RESEARCH PAPER

Prevention and Regression of WSL And Cariogenic Biofilm Using Fluoride And Xylitol Varnishes On Orthodontic Patients: A Systematic Review

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Balavenkata Bharathi Chaturvedula: Conceptualization, Methodology, Supervision, Validation, Investigation, Writing – review & editing, Project administration.
Harshinee NK: Data curation, Literature search, Study selection, Risk of bias assessment, Formal analysis, Writing – review & editing.
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Abstract

Background: Fixed orthodontic appliances increase the risk of white spot lesions (WSLs) due to plaque accumulation. Professionally applied fluoride varnish (FV) is a standard preventive measure, while xylitol varnish (XV) offers an alternative antimicrobial approach. This review synthesizes evidence on the effectiveness of both varnishes for WSL prevention and cariogenic biofilm control in orthodontic patients.

Methods: A systematic review was conducted following PRISMA 2020 guidelines. Databases (PubMed, Scopus, Ovid, Google scholar) were searched until January 2025 for randomized controlled trials (RCTs) on patients with fixed appliances comparing professional FV or XV to control/alternative agents. Outcomes were WSL incidence/prevalence/regression and biofilm parameters. Risk of bias was assessed using RoB 2.

Results: Twelve RCTs (2001-2025) were included. Fluoride varnish consistently reduced WSL incidence and severity compared to placebo/standard care, with significant preventive effects shown in multiple trials. Xylitol varnish, evaluated in two direct comparison trials, demonstrated comparable or superior efficacy to FV in reducing demineralization and improving post-orthodontic WSL regression. Biofilm-focused outcomes were rarely reported. Five studies had a low risk of bias, five raised some concerns, and two were at high risk.

Conclusion: Fluoride varnish is effective for WSL prevention during orthodontic treatment. Xylitol varnish presents a promising alternative with a unique biofilm-modifying action. Direct comparative evidence is limited, highlighting the need for future RCTs integrating both clinical and microbiological endpoints to refine preventive protocols.

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Introduction

The utilization of fixed orthodontic appliances, though highly effective for malocclusion correction, poses a substantial challenge to maintain optimal oral health. The brackets, bands, and wires create numerous plaque-retentive sites that obstruct effective cleaning, disrupt the ecological balance of the oral biofilm, and promote a cariogenic environment [1, 2]. This frequently culminates in the rapid development of enamel demineralization, clinically manifested as white spot lesions (WSLs), a prevalent iatrogenic complication of orthodontic therapy. The prevalence of WSLs at the time of appliance removal is notably high, with reports ranging from 20% to over 50% of patients [3, 4]. While some early lesions may remineralize post-treatment, more advanced WSLs can persist indefinitely, compromising aesthetic outcomes and potentially necessitating invasive restorative interventions [5]. Consequently, the implementation of effective preventive strategies during active treatment is a critical component of contemporary orthodontic practice.

Fluoride remains the cornerstone of caries prevention in dentistry, primarily through its ability to enhance remineralization and inhibit demineralization of enamel. Professional topical fluoride applications, particularly fluoride varnish (FV), are well-established interventions. Evidence from pediatric dentistry confirms the efficacy of regular FV applications in preventing caries in young permanent teeth [6], a finding that has been extrapolated to orthodontics. Recent systematic reviews focused on orthodontic patients report a protective effect of professionally applied FV against WSL development, with one meta-analysis indicating a significant reduction in WSL prevalence at debonding [7, 8]. However, the certainty of this evidence is often graded as low, attributed to methodological limitations, study heterogeneity, and the ubiquitous background use of fluoride toothpaste in control groups [8, 9]. This has spurred the search for adjunctive or alternative agents, particularly those targeting the microbial etiology of dental caries.

In this context, xylitol has emerged as a promising non-fluoride caries-preventive agent. Xylitol is a sugar alcohol that exerts an antimicrobial effect by disrupting the metabolic pathways of cariogenic bacteria, primarily *Streptococcus mutans*, thereby reducing their adhesion, acid production, and biofilm virulence [10]. Delivered via a varnish formulation, xylitol can provide sustained, localized release at high-risk sites. This introduces a compelling alternative, especially for individuals with concerns about fluoride intake, and presents a complementary, biofilm-targeting mechanism of action to fluoride's remineralization-focused activity.

Despite these advances, significant gaps persist in the evidence base. Direct comparative evaluations of fluoride and xylitol varnishes within the orthodontic context are scarce. Existing systematic reviews have either focused predominantly on fluoride agents [7, 8] or provided broader overviews with methodological limitations [11]. There is a distinct lack of synthesized evidence that concurrently evaluates the efficacy of both varnish types not only on the clinical endpoint of WSL prevention but also on their impact on the cariogenic biofilm adjacent to orthodontic appliances. Understanding this dual impact on the lesion and its microbial cause is crucial for developing optimized, mechanism-based preventive protocols. Therefore, this study aims to systematically review and synthesize the available evidence on the effectiveness of professionally applied fluoride varnish and xylitol varnish for both the prevention of white spot lesions and the control of cariogenic biofilm in patients undergoing fixed orthodontic therapy.

Methodology

This systematic review was conducted and reported in strict accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement [12]. The review protocol was registered in a prospective register of systematic reviews, PROSPERO (CRD420261381296) prior to commencement to enhance transparency and minimize duplication of effort.

Search Strategy

A comprehensive and systematic literature search was performed to identify all relevant studies published from the inception of each database until January 2025. The electronic databases searched included PubMed/MEDLINE (via NLM), Ovid and the Cochrane Central Register of Controlled Trials (CENTRAL). To ensure literature saturation, Google Scholar was also searched for the first 200 relevant hits, and the reference lists of all included studies as well as pertinent prior systematic reviews were hand-searched for additional eligible publications.

The search strategy was developed using a combination of Medical Subject Headings (MeSH) terms and free-text keywords related to the population, intervention, and outcome. The core concepts were combined using Boolean operators (AND, OR). The following

search string for PubMed is presented as an example:

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- **Outcome:** ("White Spot Lesion" OR "Dental Caries"[Mesh] OR "enamel demineralization" OR "Tooth Demineralization"[Mesh] OR "Dental Plaque"[Mesh] OR "Biofilms"[Mesh] OR "cariogenic biofilm")

No language restrictions were applied initially; however, only studies with an available abstract in English were considered for full-text retrieval. The search was confined to human studies

Eligibility Criteria:

Studies were selected based on the following PICO (Population, Intervention, Comparison, Outcome) framework:

- **Population:** Healthy children, adolescents, or adults undergoing active treatment with fixed multibracket orthodontic appliances. Studies utilizing *in situ* models (appliances on extracted teeth worn in the mouth) were also considered eligible for biofilm-related outcomes.
- **Intervention:** Professional, topical application of a fluoride varnish (FV) and/or a xylitol varnish (XV) around orthodontic bracket. The varnish could be a standalone agent or combined with other active ingredients (e.g., casein phosphopeptide-amorphous calcium phosphate). The frequency of application was not restricted *a prior* to capture the full spectrum of clinical evidence.
- **Comparison:** Placebo varnish, no intervention, standard oral hygiene care (which typically includes fluoride toothpaste use), or an alternative active agent (e.g., resin sealant, chlorhexidine gel, another fluoride formulation).
- **Outcomes:** Primary outcomes were:
    1. **Clinical Prevention of WSLs:** Measured as incidence (new lesions during treatment) or prevalence (lesions present at debonding or a follow-up point) of WSLs, assessed using a validated clinical index (e.g., Gorelick index, Enamel Decalcification Index).
    2. **Quantitative Assessment of WSLs:** Changes in lesion severity or area measured via quantitative light-induced fluorescence (QLF), laser fluorescence (Diagnodent), or similar quantitative methods.
    3. **Cariogenic Biofilm:** Changes in the composition, quantity, or metabolic activity of the dental biofilm adjacent to brackets, measured via microbiological counts (e.g., *S. mutans*, lactobacilli), standardized plaque indices, or biochemical assays (e.g., pH, fluoride release).
- **Study Design:** Randomized Controlled Trials (RCTs), including both parallel-group and split-mouth designs. For the primary prevention outcome, a minimum study duration of 6 months was required to ensure a clinically relevant observation period, aligning with evidence that the initial 6 months of treatment are critical for WSL development [13]. Studies investigating the treatment of existing WSLs had no minimum duration restriction.

Exclusion criteria: *in vitro* studies (unless directly linked to a clinical trial's mechanistic outcomes), case reports/series, narrative reviews, studies relying solely on patient-reported outcomes, and studies where the intervention was exclusively self-applied by the patient (e.g., toothpaste, mouthrinse) without a professional varnish component.

Study Selection Process

All records identified through database searching were imported into ZOTERO software for deduplication.The selection process was executed independently by two reviewers. The process involved two sequential stages:

1. **Title/Abstract Screening:** Records were screened against the eligibility criteria.
2. **Full-Text Assessment:** The full texts of all potentially eligible studies were

retrieved and independently assessed for final inclusion by the reviewers.

The entire study selection process was documented using a PRISMA flow diagram, detailing the number of records identified, screened, excluded, and the specific reasons for exclusion at the full-text stage. The PICO table and Keywords for MeSH are included in Supplementary Table 1 and 2.

Data Extraction

The following information was extracted from the included studies and standardized in the data extraction form in Microsoft Excel. Discrepancies were resolved by consensus. The extracted data included:

- **Study Characteristics:** First author, publication year, country, study design (parallel/split-mouth), sample size (per group), study duration, and setting.
- **Participant Characteristics:** Age range, mean age, gender distribution, and baseline caries risk status.
- **Intervention Details:** Type of varnish (fluoride compound and concentration, xylitol concentration, commercial name), frequency and method of application, and provider.
- **Comparison Details:** Type of control/placebo, and details of standard care provided to all participants (e.g., oral hygiene instructions, fluoride toothpaste used).
- **Outcome Measures:** Primary and secondary outcomes, method of assessment (clinical index, device), time points of measurement, and unit of analysis (patient, tooth, or site).
- **Results:** Numerical data for outcomes (e.g., mean and standard deviation for continuous outcomes, event rates for dichotomous outcomes).

Where multiple publications reported on the same trial, data were extracted and collated to avoid double-counting participants. Corresponding authors were contacted via email to request missing or unclear data.

Risk of Bias Assessment

The risk of bias in each included randomized controlled trial was independently assessed by reviewer using the revised Cochrane Risk of Bias tool for randomized trials (RoB 2) [14]. This tool evaluates bias across five domains: 1) the randomization process, 2) deviations from intended interventions, 3) missing outcome data, 4) measurement of the outcome, and 5) selection of the reported result. Each domain is judged as "low risk," "some concerns," or "high risk," leading to an overall risk of bias judgement for the study. Any disagreements in assessment were resolved through discussion.

Data Synthesis and Analysis

Extracted data were summarized narratively and presented in evidence tables, structured by intervention type (fluoride varnish vs. xylitol varnish) and outcome category (WSL prevention/treatment vs. biofilm control). All results were described and as the outcome was measured in different units, a metanalysis was not conducted

Results:

This PRISMA 2020 flow diagram illustrates systematic identification, screening, eligibility assessment, and inclusion of studies for the review. A total of 1,248 records were identified through database searches (PubMed, Ovid, and Cochrane Central, Google Scholar), with no additional records identified from other sources. After removing 215 duplicate records**,** 1,033 records remained and were screened based on titles and abstracts, of which 972 were excluded for not meeting the inclusion criteria. The full texts of 61 reports were sought out and successfully retrieved, and all were assessed for eligibility. During full-text evaluation, 49 reports were excluded for predefined reasons, including non-randomized study design (n = 25), use of self-applied interventions such as toothpaste or mouthrinse without professional varnish application (n = 10), irrelevant or non-clinical outcomes (n = 10), and duplicate publications or secondary analyses (n = 4). Following this rigorous selection process, 12 randomized controlled trials met the eligibility criteria and were included in the final systematic review [15-26]. Prisma flow diagram is given in Figure 1.

Figure 1: PRISMA Flowchart

Table 1 summarizes 12 randomized controlled trials evaluating professionally applied fluoride and xylitol varnishes for the prevention or treatment of white spot lesions (WSLs) and reduction in biofilm aggregation in patients undergoing fixed orthodontic treatment, conducted between 2001 and 2025 across seven countries (Sweden, Brazil, China, USA, Poland, Hong Kong, and Iran). All studies employed a parallel-group RCT design, with sample sizes ranging from 40 participants (20 per group) to 273 participants (137/136). WSL outcomes were assessed using validated clinical indices (Gorelick index, Enamel Decalcification Index), quantitative devices (DIAGNOdent, QLF, ΔQ), and photographic scoring systems, with several studies reporting both lesion prevalence/incidence and changes in lesion severity. Interventions primarily compare fluoride varnish against placebo,

standard care, or alternative active agents such as chlorhexidine gel, resin sealants, fluoride films, CPP-ACP (MI Paste Plus), and xylitol varnish. Early studies (Øgaard 2001) focused on WSL prevalence and progression during active treatment, while later trials increasingly incorporated quantitative fluorescence-based measures and post-orthodontic lesion regression. Notably, two Iranian trials directly compared xylitol varnish with fluoride varnish, including a recent 2025 study demonstrating post-orthodontic WSL regression. Overall, the table highlights substantial methodological consistency in study design alongside increasing sophistication in outcome assessment and expanding comparisons beyond fluoride alone.

Table 1: Characteristics of Randomized Controlled Trials Included in the Systematic Review (n = 12)

Study (Year)	Country	Study Design	Parameters of WSL Assessment	Intervention / Comparison Groups	Sample Size (per group)
Øgaard et al. (2001) [15]	Sweden	Parallel RCT	WSL prevalence & increments on maxillary incisors	Fluoride varnish ± chlorhexidine vs control	45 / 45
Du et al. (2012) [16]	China	Parallel RCT	DIAGNOdent scores; QLF parameters	Fluoride varnish vs placebo	40 / 40
Huang et al. (2013) [17]	USA	Parallel RCT	Clinical WSL scores; visual index	Fluoride varnish vs MI Paste Plus vs standard care	20 / 20 / 20
He et al. (2016) [18]	China	Parallel RCT	ΔQ values (QLF); fluorescence loss	Fluoride varnish vs fluoride film vs control	30 / 30 / 30
Restrepo et al. (2016) [19]	Brazil	Parallel RCT	Fluorescence values; WSL counts	Fluoride varnish vs CHX gel vs control	25 / 25 / 25
Turska-Szybka et al. (2016) [20]	Poland	Parallel RCT	WSL progression (%)	Fluoride varnish vs fluoride varnish + resin infiltration	38 / 38
Rechmann et al. (2018) [21]	USA	Parallel RCT	Enamel Decalcification Index (EDI); clinical exam	MI fluoride varnish vs standard care	20 / 20
Sonesson et al. (2020) [22]	Sweden	Parallel RCT	Gorelick index; photographic scoring	Ammonium fluoride varnish vs placebo	85 / 81
Zarif Najafi et al. (2021) [23]	Iran	Parallel RCT	Enamel demineralization (QLF)	Xylitol varnish (two concentrations) vs fluoride varnish	30 / 30 / 30
Flynn et al. (2022) [24]	USA	Parallel RCT	Enamel Decalcification Index; photographic scoring	Fluoride varnish vs resin sealant	20 / 20
Grocholewicz et al. (2022) [25]	Poland	Parallel RCT	Gorelick index; clinical assessment	Fluoride varnish vs standard care	30 / 30
Sardana et al. (2022) [26]	Hong Kong	Parallel RCT	Gorelick index; photographic scoring	Fluoride varnish vs standard care	33 / 33

* Trial comparing **two concentrations of xylitol varnish vs fluoride varnish**.

Table 2 summarizes the effectiveness of professionally applied fluoride and xylitol varnishes on white spot lesions (WSLs) across 12 randomized controlled trials published between 2001 and 2025**.** Early trials demonstrated that fluoride varnish significantly reduced WSL prevalence and progression compared with control, as shown by lower mean WSL scores (Øgaard et al. 2001). Quantitative assessments using DIAGNOdent and QLF further confirmed significant reductions in cariogenic activity and lesion severity with fluoride varnish (Du et al. 2012; He et al. 2016; Restrepo et al. 2016). In contrast, some studies showed no significant differences between fluoride varnish and alternative interventions or standard care, particularly for overall WSL prevalence (Huang et al. 2013; Reichmann et al. 2018), although fluoride varnish appeared more effective in preventing advanced lesions (Sonesson et al. 2020). Combination therapies demonstrated added benefit, with fluoride varnish plus resin infiltration significantly reducing lesion progression compared with fluoride varnish alone (Turska-Szybka et al. 2016). More recent trials highlighted the potential superiority of **xylitol varnish**, with two randomized studies showing significantly greater reductions in enamel demineralization and improved post-orthodontic WSL regression compared with fluoride varnish (Zarif Najafi et al. 2021;Overall, the evidence indicates consistent benefits of fluoride varnish for WSL prevention, while emerging data suggest xylitol varnish may offer additional advantages, particularly for lesion regression and biofilm-related outcomes.

Table 2: Effectiveness of Fluoride and Xylitol Varnishes on White Spot Lesions and Cariogenic Biofilm

Study (Year)	Primary Outcome Assessed	Outcome Measure (Intervention vs Control)	Effect Direction	P-value
Øgaard et al. (2001) [15]	WSL prevalence & progression	Mean WSL score: 1.13 vs 1.23 (FV vs control)	Favours FV	<0.05
Du et al. (2012) [16]	Cariogenic activity (DIAGNOdent)	DD score (6 months): 10.1 vs 13.1	Favours FV	0.004
Huang et al. (2013) [17]	Post-orthodontic WSL improvement	Visual improvement: FV ≈ MI Paste Plus ≈ control	No difference	>0.05
He et al. (2016) [18]	WSL remineralization (QLF, ΔQ)	ΔQ: −11.83 (FV) vs control	Favours FV	<0.001
Restrepo et al. (2016) [19]	WSL count & fluorescence	Active lesions: 2 vs 17 (FV vs baseline)	Favours FV	<0.001
Turska-Szybka et al. (2016) [20]	WSL progression (1 year)	Progression: 29.4% (FV) vs 7.9% (FV + resin)	FV inferior to combination	<0.001
Rechmann et al. (2018) [21]	WSL prevalence (EDI)	Prevalence: 54% vs 55%	No difference	NS
Sonesson et al. (2020) [22]	WSL prevalence; advanced WSL incidence	Advanced WSL incidence: 12% vs 26%	Favours FV (advanced lesions only)	<0.05
Zarif Najafi et al. (2021) * [23]	Enamel demineralization (QLF)	ΔQ significantly reduced with XV vs FV	Favours XV	<0.05
Flynn et al. (2022) [24]	WSL prevalence	Prevalence: 35% vs 50%	Favours FV (trend only)	NS
Grocholewicz et al. (2022) [25]	WSL prevalence & incidence	Prevalence/incidence: 17% vs 27%	Favours FV (trend only)	NS
Sardana et al. (2022) [26]	WSL prevalence	Prevalence: 10% vs 18%	Favours FV (trend only)	NS

FV = fluoride varnish; XV = xylitol varnish; NS = not statistically significant.

* Trial comparing two concentrations of xylitol varnish vs fluoride varnish

The Risk of Bias table summarizes the methodological quality of the 12 included randomized controlled trials assessed using

the Cochrane Risk of Bias 2.0 (RoB 2) **tool** across five domains: bias arising from the randomization process (D1), deviations from intended interventions (D2), missing outcome data (D3), measurement of the outcome (D4), and selection of the reported result (D5). Overall, five studies were judged to be low risk of bias, indicating robust trial design and outcome assessment, while five studies were rated as having some concerns, most commonly due to insufficient reporting related to allocation concealment or deviations from intended interventions. Two studies were classified as high risk of bias**,** primarily driven by concerns related to missing outcome data, which could have influenced the validity of the reported effects. Overall, the included studies demonstrated acceptable methodological quality, with most evidence falling within the low to moderate risk categories. (Figure 2)

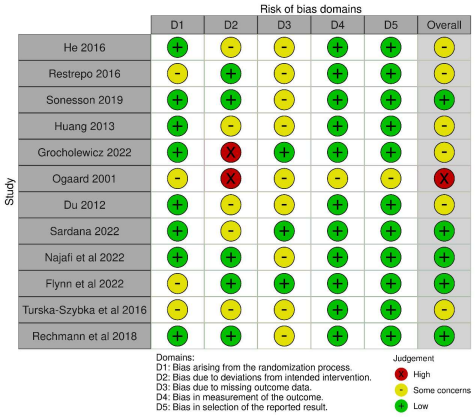

Figure 2: Risk of Bias of the included studies

Discussion:

This systematic review synthesizes evidence from 12 randomized controlled trials investigating the efficacy of professionally applied fluoride and xylitol varnishes in managing WSLs and associated biofilm in orthodontic patients. The findings indicate that FV remains a consistently effective intervention for the primary prevention of WSLs during active treatment, corroborating earlier systematic reviews [7, 8]. The majority of included studies demonstrated a significant reduction in WSL incidence, prevalence, and severity with regular FV applications compared to placebo or standard care. This effect is attributed to fluoride's well-documented role in promoting remineralization and creating a more acid-resistant enamel surface [27]. Notably, the preventive benefit appears most pronounced against the development of advanced lesions, as highlighted by Sonesson et al. (2020) [22]. However, the evidence also reveals instances where FV did not show superior efficacy to alternative agents like MI Paste Plus or chlorhexidine gel for certain endpoints, underscoring that outcome measures and comparator choices significantly influence perceived effectiveness [17, 19].

The emerging data on xylitol varnish (XV) introduces a promising alternative with a distinct mechanism of action. Unlike fluoride's predominantly enamel-focused activity, xylitol exerts its primary effect on the cariogenic biofilm. It disrupts the metabolism of *Streptococcus mutans*, reducing acid production and impairing bacterial adhesion and biofilm cohesion [28]. The two trials by Zarif Najafi and colleagues [23] provide compelling evidence that XV can be at least as effective as, and potentially superior to, FV in reducing enamel demineralization and promoting the regression of existing post-orthodontic WSLs. This suggests that targeting the microbial etiology of caries directly at the bracket-enamel interface may offer a complementary or enhanced strategy, particularly for lesion reversal. The potential synergy between fluoride's remineralization capacity and xylitol's biofilm-modifying properties presents a compelling rationale for future research into combined formulations [29].

A critical observation from this review is the relative paucity of direct, high-quality comparative studies between these two agents, with only two identified RCTs. Most evidence for FV is derived from comparisons against placebo or standard care, while XV evidence is still in its nascent stages. Furthermore, the assessment of cariogenic biofilm as a primary outcome was notably absent in most included studies. While several trials measured clinical WSL outcomes, few concurrently evaluated changes in plaque indices, microbial counts, or biofilm virulence adjacent to brackets. This represents a significant gap, as the modulation of the local biofilm ecology is a hypothesized key mechanism, especially for xylitol. Future trials should integrate standardized biofilm assessments (e.g., *S. mutans* counts, plaque pH, biofilm biomass) alongside clinical and quantitative enamel measures to provide a more holistic understanding of intervention efficacy [30].

The overall risk of bias assessment indicated generally robust methodology among the included trials, though some concerns regarding randomization and missing data were noted. The heterogeneity in outcome measurement tools—encompassing clinical indices (Gorelick, EDI), quantitative devices (QLF, DIAGNOdent), and photographic

scoring precluded a formal meta-analysis but reflects the multidimensional nature of assessing WSLs. This variability, however, complicates direct cross-trial comparisons and highlights the need for a consensus on core outcome sets in orthodontic caries prevention research [31].

Limitations of this review include those inherent to the primary studies, such as variations in FV formulation, application frequency, and follow-up duration. The strict inclusion criteria for RCTs, while ensuring a high evidence level, may have excluded other informative study designs. Furthermore, all patients likely used fluoride toothpaste, constituting a universal background exposure that may attenuate the measured effect size of professional FV applications.

Clinical Implications and Future Research: Based on the synthesized evidence, professionally applied fluoride varnish at regular intervals (e.g., every 3-6 months) can be recommended as a standard preventive measure for patients undergoing fixed orthodontic treatment. Xylitol varnish emerges as a viable non-fluoride alternative or adjunct, particularly for patients at high caries risk or with concerns about fluoride exposure. For optimal clinical decision-making, future research should prioritize well-designed, long-term RCTs that directly compare fluoride and xylitol varnishes head-to-head, using both clinical (WSL incidence/regression) and microbiological biofilm endpoints. Investigations into the cost-effectiveness, patient acceptance, and potential synergistic effects of combined fluoride-xylitol varnish formulations are also warranted [32, 33].

Conclusion

In conclusion, this systematic review affirms that professionally applied fluoride varnish is an effective mainstay for preventing white spot lesions during fixed orthodontic treatment, primarily through enhancing enamel remineralization. Concurrently, xylitol varnish emerges as a promising alternative or adjunct, offering a distinct biofilm-targeting mechanism that appears particularly beneficial for promoting lesion regression. However, the current evidence base is limited by a scarcity of direct comparative studies and a general lack of integrated biofilm assessments alongside clinical outcomes. Future high-quality, longitudinal randomized controlled trials that directly compare these agents and measure both clinical and microbiological endpoints are essential to establish clearer, evidence-based guidelines for optimizing caries prevention protocols in orthodontic practice.

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