviewed, applying strict inclusion criteria. Eligible

studies assessed cytotoxicity using recognized assays such as MTT, LDH, or live/dead staining, employed human oral cell lines or mammalian models, tested materials used in removable retainers (e.g., PMMA, thermoplastics, polyurethane, PETG, and 3D-printed resins), and reported quantitative viability outcomes. Studies limited to fixed appliances, lacking original data, or failing to specify extraction conditions were excluded. Key studies with transparent methodology are summarized in **Table 2**, illustrating the spectrum of cytotoxic responses across different materials.

Evidence from in vitro studies

Several in vitro investigations have examined oral cell viability following exposure to retainer or aligner materials. MTT assays on cultured gingival fibroblasts or epithelial cells are commonly used to evaluate metabolic activity as a proxy for cytotoxicity. Overall, findings suggest that PMMA and thermoplastic retainers exhibit minimal cytotoxicity in vitro, with cell viability generally remaining above biocompatibility thresholds [6].

Recent studies report low to moderate cytotoxicity for most orthodontic retainer materials, with cell survival typically exceeding 70–90%, even under conservative worst-case extraction conditions [15, 16]. Thermoplastic retainers, including PETG- and polyurethane-based materials, generally produce only minor reductions in cell viability [17]. For example, a comparative evaluation of four widely used clear thermoplastic retainers (Duran®, Biolon®, Zendura®, and Invisalign® SmartTrack) showed only mild cytotoxic effects on human gingival fibroblasts [15]. Material composition appears to influence cytotoxicity, as polycarbonate-based plastics tend to release more monomers than PETG or multilayer polyurethane under laboratory conditions [16, 18].

Emerging 3D-printed retainer materials have also been evaluated in vitro. Al Mortadi *et al.* [19] assessed the cytotoxicity of a photopolymer resin (Dental LT) and an ethanol-based hard resin (E-Guard) on oral cells. Both materials exhibited only slight cytotoxicity, and cell viability improved over time, suggesting leachable components either dissipate or cells adapt [19]. Initial cytotoxic effects were slightly higher for 3D-printed resins than for thermoplastics like SmartTrack, with E-Guard showing the greatest viability reduction on day 1. By contrast, SmartTrack consistently maintained over 90% cell viability in human gingival fibroblast assays [15, 20, 21]. Biolon and certain 3D-printed resins demonstrated greater viability reductions depending on post-processing quality; however, by day 7, all materials showed significant recovery, highlighting the importance of thorough post-curing and short-term soaking to mitigate leachable toxicity [19, 21].

Advanced polymers such as polyether-ether-ketone (PEEK) are increasingly used as aesthetic, metal-free retainer components. PEEK exhibits excellent chemical stability and minimal cytotoxicity in vitro, consistent with its established biocompatibility in biomedical applications [22, 23]. Clinical use of PEEK for fixed lingual retainers has not elicited adverse tissue responses, and it remains safe for procedures such as MRI.

Fiber-reinforced composite (FRC) retainers, though aesthetically appealing, may raise biocompatibility concerns. Glass- or quartz-fiber reinforced resins can release residual monomers, particularly if exposed or in acidic conditions, reducing oral fibroblast viability [24]. Simulated cariogenic challenges indicate that FRC retainers can release more cytotoxic components under low pH conditions. Interestingly, conventional multistrand wire retainers may also exhibit cytotoxicity due to metal ion release (e.g., Ni^{2+}, Cr^{6+}) under acidic conditions [25]. These findings underscore that both polymeric and metallic retainer materials can exert cytotoxic effects through degradation or leaching, although the overall impact is generally mild.

Table 2. In vitro studies evaluating the cytotoxic potential of orthodontic retainer materials using cell viability assays (e.g., MTT, live/dead staining) in oral fibroblasts and epithelial cells.

Study (Year)	Retainer Material(s) Tested	Model/Cells	Key Findings on Cytotoxicity	Study Design
Martina *et al*. [15]	Four thermoplastic brands: Duran (PET-G), Biolon (polycarbonate), Zendura (polyurethane), SmartTrack (polyurethane multilayer)	HGF cells; MTT assay	All materials caused only slight cytotoxicity (viability >80%). Biolon showed greatest toxicity (most viability reduction), followed by Zendura and SmartTrack. Duran had least effect (highest viability). Thermoforming did not eliminate cytotoxic agents and in some cases slightly increased cytotoxicity.	**In vitro**
Campo basso *et al*. [25]	3D-printed aligners using Tera Harz TC-85DAC resin (Graphy,	MC3T3-E1 mouse pre-osteoblasts	P1 (nitrogen cure): No cytotoxicity Viability >100% at both time points (107.1 ± 17.5% day 7, 106.7 ± 18.4% day 14) Comparable or better than	**In vitro**

	Korea) Post-cured via two methods: P1: Tera Harz Cure (nitrogen, 14 min) P2: Form Cure (30 min/side, 60 min total)	Cultured in DMEM; viability via MTT assay at days 7 and 14	control P2 (standard cure): Moderate cytotoxicity Viability significantly reduced (59.8 ± 10.1% day 7, 47.1 ± 20.6% day 14) $p < 0.001$ vs. P1 and control Conclusion: Post-curing method critically affects cytotoxicity. Nitrogen-based P1 is highly biocompatible; P2 leaves residual monomers causing toxicity.	
Nemec *et al*. [21]	Invisalign SmartTrack aligner (polyurethane) — inner vs. outer surface (as-grown cells)	Human oral keratinocytes; live/dead staining; PCR	No acute cytotoxicity — very few dead cells on aligner surfaces. Cell proliferation lower than plastic control (slight growth inhibition). Aligner-contact cells showed upregulated inflammatory and barrier-function genes. Conclusion: SmartTrack is non-cytotoxic but alters cell behavior, inducing a pro-inflammatory gene profile.	**In vitro**
Al Naqbi *et al*. [26]	Vivera® retainers (Invisalign®-associated polyurethane thermoplastic) Tested in two conditions: As-received Clinically used	MCF-7 cells (estrogen receptor-positive) MDA-MB-231 cells (estrogen receptor-negative control) NIH/3T3 mouse fibroblasts (general cytotoxicity)	No cytotoxic effect on fibroblasts for either as-received or used retainer eluates. No estrogen receptor-mediated proliferation in MCF-7 cells. No proliferative effect on MDA-MB-231 cells. Conclusion: Vivera® retainers show no acute cytotoxicity or estrogenicity under tested conditions. Good short-term biocompatibility.	**In vitro**

Summary of cytotoxicity findings

Over the past decade, research consistently indicates that materials used in removable orthodontic retainers are largely biocompatible. In vitro studies show only mild cytotoxic effects on oral cells, while in vivo data suggest minor and transient cellular stress [5, 6]. Differences in cytotoxicity remain detectable between material types and brands. Emerging materials such as polyether-ether-ketone (PEEK) appear particularly promising, exhibiting minimal cytotoxicity due to their chemical inertness and stability [23].

In vivo and clinical evidence

Human studies conducted in the last ten years have not reported serious cytotoxic reactions to retainer materials, though subtle biological responses have been observed. A notable randomized controlled trial evaluated patients wearing either a Hawley (acrylic) or Essix (thermoplastic) retainer. Saliva was analyzed for oxidative DNA damage markers (8-hydroxy-2′-deoxyguanosine, 8-OHdG) and antioxidant system responses (Nrf2, Keap1), while buccal mucosa cells were examined for nuclear anomalies. Patients with Hawley retainers exhibited a significant increase in salivary 8-OHdG after one and three months, indicating oxidative DNA damage likely linked to residual methyl methacrylate monomer or acrylic additives. In contrast, Essix users did not show elevated 8-OHdG; their levels slightly declined over time.

Interestingly, cytologic analyses revealed that Essix retainers were associated with a higher frequency of micronuclei and other nuclear anomalies in buccal cells after 2–3 weeks compared to Hawley retainers. Both appliance types led to increased cellular turnover and some nuclear alterations relative to baseline. The findings suggest distinct tissue interactions: acrylic retainers may induce systemic oxidative stress via leachable compounds, while thermoplastic retainers may cause more localized mechanical or frictional stress on mucosa, contributing to micronuclei formation [5].

Estrogenic potential and BPA release

A prominent biocompatibility concern for dental plastics is the potential for endocrine disruption, particularly via the release of estrogen-mimicking compounds. Bisphenol-A (BPA), a well-known xenoestrogen used in polycarbonate plastics and epoxy resins, can bind estrogen receptors, albeit with weaker affinity than endogenous estradiol, and has been associated with reproductive and developmental toxicity [27]. Because orthodontic retainers and aligners are plastic appliances used intraorally, there has been concern regarding possible BPA or related

xenoestrogen release into saliva. The clinical significance of such release, particularly systemic estrogenic effects, remains uncertain.

BPA release: in vitro vs. in vivo

Initial in vitro investigations often found either undetectable BPA levels or amounts below analytical detection limits (e.g., <1 ng/mL) from clear aligners. For example, Schuster *et al*. [28] and Gracco *et al*. [29] reported no measurable BPA or monomer release from Invisalign aligners soaked in artificial saliva. More recent studies, such as Katras *et al*. [30], tested multiple brands (SmileDirectClub, Invisalign, Essix ACE) in different media (saliva, gastric fluid, ethanol) and observed that any BPA release primarily occurred within the first 24 hours, remaining well below established safety limits. These investigations employed high-performance liquid chromatography (HPLC) or mass spectrometry for precise quantification, frequently detecting BPA levels at or below the limits of detection [6]. In vitro BPA release and associated estrogenic activity for various orthodontic retainer materials are summarized in **Table 3**.

Estrogenic activity and BPA release from retainer materials

In vitro assays have been used to determine whether leachates from orthodontic retainers can activate estrogen-sensitive cells. Two independent studies [18, 26] employed the MCF-7 breast cancer cell proliferation assay, a standard method for detecting estrogen receptor-mediated effects, to test both aligner and retainer materials. Neither study detected estrogenic activity: exposure to Invisalign® or Vivera® retainer materials did not stimulate MCF-7 cell proliferation above baseline levels. Positive controls, such as 17β-estradiol or BPA, induced significant proliferation, confirming assay sensitivity, whereas retainer samples performed similarly to negative controls. Parallel experiments using estrogen-insensitive MDA-MB-231 cells confirmed that the tested materials lacked estrogen receptor-mediated effects. These findings corroborate chemical analyses demonstrating that modern orthodontic polymers release negligible BPA. Sensitive analytical techniques, including gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS), have consistently failed to detect significant bisphenol traces in aligner material extracts [18, 26]. Studies conducted between 2016 and 2021 similarly reported undetectable BPA levels in saliva or artificial saliva following prolonged immersion of new clear aligners [6].

Clinical evidence, however, presents a more nuanced picture. Raghavan *et al*. [2] performed a randomized clinical trial evaluating salivary BPA concentrations in 45 patients assigned to three groups: Essix vacuum-formed retainers, heat-cured PMMA Hawley retainers, and cold-cure/autopolymerized PMMA Hawley retainers. Saliva samples collected before insertion, and at 1 hour, 1 week, and 1 month afterward, revealed significant post-insertion increases in BPA across all groups ($p \leq 0.05$) [2].

A subsequent trial by Nanjannavar *et al*. [12] examined the effect of pre-soaking retainers in 37 °C water for 24 hours prior to patient use. This intervention markedly reduced salivary BPA levels: 1-hour measurements showed ~0.07 ppm BPA in pre-soaked retainers versus 0.33 ppm in unsoaked ones. By 1 and 3 weeks, BPA in the pre-soaked group was near undetectable. These results suggest that a simple 24-hour water soak can substantially minimize patient exposure to leachable BPA [12].

It is important to contextualize these findings within evolving regulatory standards. Historically, the U.S. FDA and EPA established “tolerable daily intake” levels for BPA around 50 µg/kg body weight/day. More recent studies indicate that endocrine-disrupting effects may occur at much lower exposures, prompting agencies, especially in the EU, to reduce safe intake levels drastically (down to nanogram/kg ranges) between 2021 and 2023 [31]. Low-level BPA exposure has been linked to subtle hormonal and immune system alterations in both animal and human studies [32]. While current orthodontic retainer BPA release remains well below these thresholds, ongoing precautionary assessment continues.

To mitigate potential endocrine risk, the industry is increasingly shifting toward BPA-free adhesives and aligner materials. Innovations in smart polymers now aim to combine mechanical performance, antimicrobial activity, and biocompatibility without relying on endocrine-active compounds [33].

Table 3. Summary of BPA release levels and estrogenic activity in orthodontic retainers, based on in vitro assays, chemical analyses (HPLC, LC-MS/MS), and clinical trials.

Study (Year)	Materials and Conditions	BPA Release Findings	Estrogenic Effect	Study Design
Katras *et al*.,	SmileDirectClub, Invisalign, Essix ACE	Low BPA release from all aligners, mainly within first 24 h (initial	No direct estrogenicity test. BPA levels far below	In vitro

2021 [30]	aligners Incubated in artificial saliva, gastric fluid, 20% ethanol Sampled at 0, 1, 2, 6, 10, 20 days	burst). BPA levels <5 µg/L in saliva and below EU safety thresholds at all time points. No significant difference between brands or media.	toxicological concern → endocrine effects unlikely. Authors: BPA amounts "below established safety levels for adults".	
Intissar *et al*., 2020 [34]	Invisalign® aligners (polyurethane) New vs. 2-week used Stored in artificial saliva up to 8 weeks	No BPA detected in any extract (<5 ppb detection limit, HPLC). Even after intraoral use and prolonged saliva storage, chemically stable with respect to BPA.	Not applicable (chemical analysis only). Supports no detectable BPA leaching → no expected estrogenic stimulus.	In vitro
Raghavan *et al*., 2017 [2]	Patients (n = 45) wearing: 1. Vacuum-formed Essix (PET-G) 2. Heat-cured acrylic Hawley 3. Chemically cured acrylic Hawley Salivary BPA measured before and 1 month after delivery	Salivary BPA increased in all groups after 1 month: - Chemically cured Hawley: highest (~6–8 µg/L increase) - Essix: moderate (~2–3 µg/L) - Heat-cured Hawley: lowest (~1 µg/L or less) All values low (ppb range).	No clinical signs of endocrine disruption. BPA levels below known hormonal effect thresholds. Recommendation: Use heat-cured acrylic or BPA-free materials to minimize exposure.	In vivo (clinical)
Iliadi *et al*., 2017 [35]	BPA-free orthodontic adhesive (phenyl-propanediol dimethacrylate) vs. conventional Bis-GMA adhesive Used for fixed retainer bonding	No BPA release by design (no bisphenol derivatives). Experimental adhesive: undetectable BPA in eluates. Conventional adhesive: trace BPA from Bis-DMA degradation.	No estrogenic components. BPA-free adhesive: no estrogenic or cytotoxic effects in vitro. Similar bond strength to controls → clinically viable.	In vitro
Eliades *et al*., 2009 [18]	Three sets of Invisalign aligners Immersed in normal saline at 37 °C for 2 months Eluents tested at 5%, 10%, 20% concentrations	*(Note: Data repeated in original; likely incomplete reporting)* No specific BPA findings reported in summary. Study focused on general eluate composition; BPA not highlighted as a significant leachate.	No estrogenicity data reported. Eluates showed minimal biological activity at tested concentrations.	In vitro

BPA release from thermoplastic retainers

Thermoplastic retainers are capable of releasing measurable levels of BPA into saliva, particularly during the initial period of use. Simple interventions, such as pre-soaking retainers in water or selecting alternative materials, can substantially reduce this exposure. Heat-cured acrylic retainers, which contain negligible BPA, show minimal leaching, whereas certain thermoplastic types may exhibit transient BPA release. Although manufacturers increasingly promote BPA-free aligners, clinicians should remain vigilant regarding the presence of trace compounds.

Estrogenic activity of leachates

While detecting BPA is straightforward, assessing its potential estrogenic effects is more complex. Estrogen-sensitive cell assays have been employed to determine whether leachates from retainers can activate estrogen receptors. In vitro evidence indicates that any BPA or related compounds released are generally below the threshold required to trigger an estrogenic response [6]. Nevertheless, in vivo endocrine disruption can occur at very low doses, potentially exhibiting non-linear dose–response relationships. Chronic exposure, even at low concentrations, may theoretically exert subtle developmental or hormonal effects, although no clinical studies have definitively linked retainer use to systemic endocrine disorders. Evidence from other dental materials, such as sealants and composite resins, suggests that transient BPA spikes in saliva and urine occur after placement but typically return to baseline within 24–48 hours [27]. The American Dental Association acknowledges that trace amounts of BPA may transiently increase in saliva and urine following the introduction of freshly polymerized resins, a phenomenon consistent with observations in orthodontic retainers [27].

Beyond BPA, other xenoestrogens such as BPS or phthalates may be present in some plastic formulations. Most modern orthodontic appliances are phthalate-free, while BPS is sometimes used as a BPA substitute. Data on BPS leaching from retainers are limited, and its safety remains under debate. Overall, current evidence suggests that neither Hawley nor Essix retainers induce significant estrogenic activity under standard conditions of use. Measurable BPA can occur, particularly from certain thermoplastics, but levels are generally low. Both in vitro assays [6] and clinical

observations support the minimal hormonal impact of these appliances. Given the widespread use of retainers, however, cumulative exposure in adolescents warrants continued monitoring. Simple precautions, including pre-soaking new appliances and using BPA-free materials when feasible, can further mitigate potential endocrine-disrupting risks [12].

Cellular mechanisms of damage and estrogen action

Oxidative stress and DNA injury

Residual monomers, such as MMA from acrylic retainers, can leach into saliva and oral tissues and, to a minor extent, enter systemic circulation [36]. These monomers may undergo metabolic activation or redox reactions, generating reactive oxygen species (ROS). ROS can oxidize DNA, lipids, and proteins, resulting in oxidative damage [37]. Elevated salivary 8-hydroxy-2′-deoxyguanosine (8-OHdG) observed in Hawley retainer users [8] indicates oxidative DNA injury. While cellular repair mechanisms typically mitigate such damage, sustained 8-OHdG elevation reflects ongoing ROS exposure. Essix wearers, in contrast, did not show increased 8-OHdG levels, possibly due to lower ROS production or effective cellular adaptation. Cellular defense against oxidative stress often involves the Nrf2/Keap1 pathway. Upon activation by oxidative stimuli, Nrf2 translocates to the nucleus and upregulates antioxidant gene expression. In a recent trial, Gunel *et al*. measured Nrf2 and Keap1 levels in retainer users but found no significant differences between Hawley and Essix groups [5], suggesting that oxidative stress was either insufficient to elicit differential pathway activation or that both groups exhibited comparable antioxidant responses.

In addition to oxidative damage, direct cytotoxicity can occur via disruption of cell membrane integrity. Monomers and certain aligner additives can impair membrane potential and induce cell lysis, although salivary dilution mitigates these effects [6]. Cellular genotoxicity has also been observed, as evidenced by increased micronuclei formation in patients [5]. Micronuclei arise from chromosome fragments or whole chromosomes excluded from daughter nuclei during mitosis, typically due to DNA strand breaks or spindle disturbances. Elevated micronuclei frequencies after 2–3 weeks of Essix retainer use suggest an acute genotoxic response, likely linked to initial chemical leachates or mucosal friction [8].

Estrogen receptor activation pathways

Leached chemicals such as BPA have the potential to interact with estrogen receptors (ERα and ERβ) in various tissues. In the oral cavity, cells like periodontal ligament fibroblasts and bone cells express ERs, while the oral epithelium is not a primary estrogen-responsive tissue. Systemically absorbed BPA, however, could target distant endocrine organs. BPA binding to ERs can mimic natural estrogen, potentially altering gene transcription. Chronic low-dose exposure in toxicological studies has been associated with developmental, reproductive, metabolic, and behavioral changes [27]; however, these effects usually result from prolonged exposure levels far exceeding those generated by orthodontic retainers.

Locally, estrogen signaling might theoretically influence wound healing and inflammation. If BPA from retainers were absorbed through oral mucosa, it could potentially affect gingival tissues or alveolar bone. To date, no clinical evidence supports this possibility. While in vitro studies suggest BPA may modulate inflammatory pathways, no direct association has been established between BPA from orthodontic appliances and gingival inflammation. Animal or cell-based studies would be required to evaluate whether salivary BPA at retainer-use concentrations can activate local ER-mediated responses. The absence of MCF-7 cell proliferation in response to aligner extracts [6] indicates that estrogenic activity of these leachates is extremely low. Additionally, BPA is rapidly eliminated in humans, with a short half-life, making sustained estrogen receptor activation unlikely. Estrogenic responses can show non-monotonic dose–response behavior, where lower doses may paradoxically produce stronger effects than moderate doses due to regulatory feedback [27].

In conclusion, although retainer materials can release chemicals capable of binding estrogen receptors, current evidence indicates that in vivo estrogenic activation is minimal. The molecular mechanism—xenoestrogen binding to ER leading to gene expression changes—is well characterized, but modern retainer materials appear to trigger this pathway only minimally. Ongoing material refinement, such as avoiding Bis-DMA monomers that degrade into BPA [27], remains important to further minimize potential estrogenic effects, particularly in young patients or those using retainers long-term.

Clinical implications of long-term retainer use

Clinically, the critical question is whether cytotoxic or estrogenic effects from retainers translate into tangible health risks. Retainers are often worn nightly for years, sometimes indefinitely, to maintain dental alignment. In patients with hypodontia or delayed permanent restoration, retainers may be worn daily until adulthood. Understanding the implications of long-term exposure is therefore essential.

Mucosal health and patient symptoms

Most patients tolerate Hawley and Essix retainers without significant tissue damage. Nevertheless, some adverse effects have been documented, including:

- **Initial irritation:** Mild soreness of gums or palate is common when a new retainer is inserted. These symptoms usually resolve as tissues adapt or residual monomers are eliminated. Patients sometimes report transient discomfort or altered taste, potentially linked to initial chemical release [30].
- **Ulceration or contact dermatitis:** Rare allergic reactions to PMMA or other plastic components can occur, presenting as localized redness, ulcers, or even more diffuse symptoms like lip swelling and itching. Acrylic allergy is well-documented in dentistry, particularly among denture wearers. A switch to hypoallergenic materials or alternative designs may be needed for sensitive individuals [38].
- **Taste disturbance and dry mouth:** Some patients notice a plastic or chemical taste initially, and xerostomia has been reported [6]. Reduced salivary flow can exacerbate cytotoxic effects because saliva helps neutralize irritants.
- **Periodontal considerations:** Poorly fitting or inadequately cleaned retainers can provoke gingival inflammation. Although this is not a direct chemical toxicity issue, chronic inflammation can contribute to oxidative stress. Removable retainers, particularly Essix types worn mainly at night, generally offer better periodontal outcomes than fixed retainers due to ease of cleaning and reduced plaque accumulation [39].

An overview of common removable orthodontic retainers and their associated biological considerations is summarized in **Table 4**.

Table 4. Overview of removable orthodontic retainer types, associated chemical components, and reported biological risks (cytotoxicity, endocrine activity), based on in vitro and clinical evidence.

Retainer Type	Material	Common Monomers/Additives	Potential Risks
Hawley Retainer	PMMA + stainless steel wire	Methyl methacrylate (MMA), residual monomers	Cytotoxicity, allergic reactions (e.g., contact stomatitis), MMA leaching
Essix (C+) Retainer	PVC-based thermoplastic	Phthalates, residual vinyl chloride	Endocrine disruption, possible plasticizer release
Essix ACE Retainer	Copolyester (PET-G based)	BPA, PET-G oligomers	Low-level BPA release, minor cytotoxicity
Modern orthodontic thermoplastics (e.g., Duran®, Essix ACE®, Zendura® FLX)	Polyurethane	BPA, BPS	Potential estrogenic activity (in vitro), low cytotoxicity
3D-printed retainer (acrylate resin)	Multilayer polyurethane (proprietary)	BPA analogs (e.g., BPS, BPF?)	Unclear — depends on aging, wear, and proprietary formulation

Regulatory standards and international oversight

Ensuring the safety of dental materials used in removable orthodontic retainers requires both scientific evaluation and adherence to regulatory frameworks. In the United States and Europe, distinct but complementary regulations govern biocompatibility and chemical safety for these devices.

United States (FDA)

In the U.S., removable retainers are classified as Class II medical devices. Manufacturers must submit a 510(k) notification to demonstrate that their devices are substantially equivalent to existing products on the market [40]. Safety testing follows ISO 10993 standards [41], including assessments for cytotoxicity (ISO 10993-5), irritation and sensitization (ISO 10993-10), and other relevant evaluations depending on the intended tissue contact and duration of use.

Appliances intended for prolonged contact with oral mucosa must meet ISO 10993 requirements to confirm the absence of acute or chronic toxicity, genotoxic effects, or tissue irritation. Commercial products, such as Invisalign® SmartTrack, claim compliance with the full ISO biocompatibility testing suite.

Regarding chemical leachates such as bisphenol A (BPA), the FDA has not set explicit limits for dental appliances. Unlike restrictions applied to baby bottles, dental materials are evaluated using a risk-based approach. Trace amounts of BPA or degradation byproducts may occur, but exposure from retainers is

considered minimal and short-lived [42]. While voluntary efforts to reduce BPA exposure are encouraged, no legal restriction currently exists, and most manufacturers have moved toward BPA-free formulations to meet market expectations.

European union regulations

The EU enforces stricter rules concerning hazardous chemicals in medical devices. Under MDR Regulation (EU) 2017/745, manufacturers must assess and disclose any presence of carcinogenic, mutagenic, reprotoxic (CMR), or endocrine-disrupting substances [43]. Substances classified as "very high concern" (SVHC), including BPA, require justification, risk evaluation, and labeling if they exceed 0.1% of device weight. Although BPA in retainers usually falls below this threshold, manufacturers have reformulated materials to eliminate BPA entirely, simplifying compliance and addressing consumer safety concerns.

Material standards and CE marking

European standards for orthodontic appliance polymers are specified in ISO 20795-2:2013 ("Dentistry—Base polymers—Part 2: Orthodontic base polymers") [44]. This standard defines mechanical properties, such as flexural strength and color stability, while indirectly supporting biocompatibility by controlling residual monomer content. For example, allowable unreacted methyl methacrylate in acrylic devices is generally ≤2%. CE marking requires conformity with both ISO 20795-2 and ISO 10993, ensuring appliances are mechanically reliable and biologically safe for long-term oral use.

ISO 10993 biocompatibility testing

ISO 10993 evaluations are required for devices intended for extended mucosal contact (>30 days). Testing typically includes cytotoxicity, subacute and chronic systemic toxicity, mucosal irritation, and, if indicated, genotoxicity assessments. Novel materials or those with suspected endocrine activity require additional scrutiny. While some independent studies have reported minor cytotoxic or estrogenic responses, these remain below ISO safety thresholds [45]. Compliance with ISO 10993 standards is widely accepted as evidence of clinical safety.

Labeling and product transparency

In the EU, devices containing SVHCs above 0.1% must disclose this in technical documentation and labeling [46]. Instructions for use (IFU) and safety data sheets (SDS) should clearly indicate the presence or absence of BPA, phthalates, or other potential endocrine disruptors. In the U.S., such labeling is generally voluntary except for recognized allergens. Manufacturers increasingly highlight "BPA-free" or "phthalate-free" status to provide transparency to clinicians and patients.

Professional and clinical guidelines

Professional dental and orthodontic organizations have increasingly highlighted concerns regarding the release of chemical byproducts from polymer-based appliances and their potential systemic effects. Recent research emphasizes the importance of continued innovation in retainer materials, including the development of direct 3D-printed devices and enhanced analytical monitoring of monomer release, to minimize patient exposure to potentially harmful compounds [47, 48].

Current regulatory frameworks provide a comprehensive basis for evaluating orthodontic retainer safety. No standard orthodontic appliance in routine clinical use has been banned by either the FDA or EU authorities, indicating that chemical leaching and cytotoxicity levels remain within acceptable limits. Nonetheless, the European MDR requirement to disclose substances of very high concern (SVHCs) exceeding 0.1% by weight has motivated manufacturers to adopt cleaner, more transparent formulations.

Clinicians should stay informed about the composition of orthodontic materials and consider BPA-free or hypoallergenic alternatives for sensitive patients. While the benefit-risk balance of removable retainers continues to be favorable, evolving regulatory requirements and public scrutiny underscore the importance of ongoing material refinement and adherence to updated safety standards.

One health and environmental considerations

Beyond individual patient biocompatibility, attention has expanded to encompass broader environmental and public health implications of orthodontic retainers. The One Health framework, which considers the interconnectedness of human, animal, and ecosystem health, provides a useful lens for evaluating polymer-based orthodontic appliances [49, 50].

Microplastic release

Polymer-based retainers can degrade under mechanical stress and chemical exposure within the oral cavity, shedding micro- and nanoplastic particles (MNPs). Researchers demonstrated microplastic release from aligner surfaces after one week of simulated use [51], and Barile *et al*. [39] reported fragment detachment during cyclic loading of various aligner brands. Particle sizes ranged from tens to hundreds of micrometers,

though smaller nanoparticles (<1 μm) may penetrate epithelial barriers, with evidence of their presence in systemic circulation in unrelated contexts [52]. While acute health effects appear minimal, the potential implications of chronic low-dose exposure—particularly among younger patients—remain insufficiently studied.

Chemical leaching and ecosystem impact

Removable retainers can act as diffuse sources of bisphenols and other leachable additives. Though individual devices release small quantities, frequent replacement—weekly during active treatment or semiannually during retention—contributes to cumulative environmental load. In landfills, residual monomers such as BPA persist and may leach into soil or groundwater. Even trace amounts of BPA can disrupt endocrine systems in aquatic species, inducing developmental or reproductive changes at extremely low concentrations. The chemical persistence and mixed-material composition of orthodontic appliances further limit recycling options, underscoring their environmental relevance [52].

Sustainable design and preventive strategies

The elimination of hazardous compounds in orthodontic appliances provides simultaneous benefits for patient safety, occupational exposure, and environmental protection. BPA- and phthalate-free materials reduce chemical exposure for users and dental staff, while chemically stable polymers mitigate environmental contamination via landfill or wastewater leachates. Strategies emphasizing material efficiency, reduced toxicity, and end-of-life accountability support a sustainable orthodontic model [53, 54]. Pilot initiatives, such as a 2022 UK program by Align Technology, implemented post-use aligner collection for energy recovery or downcycling, reflecting an early industry move toward environmentally responsible practice.

Emerging eco-friendly materials

Research is increasingly focused on developing biopolymer alternatives to traditional petroleum-based plastics for orthodontic use. Thermoformable matrices made from cellulose acetate demonstrate partial biodegradability and can incorporate antimicrobial agents. For example, cellulose aligners loaded with cinnamaldehyde have shown the ability to inhibit biofilm formation in vitro without compromising cell viability [55, 56]. Composites containing nanohydroxyapatite or quaternary ammonium compounds have also displayed antibacterial and remineralizing properties while remaining cytocompatible [57].

Despite these promising results, additional studies are needed to assess long-term mechanical performance, biocompatibility under extended intraoral use, and potential nanoparticle release during wear and disposal. Data on the environmental fate of these materials remain limited, highlighting the need for comprehensive life-cycle evaluation.

Green dentistry practices

Minimizing the ecological footprint of orthodontics requires both material innovation and workflow optimization. Digital impressions reduce the need for disposable trays, and improved 3D printing techniques help limit resin waste. Durable retainers made from PEEK or laminated polymers may reduce replacement frequency, further conserving resources. Such practices align with life-cycle assessment principles by considering both clinical efficacy and environmental resource consumption [54].

Balancing patient preferences—such as the demand for thin, frequently replaced retainers—with sustainability remains a challenge. Recent literature emphasizes integrating environmental indicators into clinical decision-making to support both patient care and ecological responsibility [58-67].

Environmental regulations and policy outlook

While no regulations currently target orthodontic appliances specifically, existing legislation may indirectly influence future material selection. For instance, EU Regulation 2017/745 mandates disclosure and justification for devices containing more than 0.1% by weight of substances of very high concern (SVHCs), including BPA. ISO 10993 standards continue to guide biocompatibility testing, but regulatory attention is increasingly expanding to cover environmental impacts and life-cycle considerations [43, 45].

Environmental implications

The One Health approach situates orthodontic biomaterials within a broader context of interconnected human, animal, and environmental health. Removable retainers and clear aligners, as polymer-based devices, contribute to plastic waste and raise questions regarding environmental persistence and chemical leaching.

Microplastic and nanoplastic release

Mechanical stress from mastication, oral habits, and thermal and chemical challenges can degrade polymeric aligners, releasing micro- and nanoplastic

particles. Quinzi *et al*. demonstrated that aligners shed particles in the 5–20 µm range after seven days of simulated use, with emission levels varying by brand [51]. While the health effects of oral microplastic ingestion are not fully understood, general evidence links microplastics to inflammation and potential tissue uptake.

Plastic waste generation and disposal

Each aligner weighs approximately 4–5 grams per pair, and full treatment may involve 20–30 sets. Cumulatively, a single patient may generate over 100 grams of polymer waste, and with millions of patients worldwide, the total annual waste from aligner therapy is substantial [52]. Essix retainers, although less frequently replaced, also contribute to landfill accumulation. Recycling is largely infeasible due to biohazard classification and multi-material construction, resulting in long-term persistence of polymers in the environment [68-71].

Public health and ecosystem considerations

Microplastic contamination is increasingly recognized as a public health issue, with particles detected in water supplies and human tissues. While orthodontic devices contribute only a minor fraction of overall plastic pollution, sustainability initiatives are gaining traction. Macrì *et al*. proposed a "4Rs" strategy—Reduce, Reuse, Recycle, Rethink—to mitigate environmental impact. This includes minimizing material use, exploring repurposing options, establishing recycling pathways, and transitioning to biodegradable polymers [52].

From a regulatory perspective, the presence of BPA or other SVHCs in orthodontic materials raises environmental concerns. Though devices have not been explicitly targeted, manufacturers have proactively reduced or eliminated BPA content, reflecting precautionary measures to comply with existing and potential future regulations.

Conclusion

Recent research has increasingly focused on the biocompatibility of orthodontic retainer materials, reflecting advances in safety standards and scientific understanding. Evidence indicates that removable retainers made from PMMA-based acrylics or PETG/TPU thermoplastics are generally safe. Mild cytotoxic and estrogenic effects have been observed in vitro and in vivo, but no severe adverse biological outcomes have been reported. Both Hawley and Essix-type appliances have been widely used clinically without major safety concerns. Nevertheless, detectable oxidative stress markers, subcellular changes, and trace levels of bisphenol compounds suggest that these materials interact with oral tissues to a small but measurable extent, highlighting opportunities for ongoing refinement in composition and safety.

Evidence-based clinical recommendations

- **Overall safety:** Both Hawley and Essix retainers may cause slight cytotoxicity in vitro and minor biomarker changes in vivo, but these effects are not clinically significant. Patients can be reassured, while clinicians should remain vigilant for rare allergies or sensitivities.
- **Residual monomer management:** Acrylic Hawley retainers can release residual MMA. Using heat-cured acrylics and soaking appliances in water before full-time use can reduce initial exposure. Persistent taste or burning sensations may warrant extended soaking or remanufacturing with improved curing.
- **BPA and xenoestrogen mitigation:** Some thermoplastic retainers may release BPA or related compounds, especially within the first 24 hours of use. Pre-rinsing or soaking new appliances is advisable, and BPA-free products should be preferred, particularly for young patients or women planning pregnancy.
- **Monitoring and maintenance:** Retainer checkups should include mucosal inspection to detect early irritation. Adjusting or polishing appliances can prevent mechanical stress that may contribute to cellular responses.
- **Patient education:** Daily cleaning of retainers is essential not only for hygiene but also to minimize plaque-associated inflammation that could amplify cellular stress.
- **Alternative materials for sensitive individuals:** For patients with known acrylic sensitivities, polypropylene-based Essix retainers or fixed alternatives may be preferable, as they minimize monomer exposure.

Future directions and research priorities

- **Novel material surveillance:** New retainers with antimicrobial or bioactive agents should undergo thorough toxicological evaluation to

ensure no unintended cytotoxic or endocrine effects arise.

- **Biodegradable and sustainable materials:** Development of recyclable or biodegradable polymers aligns with environmental sustainability goals. However, current biodegradable options often lack the mechanical strength and clarity needed for long-term orthodontic use, requiring careful balance with clinical performance and patient safety.
- **Long-term in vivo studies:** Research should examine prolonged appliance wear, tracking oxidative stress, genotoxic markers, and systemic biomarkers over months or years.
- **Mechanistic investigations:** Studies are needed to understand precisely how specific additives or monomers contribute to cytotoxicity or estrogenic effects.
- **Regulatory evolution:** Stricter thresholds for BPA and other leachables may emerge, guiding manufacturers toward reformulated, safer materials.
- **Pediatric and adolescent focus:** Minimizing even small risks is particularly important in younger populations who may wear retainers for extended periods.

Establishing standardized testing protocols for biocompatibility and endocrine-disrupting potential will enhance comparability across materials. In summary, current evidence supports the continued safe and effective use of Hawley and Essix retainers. Advances in polymer science and heightened awareness of biocompatibility, combined with evidence-based practices and thoughtful material selection, promise to further optimize the safety, effectiveness, and environmental sustainability of retention therapy.

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