

A Review on Prevention and Treatment of Post-Orthodontic White Spot Lesions – Evidence-Based Methods and Emerging Technologies

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Abstract: *Objective:* The aim of this paper was to update the evidence for primary and secondary prevention (treatment) of white spot lesions (WSL) adjacent to fixed orthodontic appliances.

Material and methods: A search for relevant human clinical trials published in English between 2004 and March 2011 retrieved 25 publications that fulfilled the inclusion criteria. The papers were assessed for prevented fraction and/or absolute risk reduction when possible.

Results and conclusions: The findings consolidated the use of topical fluorides in addition to fluoride toothpaste as the best evidence-based way to avoid WSL. The mean prevented fraction based on 6 trials was 42.5% with a range from -4% to 73%. The recent papers provided the strongest support for regular professional applications of fluoride varnish around the bracket base during the course of orthodontic treatment. For the treatment of post-orthodontic WSL, home-care applications of a remineralizing cream, based on casein phosphopeptide-stabilized amorphous calcium phosphate, as adjunct to fluoride toothpaste could be beneficial but the findings were equivocal. For emerging technologies such as sugar alcohols and probiotics, still only studies with surrogate endpoints are available. Thus, further well-designed studies with standardized regimes and endpoints are needed before guidelines on the non-fluoride technologies can be recommended.

Keywords: Decalcification, fixed appliances, fluoride, orthodontics.

INTRODUCTION

Despite extensive research in various preventive technologies over the years, white spot lesion (WSL) development in association with orthodontic treatment with fixed appliances remains an unwanted clinical problem [1-3]. Such lesions developing during orthodontic treatment have very limited ability to improve after appliance removal [4]. A number of recent narrative and systematic reviews have failed to present sufficient evidence for most preventive measures, with topical applications of fluoride-containing products as the only exception, and the need of new approaches and further well designed clinical trials is emphasized [5-9]. Traditionally, most research has addressed the primary prevention of WSL but in recent years, also the secondary prevention, that is the control and treatment of existing WSL's after debonding, has gained interest [10]. The aim of this paper was to review and update the evidence of current methods and emerging technologies to prevent and reverse post-orthodontic WSL and to discuss the various mechanisms of action.

METHODS

A search for relevant clinical papers published in English between January 2004 and March 2011 was conducted on Medline/PubMed and the Cochrane Library with “orthodontics”, “fixed appliances”, “caries”, “white spot lesions”, “demineralization” and “decalcification” as principle search terms. Only human controlled *in vivo* studies of topical technologies with white spot lesions incidence/reversals or other relevant surrogate endpoints were accepted. Studies on extracted teeth as well as papers dealing with lingual orthodontics and various fluoride-releasing bonding materials were excluded since a very recent systematic review was available on this topic [11]. Likewise, double publications, sole abstracts and case reports were discarded. The initial search revealed 161 papers but after independent reading of the abstracts by two examiners, 38 papers were retrieved in full length and 25 were accepted for this report [12-36]. However, no formal quality grading was done. Data on design, performance and outcome was extracted. The prevented WSL fraction was calculated as the difference in mean WSL increment between the intervention and control groups, expressed as percentage of the increment in the control group. For WSL reversals, the absolute risk reduction (ARR%) was calculated when possible.

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Table 1. Clinical Trials for Primary Prevention of WSL Adjacent to Fixed Orthodontic Appliances Published in English between 2004 and March 2011

First Author [ref no.]	Design/n	Intervention vs. Control	WSL Incidence Test/Control (%)	PF
Øgaard [12]	RCT/115	AmF/SnF ₂ -tp+rinse vs. NaF-tp+rinse	4/7	43% (NS)
de Moura [13]	RCT/14	F-tp vs. anti-plaque/F-tp	NR	NR
Vivaldi-Rodrigues [14]	SM/10	F-varnish vs. no treatment	0.34/0.51*	33% (S)
Gontijo [15]	SM/16	F-varnish vs. no treatment	NR	NR
Stecksén-Blicks [16]	RCT/273	F-varnish vs. placebo	7/26	70% (S)
Farhadian [17]	SM/15	F-varnish vs. no treatment	57/93 [§]	40% (S)
Benham [18]	SM/60	buccal sealants vs. no treatment	10/7	73% (S)
Kronenberg [19]	SM/20	Ozone vs. CHX+F-varnish	3.2/0.7	-4% (NS)
Al-Mulla [20]	RCT/20	F-tp (5000 ppm) vs. F-tp (1450 ppm)	ΔF -10/-15.8	NR

* decalcification index

[§] lesion depth, micrometer

Abbreviations: PF=prevented fraction; RCT = randomized controlled trial; SM = split-mouth; S = statistically significant difference between groups; NS = no statistically significant difference; NR = data not reported; F-tp = fluoride toothpaste; ΔF = change in fluorescence

RESULTS

Of the included papers, 9 dealt with primary prevention, 8 with WSL reversals and 6 utilized a surrogate measure such as bacterial counts, plaque amount or pH-values. The most common intervention was fluoride (9 papers) followed by antibacterial agents (5 papers), remineralization with casein phosphopeptide-stabilized amorphous calcium phosphate (CPP-ACP) (5 papers), and various other methods (6 papers).

Primary Prevention of WSL Adjacent to Fixed Appliances

In the Cochrane review [7], it was concluded that there was some evidence that daily NaF mouth rinses could reduce the occurrence and severity of WSL during orthodontic treatment albeit more high quality research was required. The recent publications are compiled in Table 1. The mean prevented fraction was 42.5% with a range from -4% to 73%. Seven out of nine papers were assessing a fluoride intervention, while the remaining evaluated the impact of sealants and ozone. Four papers dealt with fluoride varnish applications but three of them were small split-mouth studies. Thus, the only large placebo-controlled trial with parallel groups was most interesting [16]. This was a fully powered double-blind randomized controlled trial with fluoride varnish (Fluor Protector, Ivoclar-Vivadent) containing 0.1% fluoride in a homogeneous solution but the concentration is approximately 10 times higher after the varnish has dried. The result immediately after debonding displayed a prevented fraction of 70% when the varnishes were applied around the bracket base every 6th week at the regular, scheduled follow-ups. The results of the three smaller split-mouth studies pointed in the same direction, albeit with various endpoints. In two papers, fluoride mouth rinses and/or fluoride toothpaste was tested against another positive fluoride control [12, 13]. No significant differences were found indi-

cating that the presence of fluoride *per se* in the oral environment might be more important than the actual formulation. The concentration of fluoride in toothpaste may however play a role. A recent study by Al-Mulla et al. [20] showed that high sodium fluoride toothpaste (5,000 ppm), available on prescription in some countries, had a greater anti-carries potential than standard 1,450 ppm formula in patients with orthodontic bands.

The impact of non-fluoride measures was reported in two trials, one on resin sealants [18] and one on ozone [19]. Impregnation of the buccal surfaces with resin sealants seemed to effectively prevent the development of WSL in comparison with no treatment while the use of ozone was inferior to chlorhexidine varnish in controlling the microflora and prevent lesions. Both studies were however limited in size but the sealant approach merits to be repeated in a larger setting. To date, only case reports are available.

Secondary Prevention (Treatment) of Post-Orthodontic WSL After Debonding

As mentioned earlier, the interest in secondary prevention of WSL has increased. Eight new papers were identified of which five publications investigated the novel casein phosphopeptides-amorphous calcium phosphate (CPP-ACP) system (Table 2). Collectively, the CPP-ACP studies with Tooth Mousse™ (GC) or similar products retrieved clinical evidence that daily applications of the remineralizing cream could reverse the severity and visual appearance of post-orthodontic WSL more effective than, or at least as good as, fluoride toothpaste [21-25]. One study displayed reduced demineralization when ACP was incorporated in the orthodontic composite [24]. The reversals were assessed with clinical scoring and/or laser fluorescence and the mean ARR was estimated to 14%. It should however be noted that all the CPP-ACP protocols were supplements to regular use of fluoride toothpaste and that two of the studies could not display any clear clinical benefits of daily applications of the

Table 2. Clinical Trials for Secondary Prevention (Treatment) of Post-Orthodontic WSL Published in English Between 2004 and March 2011

First Author, [ref no.]	Design/n	Treatment vs. Control	Endpoint	Test/Control	ARR
CPP-ACP					
Andersson [21]	RCT/26	CPP-ACP vs. NaF-rinse	regression	55%/18%	37%
Bailey [22]	RCT/45	CPP-ACP vs. placebo	regression	72%/59%	13%
Uysal [23]	RCT/14	CPP-ACP vs. composite	micro hardness	--	S
Beerens [24]	RCT/54	CPP-ACP vs. NaF	QLF	decreased	NS
Bröcher [25]	RCT/60	CPP-ACP vs. NaF-paste	regression	33%/38%	-4% (NS)
Other methods					
Willmot [26]	RCT/26	NaF-rinse vs. placebo	regression	54%/66%	NS
Knösel [27]	NT/10	bleaching	color	improved	NA
Murphy [28]	NT/8	microabrasion	regression	83%/NA	NA
Du [29]	RCT/110	F-varnish vs. saline	LF scores	-7.6/-3.1	S

Abbreviations: RCT=randomized controlled trial; NT = non-randomized, non-controlled trial; S = significant difference between groups; NS = not statistically different; NA = not applicable; QLF = Quantitative light-induced fluorescence; LF score = laser fluorescence measurements (DIAGNOdent)

remineralizing paste [23, 25]. Furthermore, Willmot [26] failed to demonstrate an additional effect of sodium fluoride mouth rinses compared with no rinses which indicates that remineralization of WSL with normal oral hygiene procedures and fluoride toothpaste after debonding may be considered “good clinical practice” in cases with normal salivary function. A recent study suggested that weekly applications of fluoride varnish (Duraphat) during the first month after debonding were effective in bringing post-orthodontic WSL lesions to an inactive state [29].

The remaining papers on WSL treatment were not addressing the biologic repair process. Instead, a cosmetic improvement of the lesions through bleaching or microabrasion was suggested [27, 28]. As expected, both techniques did obviously improve the aesthetic appearance of WSL and should be included in the therapeutic toolbox as “plan B” in non-responders to the available biological strategies. Recently, an infiltration technique has been suggested that fills the non-cavitated pores of an incipient lesion with a low-viscosity resin by capillary action, creating a barrier that blocks further bacterial diffusion and lesion development. This micro-invasive method eliminates opaqueness and may blend existing WSL with surrounding natural teeth [30].

Antibacterial Methods

The recent literature has suggested that daily consumption lozenges containing the sugar-substitute xylitol may have a beneficial impact on the ecological environment adjacent to fixed orthodontic brackets [31, 32]. Other antibacterial measures investigated are topical applications of chlorhexidine-varnish [33], essential oils [34], ozone [19], amorphous calcium phosphate [35] and probiotics [36]. Although the findings definitely are promising, all studies except one [19] were reporting surrogate or intermediate endpoints such as mutans streptococci reductions, plaque acidogenicity or pH-recovery. As such endpoints do not allow either evidence-based conclusions or treatment recommendations,

further research of the antibacterial strategies for WSL prevention and control in orthodontic patients are needed.

DISCUSSION

It was somewhat disappointing to find that most of the recent studies dealing with prevention and treatment of WSL adjacent to fixed orthodontic still were of mixed quality. Most studies were limited in size, few were double-blind and placebo-controlled with parallel arms and the minority were reporting endpoints of true relevance for the patient. Indeed, statistically significant differences concerning surrogate endpoints may not necessarily be of clinical significance or importance for the patient. Furthermore, no paper provided data on the health-economic aspects of the various interventions. Thus, the need for more high quality research, as firmly stated in previous reviews, is unchanged. However, the tested interventions seemed to be well accepted by the patients with few dropouts and no side-effects or severe adverse events were reported for any intervention. It must also be mentioned that lingual orthodontics represents a totally different concept for reducing WSL in connection with orthodontic treatments [37].

The findings of the present review reinforced the use of topical fluorides in addition to fluoride toothpaste as the most effective way to reduce the incidence of WSL in patients undergoing orthodontic treatment with fixed appliances. The novel information was that regular topical applications of fluoride varnish around the bracket base have emerged as the most effective topical method. Fluoride varnish programs have the advantage of being independent of patient compliance through its quick and simple professional applications. Moreover, applications of fluoride varnish during the first 4 weeks after debonding seemed to be an option to create lesion inactivity [29]. Concerns have been raised against the use of highly concentrated fluoride to assist remineralization since it may lead to unsightly staining [10].

There were unfortunately no recent trials available that could either confirm or reject this important research question.

Due to its paramount role in WSL prevention, it could be of interest to examine the mechanisms of action for fluoride in combination with the other measures in the light of the ecological plaque hypothesis. Dental plaque is an example of a biofilm and biofilms with a diverse and stable microbial community are generally associated with oral health [38]. Demineralization of enamel is a result of an adaption of the biofilm to environmental pH-stress. Prolonged low pH conditions promotes the growth of aciduric bacteria resulting in a reduced diversity and a selection of species associated with a cariogenic environment, such as mutans streptococci, lactobacilli, actinomyces and veillonella. A WSL will occur when the local demineralization is larger than remineralization over time. Consequently, any intervention that counteract the acidic conditions in the oral environment and in the oral biofilm is likely beneficial for the caries balance. It is well known that very low fluoride concentrations in the plaque fluid (0.03 ppm) can reduce demineralization and enhance remineralization [39]. In higher concentrations, such as those achieved with fluoride varnish (22,500 ppm), fluoride may also hamper bacterial metabolism and acid production through interference with the enzyme enolase [40]. Likewise, xylitol is a microbial metabolic inhibitor that counteracts the pH-drop in the biofilm and reduces the selection of aciduric bacteria [31]. CPP-ACP agents may, on top of the remineralizing properties, improve the salivary buffer capacity and thereby elevate pH more rapidly after food intake [41]. The rationale behind a daily intake of health-promoting probiotic bacteria is that probiotic (health-promoting) bacteria may address the biofilm imbalance, locally by competitive inhibition of pathogens and systemically by regulating the host immune response via the guts [42]. Consequently, all the preventive strategies mentioned above can contribute to maintain the stability of the oral biofilm in various ways during the low-pH challenge caused by fixed orthodontic appliances.

CONCLUSIONS

The recent clinical trials on WSL prevention provided the strongest support for regular topical applications of fluoride varnish around the bracket base during treatment with fixed appliances. For the treatment of post-orthodontic WSL, a remineralizing cream with casein phosphopeptide-stabilized amorphous calcium phosphate as adjunct to fluoride toothpaste seemed to be beneficial with some mineral and aesthetic improvements. Conflicting results were however reported and along with emerging technologies such as sugar alcohols, antibacterial peptides and probiotics, further high-quality clinical trials with standardized regimes and endpoints are needed.

ACKNOWLEDGEMENT & CONFLICTS OF INTEREST

Dr. Bergstrand is a consultant for 3M-Unitek, Stockholm, Sweden. The authors report no conflicts of interest.

REFERENCES

- [1] Chapman JA, Roberts WE, Eckert GJ, Kula KS, González-Cabezas C. Risk factors for incidence and severity of white spot lesions during treatment with fixed orthodontic appliances. *Am J Orthod Dentofacial Orthop* 2010; 138: 188-9.
- [2] Shungin D, Olsson AI, Persson M. Orthodontic treatment-related white spot lesions: a 14-year prospective quantitative follow-up, including bonding material assessment. *Am J Orthod Dentofacial Orthop* 2010; 138: 136.e1-8.
- [3] Richter AE, Arruda AO, Peters MC, Sohn W. Incidence of caries lesions among patients treated with comprehensive orthodontics. *Am J Orthod Dentofacial Orthop* 2011; 139: 657-64.
- [4] Mattousch TJ, van der Veen MH, Zentner A. Caries lesions after orthodontic treatment followed by quantitative light-induced fluorescence: a 2-year follow-up. *Eur J Orthod* 2007; 29: 294-8.
- [5] Bergstrand F, Twetman S. Evidence for the efficacy of various methods of treating white-spot lesions after debonding of fixed orthodontic appliances. *J Clin Orthod* 2003; 37:19-21.
- [6] Derks A, Katsaros C, Frencken JE, van't Hof MA, Kuijpers-Jagtman AM. Caries-inhibiting effect of preventive measures during orthodontic treatment with fixed appliances. a systematic review. *Caries Res* 2004; 38: 413-20.
- [7] Benson PE, Parkin N, Millett DT, Dyer FE, Vine S, Shah A. Fluorides for the prevention of white spots on teeth during fixed brace treatment. *Cochrane Database Syst Rev* 2004 (3): CD003809.
- [8] Chadwick BL, Roy J, Knox J, Treasure ET. The effect of topical fluorides on decalcification in patients with fixed orthodontic appliances: a systematic review. *Am J Orthod Dentofacial Orthop* 2005; 128: 601-6.
- [9] Sudjalim TR, Woods MG, Manton DJ. Prevention of white spot lesions in orthodontic practice: a contemporary review. *Aust Dent J* 2006; 51: 284-9.
- [10] Willmot D. White spot lesions after orthodontic treatment. *Semin Orthodont* 2008; 14: 209-19.
- [11] Rogers S, Chadwick B, Treasure E. Fluoride-containing adhesives and decalcification in patients with fixed appliances: A Systematic review. *Am J Orthod Dentofacial Orthop* 2010; 138: 390.e1-8.
- [12] Øgaard B, Alm AA, Larsson E, Adolfsson U. A prospective, randomized clinical study on the effects of an amine fluoride/stannous fluoride toothpaste/mouthrinse on plaque, gingivitis and initial caries lesion development in orthodontic patients. *Eur J Orthod* 2006; 28: 8-12.
- [13] deMoura MS, de Melo Simplício AH, Cury JA. In-vivo effects of fluoridated antiplaque dentifrice and bonding material on enamel demineralization adjacent to orthodontic appliances. *Am J Orthod Dentofacial Orthop* 2006; 130: 357-63.
- [14] Vivaldi-Rodrigues G, Demito CF, Bowman SJ, Ramos AL. The effectiveness of a fluoride varnish in preventing the development of white spot lesions. *World J Orthod* 2006; 7: 138-44.
- [15] Gontijo L, Cruz Rde A, Brandão PR. Dental enamel around fixed orthodontic appliances after fluoride varnish application. *Braz Dent J* 2007; 18: 49-53.
- [16] Stecksén-Blicks C, Renfors G, Oscarson ND, Bergstrand F, Twetman S. Caries-preventive effectiveness of a fluoride varnish: a randomized controlled trial in adolescents with fixed orthodontic appliances. *Caries Res* 2007; 41: 455-9.
- [17] Farhadian N, Miresmaeili A, Eslami B, Mehrabi S. Effect of fluoride varnish on enamel demineralization around brackets. an *in-vivo* study. *Am J Orthod Dentofacial Orthop* 2008; 133: S95-S98.
- [18] Benham AW, Campbell PM, Buschang PH. Effectiveness of pit and fissure sealants in reducing white spot lesions during orthodontic treatment: A Pilot study. *Angle Orthod* 2009; 79: 338-45.
- [19] Kronenberg O, Lussi A, Ruf S. Preventive effect of ozone on the development of white spot lesions during multibracket appliance therapy. *Angle Orthod* 2009; 79: 64-9.
- [20] Al-Mulla A, Karlsson L, Kharsa S, Kjellberg H, Birkhed D. Combination of high-fluoride toothpaste and no post-brushing water rinsing on enamel demineralization using an in-situ caries model with orthodontic bands. *Acta Odontol Scand* 2010; 68: 323-8.
- [21] Andersson A, Sköld-Larsson K, Hallgren A, Petersson LG, Twetman S. Effect of a dental cream containing amorphous cream phosphate complexes on white spot lesion regression assessed by laser fluorescence. *Oral Health Prev Dent* 2007; 5: 229-33.
- [22] Bailey DL, Adams GG, Tsao CE, *et al.* Regression of post-orthodontic lesions by a remineralizing cream. *J Dent Res* 2009; 88: 1148-53.
- [23] Uysal T, Amasyali M, Ozcan S, Koyuturk AE, Akyol M, Sagdic D. *In vivo* effects of amorphous calcium phosphate-containing ortho-

- dontic composite on enamel demineralization around orthodontic brackets. *Aust Dent J* 2010; 55: 285-91.
- [24] Beerens MW, van der Veen MH, van Beek H, ten Cate JM. Effects of casein phosphopeptide amorphous calcium fluoride phosphate paste on white spot lesions and dental plaque after orthodontic treatment: a 3-month follow-up. *Eur J Oral Sci* 2010; 118: 610-7.
- [25] Bröchner A, Christensen C, Twetman S, *et al.* Treatment of white spot lesions with casein-phosphopeptide-stabilized amorphous calcium phosphate. *Clin Oral Invest* 2011; 15: 369-73.
- [26] Willmot DR. White lesions after orthodontic treatment: does low fluoride make a difference? *J Orthod* 2004; 31: 235-42.
- [27] Knösel M, Attin R, Becker K, Attin T. External bleaching effect on the color and luminosity of inactive white-spot lesions after fixed orthodontic appliances. *Angle Orthod* 2007; 77: 646-52.
- [28] Murphy TC, Willmot DR, Rodd HD. Management of postorthodontic demineralized white lesions with microabrasion: a quantitative assessment. *Am J Orthod Dentofacial Orthop* 2007; 131: 27-33.
- [29] Du M, Cheng N, Tai B, Jiang H, Li J, Bian Z. Randomized controlled trial on fluoride varnish application for treatment of white spot lesion after fixed orthodontic treatment. *Clin Oral Investig* 2011 [Epub ahead of print].
- [30] Kugel G, Arsenault P, Papas A. Treatment modalities for caries management, including a new resin infiltration system. *Compend Contin Educ Dent* 2009; 30 (Spec No 3):1-10.
- [31] Stecksén-Blicks C, Lif Holgerson P, Olsson M, *et al.* Effect of xylitol on mutans streptococci and lactic acid formation in saliva and plaque from adolescents and young adults with fixed orthodontic appliances. *Eur J Oral Sci* 2004; 112: 244-8.
- [32] Sengun A, Sari Z, Ramoglu SI, Malkoç S, Duran I. Evaluation of the dental plaque pH recovery effect of a xylitol lozenge on patients with fixed orthodontic appliances. *Angle Orthod* 2004; 74: 240-4.
- [33] Derks A, Frencken J, Bronkhorst E, Kuijpers-Jagtman AM, Katsaros C. Effect of chlorhexidine varnish application on mutans streptococci counts in orthodontic patients. *Am J Orthod Dentofacial Orthop* 2008; 133: 435-9.
- [34] Tufekci E, Casagrande ZA, Lindauer SJ, Fowler CE, Williams KT. Effectiveness of an essential oil mouth rinse in improving oral health in orthodontic patients. *Angle Orthod* 2008; 78: 294-8.
- [35] Marchisio O, Esposito MR, Genovesi A. Salivary pH level and bacterial plaque evaluation in orthodontic patients treated with Recaldent products. *Int J Dent Hyg* 2010; 8: 232-6.
- [36] Cildir SK, Germec D, Sandalli N, *et al.* Reduction of salivary mutans streptococci in orthodontic patients during daily consumption of yoghurt containing probiotic bacteria. *Eur J Orthod* 2009; 31: 407-11.
- [37] van der Veen MH, Attin R, Schwestka-Polly R, Wiechmann D. Caries outcomes after orthodontic treatment with fixed appliances: do lingual brackets make a difference? *Eur J Oral Sci* 2010; 118: 298-303.
- [38] Marsh PD. Microbiology of dental plaque biofilms and their role in oral health and caries. *Dent Clin North Am* 2010; 54: 441-54.
- [39] ten Cate JM. Fluorides in caries prevention and control: empiricism or science. *Caries Res* 2004; 38: 254-7.
- [40] Marquis RE. Antimicrobial actions of fluoride for oral bacteria. *Can J Microbiol* 1995; 41: 955-64.
- [41] Reynolds EC. Casein phosphopeptide-amorphous calcium phosphate: the scientific evidence. *Adv Dent Res* 2009; 21: 25-9.
- [42] Twetman S, Stecksén-Blicks C. Probiotics and oral health effects in children. *Int J Paediatr Dent* 2008; 18: 3-10.

Received: May 26, 2011

Revised: July 10, 2011

Accepted: July 12, 2011

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