CATÓLICA
FACULDADE DE MEDICINA DENTÁRIA
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DO CLEAR ALIGNERS RELEASE TOXIC CHEMICALS? –

A SYSTEMATIC REVIEW

Dissertação apresentada à Universidade Católica Portuguesa para obtenção do grau de Mestre em Medicina Dentária

Por: Mariana de Almeida Baraças Ferreira

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Por: Mariana de Almeida Baraças Ferreira

Orientador: Professor Doutor Pedro Lopes

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Viseu, 2025

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DEDICATÓRIA

Dedico esta tese à memória do meu avô, cuja presença continua viva em cada conquista. E à minha família, por ser alicerce e apoio incondicional.

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RESUMO

Os alinhadores transparentes são uma solução ortodôntica recente concebida para tratar as más oclusões dentárias de forma discreta e eficaz. Em termos de efeitos secundários clínicos, os problemas mais relatados entre os utilizadores de alinhadores são uma ligeira irritação da mucosa oral, inflamação localizada e hipersensibilidade. A utilização de materiais poliméricos avançados nestes alinhadores transparentes, como o poliuretano e o PET-G, alcançou uma aceitação generalizada devido às suas propriedades mecânicas, biocompatibilidade e atrativo estético. No entanto, persistem preocupações relativamente ao seu potencial de libertação de compostos químicos. O objetivo deste trabalho é compreender o impacto da toxicidade dos alinhadores transparentes na cavidade oral de pacientes ortodônticos. Para tal, foi realizada uma extensa pesquisa sistemática nas bases de dados electrónicas PubMed, Scopus e Cochrane, para identificar artigos com dados relevantes. Esta revisão sistemática foi realizada de acordo com as diretrizes *Preferred Reporting Items for Systematic reviews and Meta-analysis* para responder a uma pergunta formulada de acordo com a estratégia População, Intervenção, Comparação e Resultados (PICO). Foram identificados quatrocentos e treze artigos potencialmente relevantes e, após a aplicação das diretrizes PRISMA e dos critérios de inclusão/exclusão, foram incluídos sete artigos nesta revisão. Os resultados sugerem que os alinhadores transparentes são geralmente seguros, mas subsistem preocupações relativamente à lixiviação química dos materiais termoplásticos, à acumulação de bactérias devido à redução do fluxo salivar e a respostas inflamatórias ligeiras. Esta revisão enfatiza que, embora a maioria dos materiais seja biocompatível, alguns apresentam citotoxicidade moderada, com o potencial de afetar a saúde oral destes pacientes, sublinhando a necessidade de investigação contínua e de melhorias na composição dos materiais.

Palavras-chave: alinhadores transparentes; materiais poliméricos avançados; citotoxicidade; ortodontia

ABSTRACT

Clear aligners are a modern orthodontic solution designed to address dental malocclusions discreetly and effectively. In terms of clinical side effects, the most reported issues among aligner users are mild irritation of the oral mucosa, localized inflammation, and hypersensitivity. The use of advanced polymer materials in clear aligners, such as polyurethane and PET-G, has gained widespread acceptance due to their mechanical properties, biocompatibility and aesthetic appeal. However, concerns persist regarding their potential to release chemical compounds. The goal of this work is to understand the impact of the toxicity of clear aligners on oral cavity of orthodontic patients. An extensive systematic search was conducted in electronic databases PubMed, Scopus and Cochrane, to identify articles with relevant data. This systematic review was conducted following the Preferred Reporting Items for Systematic reviews and Meta-analysis guidelines to answer a question formulated according to the Population, Intervention, Comparison and Outcomes strategy. Four hundred and thirteen articles potentially relevant were identified and after applying PRISMA guidelines and inclusion/exclusion criteria, seven articles were included in this review. The results suggest that clear aligners are generally safe, but concerns remain regarding the chemical leaching of thermoplastic materials, bacterial accumulation due to reduced saliva flow, and mild inflammatory responses. This review emphasizes that although most materials are biocompatible, some exhibit moderate cytotoxicity, with the potential to impact these patients' oral health, underscoring the need for continued research and improvements in material composition.

Keywords: clear aligners; advanced polymer materials; cytotoxicity; orthodontics

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INTRODUCTION

1. INTRODUCTION

1.1 Orthodontics: Definition and purpose

Orthodontics is a specialized branch of dentistry focused on the diagnosis, prevention, and correction of misaligned teeth, jaw irregularities, and malocclusion patterns (1). Orthodontic interventions are essential not only for aesthetic improvement but also for enhancing functional aspects of dental occlusion and alignment. These treatments, which include both traditional and modern orthodontic devices, play a critical role in overall oral health by preventing complications such as periodontal disease, temporomandibular joint dysfunction (TMJ), and dental decay that can result from difficulty in cleaning crowded teeth (2).

Beyond aesthetics, orthodontic treatment aims to improve fundamental oral functions. Proper alignment of teeth contributes to effective biting and chewing, essential for nutrition and digestive health, as well as clear speech. Additionally, studies underscore the significant psychosocial impact of orthodontics, noting that patients often experience enhanced self-esteem and confidence with well-aligned teeth, which are closely tied to an improved quality of life (3).

The field of orthodontics has seen substantial advancements, particularly with the development of clear aligners, which offer patients a discreet alternative to traditional metal braces. Clear aligners are increasingly popular for treating mild to moderate malocclusions, and they are associated with fewer adverse effects on oral health compared to fixed appliances (4,5). These aligners are especially appealing to adults who seek effective yet inconspicuous treatments, reflecting a shift in orthodontic approaches that prioritize patient comfort and lifestyle compatibility (5).

Clear aligners and other advanced orthodontic materials are designed with biocompatibility and safety in mind, meeting stringent requirements to ensure they do not induce cytotoxic effects or release harmful substances during use (6,7). Moreover, aligners with antibacterial properties, such as those enhanced with fluoride coatings, can reduce enamel demineralization, further contributing to long-term oral health (8,9).

1.2 Fixed orthodontic appliances: Characteristics, plaque accumulation, and caries risk

Fixed orthodontic appliances, commonly referred to as braces, are a widely used orthodontic treatment modality designed to correct dental misalignments by applying continuous, gentle forces to teeth. These appliances typically consist of metal brackets, arch wires, and bonding materials that remain affixed to the teeth throughout the treatment duration. Due to their non-removable nature, fixed appliances offer a high degree of control over tooth movement, making them suitable for a broad range of complex dental malocclusions (10). While fixed appliances are highly effective, they introduce significant challenges in maintaining oral hygiene, which can lead to increased plaque accumulation and elevate the risk of dental caries, gingivitis, and other oral health issues (3,11).

The configuration of fixed appliances, specifically the presence of brackets, bands, and wires, creates multiple retentive areas that make tooth cleaning more difficult. Studies indicate that these retentive sites allow for food debris and plaque to accumulate more readily than on unencumbered tooth surfaces (12). Plaque, a biofilm composed of oral bacteria and other organic materials, thrives in the microenvironments provided by orthodontic brackets and wires. A key concern associated with plaque accumulation is the development of dental caries, as bacteria in the plaque metabolize sugars and produce acids that can demineralize the enamel surface, potentially leading to white spot lesions and cavities (13). Furthermore, the inability to effectively remove plaque around fixed appliances is associated with an increased risk of gingival inflammation and periodontal disease (2,6,14).

White spot lesions, an early sign of enamel demineralization, are commonly observed around brackets in patients undergoing orthodontic treatment (15). These lesions, which often develop within the first few months of treatment, can be irreversible and pose a significant aesthetic concern once the braces are removed (16). According to Chapman et al. (17), the prevalence of white spot lesions in orthodontic patients can be as high as 50%, underscoring the need for strict oral hygiene practices and preventive strategies (15). The likelihood of caries development is exacerbated by poor dietary habits, such as high consumption of sugary foods and beverages, which contribute to an acidic oral environment favorable to cariogenic bacteria (16). Consequently, orthodontic patients are encouraged to adopt a low-sugar diet and to use fluoride-based products to reinforce enamel resistance to acid attacks (8).

In addition to caries risk, plaque accumulation around fixed appliances contributes to a heightened risk of gingivitis. Gingivitis, characterized by inflammation of the gingival tissues, often results from the presence of microbial plaque near the gumline (18). The inflammatory response is initiated by bacterial byproducts, which irritate the gum tissue, leading to redness, swelling, and bleeding upon brushing or flossing. In the context of fixed orthodontic appliances, gingival inflammation is particularly concerning due to the difficulty of plaque control, especially for younger patients who may struggle with comprehensive oral hygiene routines. As noted by Ristic *et al*. (19), orthodontic patients with fixed appliances demonstrate higher gingival index scores than those without appliances, indicating a greater incidence and severity of gingivitis in this population (9).

To mitigate the risks associated with fixed appliances, various preventive measures are recommended. These include the use of fluoride mouth rinses, which can aid in remineralizing enamel, and the application of antimicrobial agents like chlorhexidine to control bacterial proliferation in plaque biofilms (20). Additionally, regular professional cleanings and the use of interdental brushes and water flossers can enhance plaque removal in areas that are challenging to access with standard toothbrushes alone (21). Recent advancements in orthodontic bracket designs, such as self-ligating brackets, have shown promise in reducing plaque accumulation by minimizing the contact area between the bracket and the tooth surface, although evidence remains inconclusive (4,22).

1.3 Microbiome associated with fixed appliances

The oral microbiome is a complex ecosystem, comprising various microorganisms that maintain a delicate balance crucial to oral health (23). However, the introduction of fixed orthodontic appliances disrupts this balance, creating a microenvironment that facilitates the proliferation of specific bacteria associated with oral diseases (24). Unlike other dental interventions, fixed appliances introduce non-shedding surfaces and micro-niches that significantly alter microbial dynamics. This shift is often marked by an increase in pathogenic bacteria and a decrease in microbial diversity, leading to a heightened risk of oral infections and inflammatory responses (3,25).

The alteration in microbial composition associated with fixed appliances is largely driven by the surfaces introduced, such as brackets and bands, which provide additional adhesion sites for bacteria. Studies demonstrate that orthodontic brackets increase the prevalence of *Streptococcus mutans* and *Lactobacillus* species, both of which are key contributors to cariogenic biofilms (9,26). This is further exacerbated by the reduced mechanical clearance of bacteria from these surfaces, as the structural complexity of fixed appliances impedes effective oral hygiene. Moreover, these bacterial shifts are not limited to cariogenic species but also include periodontopathogens like *Porphyromonas gingivalis*, which are associated with gingival inflammation and periodontal complications (27).

In addition to promoting specific bacterial species, fixed appliances are known to reduce the overall diversity of the oral microbiome. A balanced, diverse microbial community is essential for the resilience of the oral environment against environmental stresses, such as pH fluctuations due to dietary acids or sugar intake. Research employing next-generation sequencing (NGS) highlights that orthodontic patients experience a marked reduction in microbial diversity during treatment, which may weaken the microbiome’s resistance to pathogenic invasion (2,28). Consequently, orthodontic patients are more susceptible to rapid microbial shifts in response to changes in oral hygiene practices or dietary habits (8,29).

The interplay between fixed appliances and the oral microbiome also extends to biofilm maturation and resilience. Biofilms associated with orthodontic appliances tend to mature faster and exhibit increased resistance to antimicrobial treatments, as compared to biofilms in non-orthodontic patients (30). The resilience underscores the challenge of managing oral health in orthodontic patients, as standard preventive measures may be insufficient to disrupt the robust biofilms that form around orthodontic components (6).

To mitigate the adverse effects of these microbial changes, targeted preventive strategies are recommended. Fluoride applications and antimicrobial mouth rinses, such as chlorhexidine, can help reduce bacterial proliferation and acid production. Furthermore, emerging research suggests that probiotics may offer a promising approach by enhancing beneficial bacterial populations within the oral cavity, potentially counteracting the pathogenic shifts associated with fixed appliances (31). Regular dental check-ups, combined with specialized cleaning tools like interdental brushes, can also aid in maintaining microbial balance and minimizing the risks posed by pathogenic biofilms in orthodontic patients (9).

1.4 Introduction of thermoplastic materials with clear aligners

1.4.1 Characterization of clear aligners

Clear aligners, also referred to as invisible aligners, represent an innovative approach to orthodontic treatment, providing an alternative to traditional fixed braces. These devices consist of custom-made, clear, removable trays that fit snugly over the teeth and are used to incrementally reposition them into the desired alignment (32). The increasing popularity of clear aligners stems from their aesthetic appeal, convenience, and adaptability, making them particularly appealing to adult patients who seek a discreet method for correcting dental malocclusions (5,33). By allowing patients to maintain their regular dental hygiene routines and reducing the social stigma often associated with visible orthodontic appliances, clear aligners align with modern demands for unobtrusive, patient-friendly treatments (31).

The design and function of clear aligners are rooted in digital orthodontic technology, where the treatment process begins with a digital scan or impression of the patient’s teeth. This image is then used to create a precise 3D model of the dental arches, enabling the orthodontist to develop a comprehensive treatment plan (34). Using computer-aided design/computer-aided manufacturing (CAD/CAM) technologies, a series of aligners is fabricated to guide the teeth through progressive movements toward optimal alignment. Each aligner in the sequence is typically worn for one or two weeks, applying gentle, controlled pressure on specific teeth to achieve the planned movements (35). Compared to conventional braces, this staged process allows for more predictable and controlled adjustments, contributing to the popularity and clinical acceptance of aligners in cases of mild to moderate malocclusions (36).

One of the key distinguishing features of clear aligners is their transparency, which is achieved through the use of advanced thermoplastic materials such as polyurethane or polyethylene terephthalate glycol-modified (PET-G). These materials are selected for their durability, flexibility, and biocompatibility, allowing aligners to withstand the oral environment and maintain their shape and clarity over time (37,38). The material properties also contribute to patient comfort, as the aligners are custom fitted to each individual's dental morphology and are free from the metal brackets and wires associated with traditional braces. As a result, aligners generally cause fewer oral abrasions and require less frequent adjustments, offering a comfortable and practical solution for patients (4,39).

In terms of effectiveness, research indicates that clear aligners can produce results comparable to traditional fixed appliances for certain types of malocclusions. For example, studies by Miller *et al*. (40) suggest that aligners are particularly effective for resolving minor crowding and spacing issues, as well as for performing controlled tipping and limited tooth rotations. However, the limitations of aligners become apparent in more complex orthodontic cases, such as those requiring significant root movement or correction of severe malocclusions, where fixed appliances may still offer superior outcomes (41). Therefore, while aligners represent a versatile option for many patients, careful case selection remains crucial for achieving optimal results (41).

Clear aligners also allow for improved oral hygiene during treatment, as they are removable and do not create additional plaque-retentive areas on teeth (42). Patients are encouraged to remove the aligners during meals and to brush and floss regularly, thereby reducing the risk of plaque accumulation, gingivitis, and enamel demineralization commonly associated with fixed orthodontic appliances (9,43). This removability, however, places responsibility on the patient to adhere to the recommended wear time, generally between 20-22 hours per day, to ensure the efficacy of the treatment (10).

Furthermore, while the benefits of clear aligners are significant, understanding the potential for these materials to degrade over time and the subsequent health risks is critical for patient safety. The degradation of the aligner material could potentially lead to the release of toxic substances that may have harmful effects, ranging from mild irritation to more severe systemic health concerns.

1.4.2 Manufacturing process of clear aligners

The manufacturing process of clear aligners combines digital imaging, CAD, and thermoforming techniques to create precise, customized appliances. This begins with high-resolution digital scans or impressions of the patient's dentition, which generate a 3D model of the dental arches. These digital impressions allow orthodontists to design a detailed treatment plan, mapping out the incremental tooth movements needed over time (37,44). CAD software is then used to develop a sequence of aligners, each slightly modified to apply controlled forces that guide the teeth gradually toward alignment (5,45).

Once the digital model is complete, the aligners are produced through a process known as thermoforming. In this stage, 3D-printed molds representing each treatment step are used to shape aligners from thermoplastic materials such as polyurethane or PET-G, chosen for their strength and transparency (7,30). The thermoplastic sheets are heated and vacuum-formed over each mold, ensuring a snug fit to the teeth's contours. After cooling, each aligner is carefully trimmed and polished for comfort and appearance (46).

Quality control is integral throughout manufacturing, as each aligner must match precise specifications to ensure proper fit and function. This includes inspecting for any material inconsistencies or dimensional inaccuracies that could affect the appliance's effectiveness. Additionally, advancements in 3D printing have further streamlined this process, enhancing both production speed and cost-efficiency (6,35). For instance, thermoplastic materials must be assessed for biocompatibility and resistance to deformation over time, as slight changes in properties can impact the sequential alignment process (3).

1.4.3 Composition and material of clear aligners

The materials must meet strict criteria, such as biocompatibility, resistance to deformation, and the ability to maintain clarity throughout the treatment. Given the close and prolonged contact with the oral environment, the materials used in aligner fabrication are chosen for their non-toxic, non-allergenic properties, ensuring patient safety and comfort (5,40). Additionally, they are engineered to withstand the mechanical forces exerted during tooth movement, maintaining their structural integrity over weeks or months of wear (6,47).

One of the most common materials used in clear aligners is polyurethane, a versatile polymer that combines flexibility with resilience. Polyurethane’s thermoplastic nature allows it to be shaped easily during manufacturing, making it ideal for the vacuum-forming processes involved in aligner production (46). Polyurethane aligners demonstrate significant strength and are resistant to fractures and tears, which are critical qualities for maintaining force on teeth and ensuring the aligner’s longevity. Studies indicate that polyurethane aligners can exert consistent forces over the intended wear period without substantial degradation in performance (4,41).

Another widely used material in aligners is PET-G, which is known for its rigidity and optical clarity, contributing to the aligners’ aesthetic appeal and ability to maintain shape under stress. Unlike other plastics, PET-G offers greater resistance to stains and discoloration, which can be beneficial for patients concerned with appearance during long-term treatment (48). PET-G is also less prone to stress-induced deformation, making it suitable for aligners that need to maintain precise fit and alignment capabilities over time (30). Moreover, PET-G’s hydrophobic properties reduce the tendency of bacterial adhesion, lowering the risk of biofilm formation on aligners (9,49).

Each of these thermoplastic materials is often modified or combined to optimize performance. Some manufacturers employ multilayered aligners that combine softer inner layers with more rigid outer layers, creating aligners that balance comfort with force application. These composite materials allow for an inner layer that adapts to the teeth comfortably while the outer layer provides the necessary rigidity for effective tooth movement (50). This layered approach enhances the aligner’s functional properties and durability, addressing limitations associated with single-material designs (37).

However, despite the benefits of these materials, there are some considerations regarding potential health risks associated with thermoplastics. Studies have raised concerns over the potential release of bisphenol A (BPA) and other chemical compounds during use, especially under high temperatures or acidic conditions that may occur in the oral environment. BPA, a known endocrine disruptor, has been detected in certain polycarbonate plastics, which raises concerns about its presence in dental materials (51). Although many manufacturers now use BPA-free plastics, the potential for chemical leaching remains a subject of research. For example, polyurethane and PET-G are generally regarded as safer alternatives to polycarbonate due to their lower risks of leaching harmful substances. Nevertheless, ongoing studies continue to monitor the long-term effects of these materials in the oral environment (2,52).

Beyond their chemical stability, thermoplastic materials in aligners are also evaluated for their mechanical properties, such as elasticity and hardness. These factors determine the aligner's ability to apply sustained forces while conforming comfortably to the patient's dentition. High elasticity allows the aligner to adjust slightly as teeth move, maintaining consistent pressure and reducing the risk of material fatigue or fractures. Similarly, the hardness of the material affects how well it can resist deformation, ensuring that each aligner maintains the intended force over time. This is especially important in longer treatment durations, where any loss of force could compromise the planned tooth movement (8,35).

Moreover, advancements in material science continue to influence the development of aligners. For instance, some manufacturers are exploring shape-memory polymers (SMPs), which can return to a pre-set shape when exposed to certain stimuli, such as heat. SMPs have shown promise for their potential to adjust aligners progressively without the need for new sets, potentially reducing costs and treatment duration (53). Similarly, biodegradable materials are being investigated to improve the environmental footprint of aligners, addressing concerns about waste from single-use plastics in orthodontics (5).

1.4.4 Cytotoxicity and secondary effects of thermoplastic aligners

The cytotoxicity and potential secondary effects of thermoplastic aligners are areas of growing concern in orthodontics, particularly due to the prolonged contact of these devices with oral tissues. Clear aligners are typically made from thermoplastic materials such as polyurethane and PET-G, which have shown general compatibility with the oral environment (46,52). However, research indicates that certain conditions, such as temperature fluctuations and pH changes in the oral cavity, may trigger the release of chemical substances from these materials, raising questions about their long-term safety (2,51).

While many aligners today are manufactured with BPA-free plastics, concerns remain about the possibility of leaching other bisphenol derivatives or additives under certain conditions, such as exposure to high temperatures or acidic environments (51). Studies indicate that repeated exposure to acidic or hot beverages while wearing aligners may increase the risk of such chemical release, suggesting the need for patient education on proper aligner care (4,48).

Beyond BPA, other additives and stabilizers in thermoplastic aligners may also contribute to cytotoxicity. These substances are often used to enhance the flexibility, durability, and clarity of aligners but can sometimes induce adverse cellular responses. A study by Ryokawa *et al*. (47) found that certain orthodontic thermoplastics could elicit inflammatory responses when tested on human gingival fibroblasts. Although these effects were mild and generally below clinically significant levels, they highlight the importance of understanding the cellular interactions between aligners and oral tissues, particularly for patients with pre-existing sensitivities (7).

In terms of clinical side effects, the most commonly reported issues among aligner users are mild irritation of the oral mucosa, localized inflammation, and hypersensitivity. These symptoms are typically attributed to friction between the aligner and the gingival tissue, rather than chemical toxicity (40). However, in some cases, patients have reported experiencing more persistent symptoms, such as soreness and gum swelling, possibly linked to localized inflammatory responses triggered by minor chemical releases (54). These reactions vary significantly among individuals, and further research is necessary to determine the underlying causes and to identify potential biomarkers for susceptibility (3).

Thermoplastic aligners may also impact on the oral microbiome, indirectly affecting patient health. As aligners cover the teeth and create a microenvironment that can trap saliva and food particles, they may contribute to biofilm formation and bacterial proliferation, particularly if not cleaned thoroughly.

Studies indicate that biofilms on aligners can harbor pathogenic bacteria, increasing the risk of gingivitis and other periodontal issues (9,54). While this effect is primarily mechanical rather than chemical, it underscores the need for regular aligner cleaning to prevent secondary health issues.

In response to these concerns, manufacturers have taken steps to reduce the potential cytotoxicity of aligners. The development of BPA-free materials and the use of alternative plasticizers have contributed to significant improvements in aligner safety (55). Additionally, some companies are exploring the use of biocompatible coatings to reduce direct contact between the plastic and the oral mucosa, potentially lowering the risk of both chemical leaching and mucosal irritation. These innovations reflect a shift towards safer and more biocompatible aligners, though rigorous testing remains essential to validate the efficacy of these new materials under clinical conditions (5,48).

Future research in this area may focus on assessing the long-term cytotoxicity of aligners under realistic usage conditions, particularly over extended treatment periods. Some studies are beginning to examine the potential cumulative effects of low-dose chemical exposure from aligners, aiming to better understand how these exposures might contribute to systemic health concerns. Given the wide range of variables, including individual oral pH levels, diet, and hygiene practices, a personalized approach to aligner safety could be beneficial, allowing orthodontists to tailor aligner recommendations to each patient's specific risk profile (6,51).

1.4.5 Health considerations

The use of clear aligners, while generally regarded as safe and effective, is associated with various secondary effects that can impact both oral and systemic health. These secondary effects range from minor discomfort to potential issues like soft tissue irritation, hypersensitivity, and microbiome changes within the oral cavity (3,40). Additionally, as thermoplastic aligners are worn for extended periods, they create a unique environment in the mouth that can influence bacterial growth and potentially lead to other health considerations (9,54).

One of the most common secondary effects reported by patients using aligners is soft tissue irritation, particularly affecting the gingiva and mucosal surfaces.

This irritation often occurs due to the edges of the aligner tray rubbing against sensitive areas of the gums, leading to inflammation, soreness, and, in some cases, minor ulcerations (41). Although aligners are typically polished to minimize sharp edges, slight variations in fit or patient-specific anatomy can result in localized discomfort. Orthodontists usually recommend using dental wax or requesting adjustments to alleviate such issues, through irritation may persist for some patients, especially during the initial phase of treatment (36).

Another significant health consideration associated with aligner use is the potential for increased tooth sensitivity. This sensitivity can be attributed to the constant, albeit gentle, pressure exerted by the aligners on the teeth, particularly during initial wear periods or with each new set of aligners (40). Patients commonly report heightened sensitivity to temperature extremes in food and beverages, which can sometimes persist for the duration of the treatment. While this sensitivity typically subsides after each adjustment period, it can be an ongoing concern, especially for patients with pre-existing dental conditions like enamel erosion or gingival recession. In such cases, desensitizing toothpaste or fluoride treatments may be recommended to mitigate discomfort (8).

In addition to irritation and sensitivity, changes in the oral microbiome are a noteworthy consideration in aligner therapy. Aligners cover the dental arches continuously, creating a closed environment that may contribute to the growth of anaerobic bacteria. Studies have shown that prolonged use of aligners can lead to increased accumulation of bacterial plaque on teeth and aligner surfaces, potentially elevating the risk of gingivitis, caries, and periodontal disease if oral hygiene is not rigorously maintained (2,43). Aligners can trap food particles and create acidic conditions that favor the growth of cariogenic bacteria such as *S. mutans*, which can contribute to enamel demineralization over time (31). To counteract these risks, patients are advised to follow strict oral hygiene practices, including thorough brushing, flossing, and cleaning of aligners, as well as to avoid sugary foods and beverages while wearing aligners (6).

The systematic implications of long-term aligner use have also garnered attention, primarily concerning chemical leaching from thermoplastic materials. Although many aligners are now manufactured from BPA-free plastics, some research has indicated that certain compounds used in aligners, such as plasticizers, can leach out, especially when exposed to high temperatures or acidic conditions (4,56). While the levels of chemical release are generally low and within safety thresholds, there is still concern regarding the cumulative exposure to these chemicals over extended treatment periods, particularly for patients with prolonged aligner use. Further research is necessary to fully assess the long-term systemic impacts of such exposure and to develop materials that further minimize chemical leaching (5).

Dry mouth, or xerostomia, is another reported side effect, particularly among patients who wear aligners for the recommended 20-22 hours per day. The constant presence of aligners can interfere with natural saliva flow, which is essential for neutralizing acids, providing minerals for enamel remineralization, and facilitating the natural cleansing of teeth (48). Reduced salivary flow may not only increase the risk of dental caries and bad breath but can also exacerbate oral discomfort, especially for patients prone to dry mouth. Orthodontists may suggest using saliva substitutes, staying hydrated, or chewing sugar-free gum (if compatible with aligner use) to stimulate saliva production and alleviate symptoms of xerostomia (7).

Finally, compliance and patient education play a critical role in managing these secondary effects and promoting optimal health outcomes. Since aligners are removable, treatment success and the minimization of adverse effects depend heavily on patient adherence to the recommended wear schedule and maintenance routines. Patients are advised to clean aligners daily with mild soap or specialized cleaning solutions to prevent bacterial buildup and to avoid high-temperature cleaning methods, such as boiling water, which could compromise the integrity of the thermoplastic material (37,47). Furthermore, educating patients on the importance of wearing aligners for the recommended hours, as well as removing them for eating and drinking (except water), can significantly reduce the risk of both oral and systemic side effects.

1.5 Aim of the study

Aligners have been used by an increasing number of individuals and information on the devices is still insufficient to formulate a basic idea of their safety, biocompatibility and toxicity. The aim of this systematic review is to aggregate all available and relevant information on the subject to assess and understand the impact of the toxicity of invisible aligners on the oral cavity of patients who use invisible dental aligners and consequently to understand how the materials that make up the aligners are related to possible toxicity. Therefore, this systematic review stands out for the fact that it only describes information about invisible aligners and their side effects. The existing systematic reviews include aligners, retainers and other types of orthodontic devices which, in addition to acrylic, use other materials such as metals.

MATERIALS AND METHODS

2. MATERIALS AND METHODS

In order to organize current scientific knowledge on the toxicity of invisible aligners, a systematic search of studies available in the medical literature was carried out in the Pubmed, Scopus and Cochrane electronic databases to identify relevant articles. To reach the goals, the literature search was conducted following the Preferred Reporting Items for Systematic reviews and Meta-analysis (PRISMA) guidelines to answer a Population, Intervention, Comparison and Outcomes (PICO) question: "In patients undergoing orthodontic treatment (P), with clear aligners (I), what are the changes or effects observed in the oral cavity (O) when comparing before and after treatment (C)." This review will also be registered in the OSF database with the registration DOI: https://doi.org/10.17605/OSF.IO/HB2WQ (accessed on 7 November 2024).

The search results were imported into Rayyan (413) to help visualize and operationalize the selection of articles and the Joanna Briggs Institute (JBI) (57) evaluation tool will be used to assess the methodological quality of the studies.

2.1. Search strategy

The PICO question for this review was as follows: In patients undergoing orthodontic treatment (P), with clear aligners (I), what are the changes or effects observed in the oral cavity (O) when comparing before and after treatment (C).

The following terms were used in the searches: (clear aligners OR invisalign) AND (biocompatibility OR toxicity OR (biological effects)). For years, Invisalign dominated the clear aligner industry, to the point where its name became synonymous with the product itself. Many patients refer to clear aligners simply as "Invisalign" rather than using the generic term. Research from the past decade suggests that the term "Invisalign" gained widespread popularity because people prefer it over the general name for the treatment. For this, the term "Invisalign" was added in the key search. The following search equations were used for the different databases, and the following results were obtained:

PubMed/Medline: (clear aligners OR Invisalign) AND (biocompatibility OR toxicity): Filters: Human, English, in the last 10 years; Retrieved: 367 results.

Cochrane: (clear aligners OR Invisalign) AND (biocompatibility OR toxicity): Filters: Human, English, in the last 10 years; Retrieved: 15 results.

Scopus database: (clear aligners OR Invisalign) AND (biocompatibility OR toxicity): Filters: Human, English, in the last 10 years; Retrieved: 31 results.

2.2. Sources of information

A systematic search was carried out on 21 September in the National Institute of Health database. The search was completed on 22 September in the following databases: Pubmed, Cochrane Library and Scopus.

The articles obtained were imported into bibliographic reference software. The searches were carried out with filters for humans, with text in English and Portuguese and published between 2014 and 2024.

To prepare this review, the search strategy used consisted of applying MeSH terms, which resulted in the articles summarized in Table I.

Table I. Summary of the search in the various databases using MeSH terms

Database	Descriptors	Time period	Filters	Results
MEDLINE (PubMed)	(clear aligners OR invisalign) AND (biocompatibility OR toxicity OR (biological effects))	10 years (published between 2014-2024)	written in english, have full text available, not be a systematic review, studies resulting from scientific research regarding clear aligners and describing side effects related to their use	367
Cochrane Library	(clear aligners OR invisalign) AND (biocompatibility OR toxicity OR (biological effects))	10 years (published between 2014-2024)	written in english, have full text available, not be a systematic review, studies resulting from scientific research regarding clear aligners and describing side effects related to their use	15
Scopus	(clear aligners OR invisalign) AND (biocompatibility OR toxicity OR (biological effects))	10 years (published between 2014-2024)	written in english, have full text available, not be a systematic review, studies resulting from scientific research regarding clear aligners and describing side effects related to their use	31

2.3. Eligibility criteria

To present relevant results in this review and to address the question, it was necessary to establish well-defined inclusion criteria. In this way, articles dealing with orthodontic treatment with transparent aligners, without restricting the age or gender of the participants were included.

Therefore, this systematic review was based on aggregating all information on toxicity, biocompatibility and side effects of clear aligners and the following inclusion criteria were defined for the selection of publications: (1) written in English, (2) published between 2014-2024, (3) studies resulting from scientific research on clear aligners, (4) studies describing side effects related to the use of clear aligners, (5) *in vitro* and *in vivo* studies and (6) with full text available.

After the initial assessment, the articles that actually met the inclusion criteria were read in full and those that did not meet at least one of the following exclusion criteria were excluded: (1) case reports, (2) systematic reviews, (3) articles without information on the toxicity and adverse effects of clear aligners, (4) studies that used culture methods as a method of assessing the microbiota, and (5) orthodontic appliances such as retainers, breaker appliances, and expander appliances.

The articles selected from the included studies were read in full to ensure that they only dealt with studies on the toxicity of clear aligners and their side effects.

- Studies evaluating removable appliances other than transparent aligners;
- Wrong outcome, wrong population, wrong study design;
- Availability of full-text article.

2.4. Study selection

After searching the literature, two independent researchers (PL and MF) filtered the relevant articles that fit the study, analyzing the title and abstract to select the studies. Any disagreements between reviewers will be discussed with a third author (AG). Cohen's Kappa test will be used to assess the agreement between the reviewers. The Rayyan Intelligent Systematic Review Platform was used to assist in the systematic review process (58).

Reviewers independently extracted data from the articles selected for analysis. The information collected during data collection was as follows: authors, year of publication, title of the article, study design, type of participants, number of participants, age of participants, type of diagnosis and treatment, period of clinical follow-up, materials and substances used, and the outcome assessed.

2.5. Methodological quality analysis

The risk of bias was assessed using the Quin Tool 2.0 (59). This analysis is based on five domains: D1 (Bias arising from the randomized process), D2 (Bias due to deviations from the intended intervention), D3 (Bias due to lack of outcome data), D4 (Bias in the measurement of the outcome) and D5 (Bias in the selection of the reported outcome). In each article, the five domains were assessed and, in each domain, one of the following judgments was applied: low risk, some concern and high risk. In the end, the most prevalent judgment was the one that determined the final evaluation of each article.

RESULTS

3. RESULTS

In September 2024, data was collected and a systematic literature search was carried out which identified 413 potentially relevant references, 367 of which came from the PubMed/Medline database, 31 from Scopus, and 15 from Cochrane. Nineteen duplicates were found and excluded. Based on the information provided in the title and abstract, 360 were considered ineligible. The main reasons for not including the articles were as follows: (1) unrelated topic; (2) wrong result; (3) review; (4) wrong population; (5) being in humans; (6) wrong study; (7) wrong study duration. Thirty-four articles were analyzed in full to gather more detailed information. Twenty-seven articles were excluded for the following main reasons: (1) wrong result; (2) wrong population; (3) wrong study; (4) no full text available.

Finally, seven articles were included in the following review. The study selection process is shown in Figure 1 and the characteristics of the seven studies included in this systematic review are presented in Table 1.

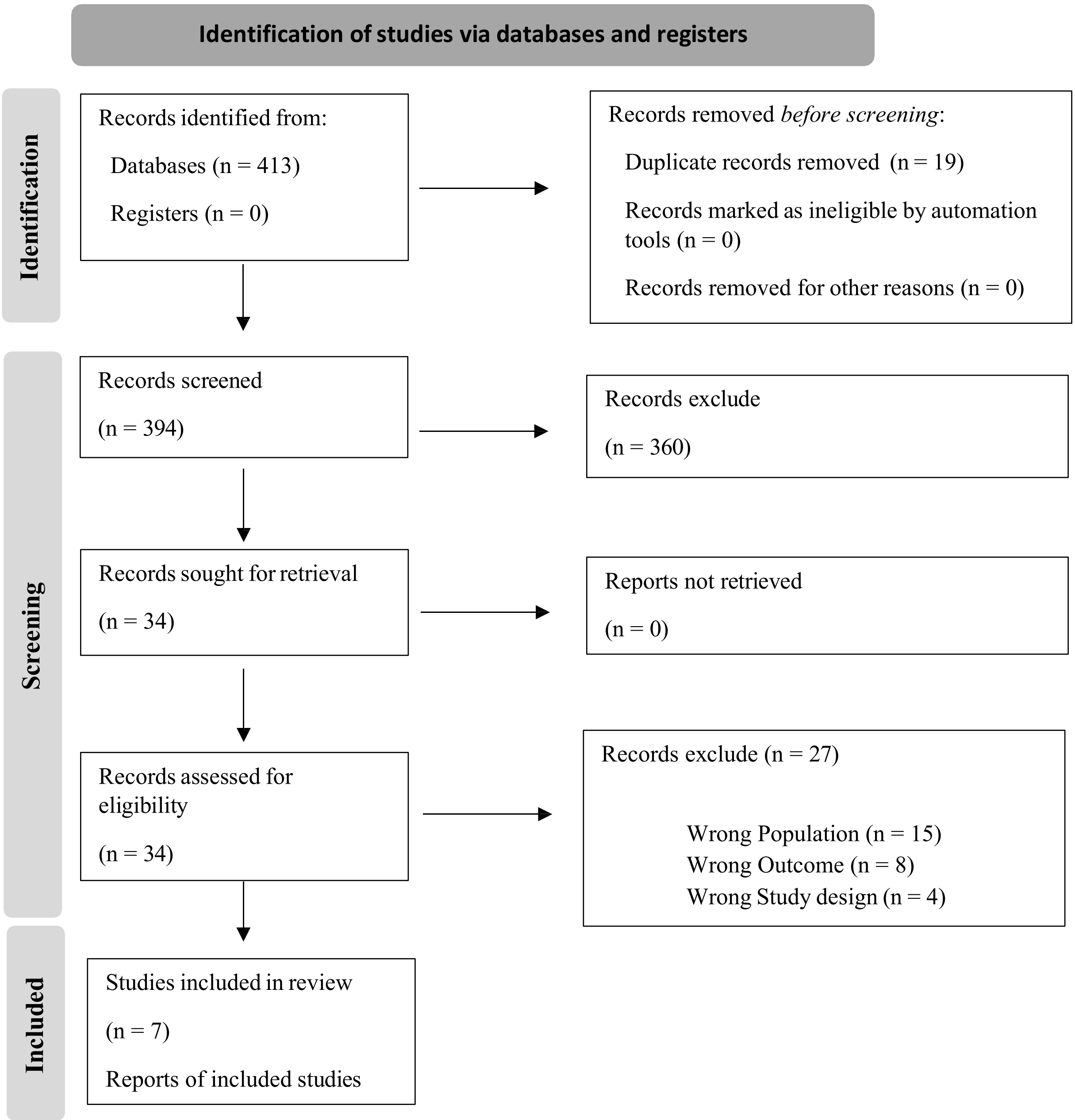

Figure 1. Article selection according to PRISMA guidelines

Table 2. Characteristics of the 7 studies included in this systematic review

Author/ Year	***Study Design***	***Appliance Studied /Materials***	***Interventio n Time***	***Goals***	***Substances Analyzed***	***Outcomes***	***Conclusions***
Nemec, M. et al., 2023 [3]	In vitro/ In vivo	Invisalign aligners (SmartTrack)	First 6 months of treatment	Compare the effects of saliva from patients with Invisalign aligners and brackets on human gingival fibroblasts and oral epithelial cells.	Pro-inflammatory mediators; IL-8 production	Increase in the expression of pro-inflammatory mediators (IL-6, IL-8, MCP-1) in the analyzed cells in saliva samples of bracket patients. With the progression of orthodontic treatment (from 0 to 6 months), there was a tendency for a decrease in IL-8 production.	The type of orthodontic treatment (Invisalign vs. brackets) does not significantly influence the saliva's effect on the inflammatory or barrier properties of oral cells. The changes seen during treatment were minor and transient.
Dinu, S. et al., 2024 [5]	In vitro	Two clear aligner types (Thermoplastic)	24h	Assess the cytocompatibility and safety profile.	HaCaT keratinocytes and HGF fibroblasts (in vivo) in artificial saliva; chorioallantoic membrane	No relevant changes were observed in the morphology of HaCaT cells, except for a slight decrease in confluence in the more concentrated acidic saliva. Testing on a 3D reconstructed human epidermis (EpiDerm™ model) showed viability above 82% with all samples (threshold for irritation is <50%).	The results suggest that CA1 and CA2 aligners are suitable materials for use, as they did not present significant cytotoxicity and were not irritating, confirming the safety and compatibility of these materials.
Yu, X. et al., 2022 [61]	In vitro	Angelalign (Thermoplastic polyurethane)	No data	Feasibility of using 'transparent' polymeric aligners molded into personalized 3D printed dental models.	Poliuretan (polyurethane films were molded via thermoforming into custom 3D printed dental models)	Thermoplastic polyurethane demonstrated excellent biocompatibility: low cytotoxicity, hemolysis <5%, and preserved cell morphology.	The aligner material had good mechanical properties.
Yan, J. et al., 2023 [8]	In vitro	Fluoride-coated clear aligner plastic	26h	Assess the physicochemical properties and biocompatibility of a fluoride-coated clear aligner plastic.	Human fibroblasts were placed in a Minimum Essential Medium with 10% Fetal Bovine Serum and cell counts were evaluated.	Fluoride-coated clear aligners did not compromise the cell viability of human fibroblasts.	FCAP had antibacterial, fluoride recharge, and enamel remineralization capabilities with appropriate physicochemical properties and biocompatibility.

Table 2 (cont). Characteristics of the 7 studies included in this systematic review

Author/ Year	***Study Design***	***Appliance Studied /Materials***	***Intervention Time***	***Goals***	***Substances Analyzed***	***Outcomes***	***Conclusions***
Martina, S. et al., 2019 [7]	In vitro	Duran, Biolon, Zendura, and SmartTrack aligners	14 days	Assess the in vitro cytotoxicity of different thermoplastic materials for clear aligners on human primary gingival fibroblasts incubated in Dulbecco's Modified Eagle's Medium.	Aligners with plastic materials (Duran, Biolon, Zendura, and SmartTrack)	All the materials showed mild cytotoxicity after 14 days of incubation with HGFs. Observed cell viability: Duran: 84.6% ± 4.0 (lowest cytotoxicity); SmartTrack: 78.8% ± 6.3; Zendura: 74.4% ± 2.3; Biolon: 64.6% ± 3.3 (highest cytotoxicity). Thermoforming significantly increased the cytotoxicity of Duran, Biolon, and Zendura materials.	All the materials showed some level of cytotoxicity, with Biolon being the most cytotoxic. Although they are generally safe for clinical use, caution is advised, especially with materials such as Biolon and Zendura.
Alhendi, A. et al., 2022 [52]	In vitro	Invisalign, Eon, SureSmile, and Clarity	30 days	Evaluate and compare the cytotoxicity of multiple clear aligner systems.	HGFs were incubated in a saline solution.	The cell viability of the HGFs decreased with increasing solution concentration, indicating a dose-dependent relationship.	The thermoplastic materials used by all the tested systems presented some degree of toxicity (slight to moderate)
Lo, I. et al., 2024 [60]	In vitro	Duran, Keystone, Zendura, Essix C+, Maxflex, and Leone (Thermoplastic polyurethane and copolyester polyethylene terephthalate)	14 days	Evaluating the cytotoxicity of different clear aligner materials to ensure safety and biocompatibility.	Human periodontal ligament cells (HPDL) were incubated in a Minimum Essential Medium with 10% Fetal Bovine Serum.	The HPDL cells maintained high cell viability after exposure to most of the materials tested, indicating low toxicity of the aligners, especially those composed of PETG (polyethylene terephthalate co-1,4-cyclohexylenedimethylene terephthalate) and PET (polyethylene terephthalate copolyester), especially in their original form, before thermoforming. Some thermoformed TPU (thermoplastic polyurethane) materials showed a more significant reduction in the viability of the HPDL cells.	The results indicate that under the conditions tested, these materials are generally safe for use in orthodontic treatments.

A total of 413 articles were obtained: 367 of which came from the PubMed/Medline database, 31 from Scopus and 15 from Cochrane. 19 duplicates were excluded and based on the information provided in the title and abstract, 360 were found to be ineligible. 34 articles were reviewed in full to gather more detailed information and 27 were excluded by applying the inclusion and exclusion criteria. Finally, 7 articles were included in this review.
We analyzed the articles and from them relevant data was collected and organized on an Excel table (Table 1). Author/Year; Article title; Study design; Appliance studied; Material; Objectives; Substances analyzed and Conclusions. In one of the articles that presented toxicity [1], four clear aligner materials were considered in the study: Duran, Biolon, Zendura (which were evaluated as thermoformed and non-thermoformed) and SmartTrack (evaluated as non-thermoformed). The constituent thermoplastic materials are Polyethylene Terephthalate Glycol (PET-G), polyurethane resin and thermoplastic polyurethane. Duran was considered the least toxic. Biolon, Zendura and SmartTrack showed mild cytotoxicity. It is concluded that thermoformed materials showed greater cytotoxic behavior. Materials such as PET-G tend to release more substances as the temperature increases, meaning that thermoforming can increase the release of monomers and consequently increase cytotoxicity. In Alhendi, A. et al, 2022 [24] a cytotoxicity evaluation was also performed by immersing 3 sets of the 4 systems (Invisalign, Eon, SureSmile, Clarity) included in saline solution. The solution was diluted to 3 different concentrations (5%, 10%, 20%). The thermoplastic materials of these systems demonstrated mild to moderate levels of cytotoxicity, with statistically relevant differences compared to the control group. It was concluded that the higher the solution concentration, the lower the cell viability and the greater the cytotoxicity, except for the Eon system. We therefore conclude that, of the 7 articles selected, 5 consider the material safe and 2 present some level of toxicity.

The studies selected were analyzed regarding the quality of the study according to the (QUIN Tool)[2] and the results of the analysis are presented in table 2. Almost all aspects of the analysis were fulfilled except for 2 articles (Yu, X. et al, 2022[3]; Yan, J. et al, 2024 [4]).

***Table 2**. Results of the analysis of the Quin Tool appraisal checklist for critical evaluation of in vitro studies.*

Author/Year	A	B	C	D	E	F	G	H	I	J	K	L	Score	Bias Evaluation
Nemec, M. *et al,* 2023[5]	2	2	2	2	2	0	NA	2	NA	NA	2	2	16	88.9 (Low Risk)
Dinu, S. *et al,* 2024[6]	2	2	2	2	2	0	NA	2	NA	NA	2	2	16	88.9 (Low Risk)
Yu, X. *et al,* 2022[3]	2	1	2	1	2	0	NA	2	NA	NA	2	2	14	77.8 (Low Risk)
Yan, J. *et al,* 2024[4]	2	2	2	1	2	0	NA	2	NA	NA	2	2	15	83.3 (Low Risk)
Martina, S. *et al,* 2019[1]	2	2	2	2	2	0	NA	2	NA	NA	2	2	16	88.9 (Low Risk)
Alhendi, A. *et al,* 2022[7]	2	2	2	2	2	0	NA	2	NA	NA	2	2	16	88.9 (Low Risk)
Lo, I. *et al*, 2024[8]	2	2	2	2	2	0	NA	2	NA	NA	2	2	16	88.9 (Low Risk)

A: Clearly stated aims/objectives; B: Detailed explanation of sample size calculation; C: Detailed explanation of sampling technique; D: Details of comparison group; E: Detailed explanation of methodology; F: Operator details; G: Randomization; H: Method of measurement of outcome; I: Outcome assessor details; J: Blinding; K: Statistical analysis; L: Presentation of results.

DISCUSSION

4. DISCUSSION

4.1 Biocompatibility and toxicity of clear aligners

The use of thermoplastic materials in clear aligners, such as polyurethane and PET-G, has gained widespread acceptance due to their mechanical properties, biocompatibility, and aesthetic appeal (1). However, concerns persist regarding their potential to release chemical compounds under specific conditions, such as elevated temperatures, acidic environments, and prolonged wear (2,3). Understanding these materials' biocompatibility and toxicity is essential for assessing their long-term safety in orthodontic treatments.

One of the primary concerns in the biocompatibility of clear aligners is the potential release of BPA and other monomers. BPA, an endocrine disruptor with known estrogenic activity, has been detected in certain thermoplastic dental materials, raising concerns about its cumulative exposure over prolonged treatment durations (4). Studies indicate that although the levels of BPA released from aligners generally fall within regulatory safety limits, the long-term effects of prolonged exposure remain unclear, particularly for susceptible populations such as adolescents and pregnant women (5,6). Moreover, alternative bisphenol derivatives such as BPS and BPF, commonly used in BPA-free plastics, may pose similar health risks, necessitating further investigation into their systemic absorption and biological impact (7).

Several studies have assessed the cytotoxicity of clear aligners, investigating their interactions with oral tissues. For example, Nemec et al. (3) analyzed the effects of saliva from patients wearing Invisalign aligners and brackets on human gingival fibroblasts and oral epithelial cells. Their findings revealed minimal differences in inflammatory responses before and after treatment, suggesting that orthodontic therapy does not significantly alter saliva composition in terms of inflammatory mediators (8). However, the cytotoxicity of different clear aligner systems varies depending on material composition. In their study, Alhendi et al. (52) evaluated multiple aligner systems and found that all tested thermoplastic materials exhibited some degree of cytotoxicity, ranging from slight to moderate (9).

Similarly, Martina et al. (7) investigated the cytotoxicity of different thermoplastic materials used in clear aligners, including Duran, Biolon, Zendura, and SmartTrack. Their results indicated that while these materials are generally safe for clinical use, Biolon exhibited the highest cytotoxic potential, raising concerns about its long-term safety (10). Lo et al. (60) expanded on these findings by analyzing a range of thermoplastic materials, including polyurethane and PET, and concluded that under the tested conditions, these materials were generally safe for orthodontic treatments, although some variations in cytotoxicity were observed (11).

In addition to cytotoxicity concerns, some research has focused on the potential irritant properties of clear aligners. Dinu et al. (5) conducted an in vitro and in vivo biocompatibility study on two types of clear aligners (CA1 and CA2), assessing their safety profiles. Their findings confirmed that both aligner types did not induce significant cytotoxicity or irritation, supporting their suitability for orthodontic use (12). Furthermore, Yu et al. (61) explored the mechanical properties of clear aligners and found that polymeric materials exhibited good durability and flexibility when molded onto 3D-printed dental models, which is crucial for maintaining orthodontic forces without compromising safety (13).

In contrast to concerns regarding cytotoxicity, some advancements in aligner materials aim to enhance biocompatibility. Yan et al. (8) evaluated a novel fluoride-coated clear aligner material with antibacterial and enamel remineralization properties. Their study demonstrated that fluoride-coated aligners exhibited antibacterial activity, promoted fluoride recharge, and maintained appropriate physicochemical properties, suggesting a potential avenue for reducing adverse effects associated with prolonged aligner wear (14).

While current evidence suggests that clear aligners are generally safe, variations in material composition, manufacturing processes, and environmental conditions can influence their toxicity profile. Some thermoplastic materials may degrade under high temperatures or acidic conditions, increasing the release of potentially harmful substances. Patients are advised to avoid consuming hot beverages or acidic foods while wearing aligners to minimize these risks (15). Furthermore, proper aligner maintenance, including regular cleaning with non-abrasive agents, is crucial for preventing bacterial accumulation and ensuring continued safety during orthodontic treatment (16).

Given these findings, continued research is necessary to assess the long-term impact of clear aligner materials on both oral and systemic health. Standardized testing protocols for material toxicity and biocompatibility should be established to ensure the safety of emerging aligner materials. Future studies should also explore patient-specific factors, such as variations in oral pH, saliva composition, and hygiene practices, which may influence the extent of material degradation and chemical leaching (17).

4.2 Effects of aligners on oral health

The continuous use of clear aligners, typically for 20-22 hours per day, creates a unique oral environment that can influence microbial composition, saliva flow, and overall oral health. Several studies have explored these effects, identifying potential risks such as alterations in the oral microbiome, increased bacterial accumulation, xerostomia, and gingival inflammation (1). While clear aligners offer improved hygiene compared to fixed appliances, they still pose challenges that require careful patient management and preventive strategies (2).

One of the most significant concerns associated with clear aligner therapy is the alteration of the oral microbiome. The enclosed space created between the aligner and the teeth provides a favorable environment for bacterial proliferation, particularly cariogenic and periodontal pathogens. Nemec et al. (3) investigated the effects of Invisalign aligners on saliva composition and found minimal inflammatory changes before and after treatment, suggesting that aligners do not significantly alter inflammatory responses in saliva (3). However, their study did not assess bacterial shifts, which remain a critical factor in oral health during treatment.

Other research has highlighted the increase in cariogenic bacteria due to prolonged aligner wear. Alhendi et al. (52) evaluated multiple clear aligner systems and found that thermoplastic materials used in aligners facilitated bacterial adherence, leading to moderate cytotoxic effects on gingival fibroblasts

(4). These findings align with previous reports indicating that aligners may create conditions conducive to biofilm formation, increasing the risk of enamel demineralization and white spot lesions (5).

Similarly, Martina et al. (7) analyzed the cytotoxicity of different aligner materials (Duran, Biolon, and SmartTrack) and found that while they are generally safe, they still pose some risks of bacterial accumulation (6). Lo et al. (60) expanded on this by assessing polyurethane and PET aligners, concluding that they are generally safe but require strict oral hygiene to prevent bacterial colonization (7).

Beyond microbial changes, xerostomia (dry mouth) is another commonly reported side effect of aligner use. The presence of an aligner covering the dental arches reduces salivary circulation, which can compromise the oral cavity's natural defense mechanisms. Saliva plays a crucial role in buffering acids, remineralizing enamel, and cleansing bacterial deposits. Dinu et al. (5) confirmed that while certain clear aligner materials showed good biocompatibility, prolonged use can contribute to dryness and discomfort, especially in patients with pre-existing salivary deficiencies (8). Managing xerostomia involves increasing water intake, using saliva substitutes, and avoiding dehydrating substances such as caffeine and alcohol while wearing aligners (9).

Another effect of aligners on oral health is gingival irritation and mild inflammation. The mechanical irritation caused by the aligners, particularly along the gingival margins, can lead to localized inflammation and soreness. Yan et al. (8) examined a novel fluoride-coated aligner and found that its antibacterial properties reduced plaque accumulation, potentially lowering gingival inflammation risks (10). This suggests that advancements in aligner materials could mitigate some of the adverse effects currently observed in conventional aligners.

Furthermore, Yu et al. (61) explored the mechanical and chemical stability of aligners molded onto 3D-printed dental models. Their findings demonstrated that well-fitted aligners reduce irritation but emphasized that patient compliance with oral hygiene routines is essential in preventing gingival issues (11).

From a clinical perspective, maintaining optimal oral hygiene while wearing aligners is critical in preventing adverse effects. Patients should be instructed to clean aligners thoroughly using appropriate cleaning agents, avoid sugary and acidic foods that may exacerbate bacterial growth, and ensure consistent oral care practices, including brushing, flossing, and regular dental check-ups (12). In addition, orthodontists should assess individual patient risks and recommend adjunctive treatments, such as antimicrobial mouth rinses, to minimize bacterial colonization.

Future research should focus on long-term evaluations of aligner-associated microbiome shifts and the cumulative impact on periodontal and dental health. Additionally, material innovations, such as antimicrobial and fluoride-releasing aligners, may offer new strategies for mitigating oral health concerns associated with prolonged aligner use (13). Given the growing popularity of clear aligners, further clinical trials are necessary to establish best practices for maintaining oral health during orthodontic treatment.

While clear aligners remain a convenient and effective alternative to fixed appliances, their impact on oral health should not be overlooked. By implementing evidence-based preventive measures and advancing material technologies, the potential risks associated with aligners can be minimized, ensuring that patients achieve both functional and aesthetic benefits without compromising their oral health (14).

4.3 Preventive measures and alternative materials

To mitigate the risks associated with clear aligners, including cytotoxicity, microbial accumulation, and material degradation, preventive strategies and material advancements are crucial. Research has focused on improving aligner compositions and clinical recommendations to enhance safety and efficacy.

One of the most promising strategies is integrating antimicrobial and remineralization agents into aligners. Yan et al. (8) demonstrated that fluoride-coated aligners exhibit antibacterial properties and support enamel remineralization, reducing the risk of demineralization during treatment (1). Antimicrobial coatings, such as silver nanoparticles or chlorhexidine-releasing polymers, have also been explored to limit bacterial growth on aligner surfaces (2).

Patient behavior significantly influences aligner safety. Alhendi et al. (52) noted that thermoplastic materials, while generally safe, exhibit slight to moderate cytotoxicity, which may increase with exposure to high temperatures and acidic environments (3). Patients should avoid consuming hot or acidic beverages while wearing aligners and adhere to proper hygiene practices, including regular aligner cleaning with non-abrasive agents (4).

Material advancements aim to improve aligner biocompatibility. Dinu et al. (5) found that newer thermoplastics exhibited excellent safety profiles with no significant cytotoxic effects (5). Lo et al. (60) further assessed the safety of polyurethane and PET-based aligners, confirming their suitability but emphasizing the need for long-term evaluations (6). These findings suggest that continued innovation in material science can enhance aligner performance while minimizing potential risks.

Clinically, orthodontists can implement preventive measures such as recommending fluoride mouth rinses and ensuring precise aligner fit to reduce gingival irritation. Martina et al. (7) emphasized the importance of individualized care for patients prone to periodontal issues (7). Yu et al. (61) further highlighted the role of aligner design in minimizing discomfort and optimizing treatment outcomes (8).

Future research should focus on developing aligners with enhanced antimicrobial properties, improved resistance to degradation, and better biocompatibility. Innovations such as shape-memory polymers may further improve patient experience and long-term treatment success (9). By integrating advanced materials and preventive strategies, orthodontic treatments can continue to evolve towards safer and more effective solutions.

4.4 Clinical implications and future research directions

The findings of this systematic review underscore the importance of continued research into the long-term safety and biocompatibility of clear aligners. While clear aligners have transformed orthodontic treatment by providing a more aesthetic and convenient alternative to traditional braces, their impact on oral and systemic health remains an area of active investigation. The potential for chemical leaching, alterations in the oral microbiome, and risks of gingival inflammation emphasize the need for further clinical evaluation and material innovation.

One of the key clinical implications of this review is the necessity for orthodontists to consider material composition when selecting aligners for patients. Studies such as those by Alhendi et al. (52) and Martina et al. (7) have shown that while most aligner materials are generally safe, some exhibit moderate cytotoxicity, particularly Biolon and certain PET-based thermoplastics (1,2). These findings highlight the importance of material selection, particularly for patients with pre-existing sensitivities or periodontal conditions.

Another crucial consideration is the impact of aligners on the oral microbiome. As noted by Nemec et al. (3) aligner use does not significantly alter inflammatory mediators in saliva, yet their enclosed environment facilitates bacterial accumulation (3). Lo et al. (60) further emphasized that while most materials are biocompatible, they still require strict oral hygiene protocols to prevent microbial imbalances (4). These insights suggest that orthodontists should emphasize meticulous hygiene practices, possibly incorporating antimicrobial mouth rinses or recommending fluoride-coated aligners like those studied by Yan et al. (8) to mitigate bacterial proliferation (5).

Looking forward, future research should focus on optimizing aligner materials to enhance their safety profile while maintaining their mechanical properties. Dinu et al. (5) found that certain thermoplastics demonstrated excellent safety profiles with no significant cytotoxic effects, pointing towards the potential for improved materials with enhanced durability and reduced toxicity (6). Similarly, Yu et al. (61) suggested that advancements in material technology, such as 3D-printed custom aligners, may allow for better adaptation to individual dental structures, reducing mechanical irritation and improving patient compliance (7).

Finally, the regulatory landscape surrounding aligner materials must continue evolving to ensure patient safety. Standardized testing protocols for material toxicity, leaching potential, and long-term biological interactions should be implemented across manufacturers. Future studies should prioritize long-term clinical trials assessing the systemic effects of extended aligner use, as well as the cumulative exposure to potential chemical leachates.

By integrating these advancements into clinical practice, orthodontists can optimize treatment outcomes while prioritizing patient safety. Through material innovation, improved hygiene protocols, and comprehensive patient education, clear aligner therapy can continue to offer a safe and effective orthodontic solution. Future research must address the gaps identified in this review to refine aligner technology, ensuring that it remains a biocompatible and sustainable choice for patients worldwide. In the absence of such developments, it is the dentist’s responsibility to guide patients in implementing preventive measures to ensure the safe use of materials. Keeping aligners clean is crucial to avoid bacterial accumulation and prevent oral health issues. They should be brushed daily using a soft toothbrush and cleaning products specifically designed for aligners. In addition to routine cleaning, patients should take certain precautions to maintain both the aligners’ integrity and their oral health. Aligners must be removed before eating or drinking, as food and beverages can stain or damage the material. Consuming acidic or sugary drinks while wearing aligners can foster bacterial growth and elevate the risk of cavities. If such drinks are consumed, the aligners should be taken out beforehand.

Lastly, patients must adhere to the aligner replacement schedule set by their orthodontist. Natural wear over time can reduce the aligner's effectiveness if not replaced as directed.

CONCLUSION

5. CONCLUSION

The widespread use of clear aligners needs a thorough understanding of their bio-compatibility, potential toxicity, and long-term impact on oral health. While current evidence suggests that clear aligners are generally safe, concerns remain regarding the chemical leaching of thermoplastic materials, bacterial accumulation due to reduced saliva flow, and mild inflammatory responses. Our review emphasizes that although most materials are biocompatible, some exhibit moderate cytotoxicity, underscoring the need for continued research and improvements in material composition.

PUBLICATIONS

6. PUBLICATIONS

This work was published as a Sustematic Review on the peer-review *Journal of Functional Biomaterials* as:

Ferreira, M., Costa, H., Veiga, N., Correia, M. J., Gomes, A. T. P. C., & Lopes, P. C. (2025). Do Clear Aligners Release Toxic Chemicals?—A Systematic Review. Journal of Functional Biomaterials, 16(5), 173. https://doi.org/10.3390/jfb16050173

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