Randomized controlled trial

Long-term remineralizing effect of MI Paste Plus on regression of early caries after orthodontic fixed appliance treatment: a 12-month follow-up randomized controlled trial

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Summary

Background: Casein-phosphopeptide-amorphous-calcium-fluoride-phosphate (CPP-ACFP) can remineralize subsurface lesions. It is the active ingredient of MI-Paste-Plus® (MPP). The long-term remineralization efficacy is unknown.

Objective: To evaluate the long-term effect of MPP versus a placebo paste on remineralization of enamel after fixed orthodontic treatment over a 12-month period.

Design: This trial was designed as a prospective, double-blinded, placebo-controlled RCT.

Methods: Patients with subsurface lesions scheduled for removal of the appliance were included. They applied either MPP or control paste once a day at bedtime for 12 months, complementary to normal oral hygiene.

Main outcome measures: Changes in enamel lesions (primary outcome) were fluorescence loss and lesion area determined by quantitative light-induced fluorescence (QLF). Secondary outcomes were Microbial composition, by conventional plating, and acidogenicity of plaque, by capillary ion analysis (CIA), and lesion changes scored visually on clinical photographs.

Randomization: Participants [age = 15.5 years (SD = 1.6)] were randomly assigned to either the MPP or the control group, as determined by a computer-randomization scheme, created and locked before the start of the study. Participants received neutral-coloured concealed toothpaste tubes marked A or B.

Blinding: The patients and the observers were blinded with respect to the content of tube A or B.

Results: A total of 51 patients were analysed; MPP (n = 25) versus control group (n = 26); data loss (n = 14). There was no significant difference between the groups over time for all the used outcome measures. There was a significant improvement in enamel lesions (fluorescence loss) over time in both groups (P < 0.001 and P < 0.001), with no differences between groups.

Limitations: Being an in vivo study, non-compliance of the subjects could have influenced the result.

Conclusion: The additional use of MPP in patients with subsurface enamel lesions after orthodontic fixed appliance treatment did not improve these lesions during the 1 year following debonding.

Registration: This trial is registered at the medical ethical committee of the VU Medical Centre in Amsterdam (NL.199226.029.07).
Introduction

Enamel subsurface lesions, so-called white spot lesions (WSL), can form rapidly around orthodontic brackets. These WSL are vulnerable to ongoing demineralization (1–4). Individuals with elevated levels of acidogenic bacteria in saliva and plaque are at high risk for the development of WSL (5–8).

A product, MI Paste Plus® (MPP, Tooth Mousse Plus®), was developed to improve remineralization. This product contains 900 p.p.m. fluoride with added calcium and phosphate, in a composition ideal for depositing fluorapatite into enamel (9–11). A crucial component of the product is the milk-derived protein casein phosphopeptide (CPP), which stabilizes amorphous calcium phosphate (ACP). This is converted to fluorapatite deposited in enamel by the available fluoride (10, 12).

The efficacy of CPP-ACP for the treatment of post-orthodontic WSL in vivo (15, 16). Also the long-term effect of this remineralizing agent is unclear (17).

In this prospective double-blinded randomized placebo-controlled superiority trial, we assessed the long-term (12 months) remineralization effect of MPP on existing WSL immediately after fixed orthodontic appliance treatment in vivo, to be used in addition to normal oral hygiene. The primary outcome is fluorescence loss and lesion area as assessed by quantitative light-induced fluorescence (QLF). Secondary outcome was microbial composition, by conventional plating, and acidogenicity of plaque, by capillary ion analysis (CIA). Additionally, lesion changes were assessed visually on clinical oral photographs.

The null hypothesis to be tested was that the in vivo use of commercially available MPP, in addition to normal oral hygiene, does not have an effect on (i) the remineralization of WSL over time, (ii) plaque composition assessed as number of colony-forming units (CFUs) and percentage of aciduric bacteria, Streptococcus mutans, Lactobacillus spp., and Candida albicans, as well as plaque acidogenicity over time, (iii) the visual changes in WSL over time in patients during 1 year after the removal of fixed orthodontic appliances.

Materials and methods

The medical ethical committee at the University Medical Centre of the Free University Amsterdam, The Netherlands, approved this study protocol (NL.199226.029.07). Treatment of WSL in former orthodontic patients was assessed during 12 months directly post-debonding. This study determined the long-term effect of MPP versus a control paste on caries lesion extent and microbial parameters.

Trial design

This trial was performed as a prospective, double-blinded, randomized, placebo-controlled superiority trial. The allocation of subjects followed a randomization scheme with stratification for gender. This resulted in an allocation ratio of 6:7 for MPP and control paste. No changes to the original protocol were made during or after the trial. It was initially intended to also assess microbial diversity by denaturing gradient gel electrophoresis (DGGE). Due to improvements in molecular biology techniques used (18–20) as well as limitations experienced with DGGE (19), this method was not utilized.

Participants

Eligible subjects had been treated with orthodontic multiple fixed bracket appliances in both arches at the Department of Orthodontics of ACTA. Subjects were enrolled after debonding and signing informed consent. All participants fulfilled the following requirements: (i) healthy adolescent males or females between 12 and 19 years of age; (ii) two or more buccal WSL on former bracketed surfaces, seen without prolonged air drying as a distinct visual change in enamel and/or localized enamel breakdown without clinical visual signs of dentinal involvement (International Caries Detection and Assessment System (ICDAS) code 2); (iii) no systemic diseases or syndromic abnormalities and (iv) no proven or suspected milk protein allergy and/or sensitivity, or allergy to benzoate preservatives, as both are components of the MPP product.

Eligible subjects were invited to participate in this study and were screened by MWB for WSL directly after debonding. The study group consisted of 65 participants: 27 male and 24 female subjects with a mean age of 15.5 years (SD = 1.6).

Study settings

This single-centre trial took place at the Department of Orthodontics, Academic Centre of Dentistry Amsterdam, The Netherlands from January 2008 to August 2010. Amsterdam is the capital of The Netherlands with a population of 756 000 at the time in 2009, having 156 000 children between the ages of 0–19 years (21). There is a broad range in socioeconomic status for children undergoing orthodontic treatment, as orthodontics is mostly accessible for all children until the age of 18, as a result of the social health service structure. In Amsterdam, the community tap water is not fluoridated.

Randomization, intervention procedure, and blinding

Participants, complying with the inclusion criteria as determined by MWB, were randomly assigned by MHV to group A or B, i.e. the MPP or the control group, as determined by a computer-randomization scheme, created and locked before the start of the study. Participant allocation was kept separate from the data recording files in a locked closet. Data were collected and coded based on participants’ ID number and a sequential number in order of date of study visits. Data analysis was performed blind for group allocation.

Participants received neutral-coloured concealed toothpaste tubes marked A or B, which contained either CPP-ACP + sodium fluoride [0.2 per cent w/w; 900 p.p.m.; [MPP 35 ml, Recaldent; GC Benelux Europe, Leuven, Belgium] or fluoride-free control paste + calcium [Ultradent 100 ml; Kruidvat NL, Renswoude, The Netherlands] for home use. Participants were instructed to use their respective paste once a day at bedtime after tooth brushing. Participants received verbal and written instructions on product use and oral hygiene by a dental hygienist. They were advised how to brush properly using a fluoridated dentifrice (i.e. at least twice a day, either with a hand toothbrush or an electric toothbrush for at least 2 minutes). No additional fluoride was to be applied. Participants were informed to apply at least a pea-size amount to the tooth surfaces in each arch using a clean, dry finger and keep the study product in the mouth for as long as possible. Participants were instructed not to rinse afterwards. Compliance was checked by questions regarding product use at each visit. Furthermore, participants were asked to bring their study paste to each visit. Prior to each study visit, they were asked to refrain from tooth brushing from the evening before the visit and from eating and drinking 2 hours prior to the visit. Each visit started with plaque sampling. After plaque sampling, the tooth surfaces were cleaned and polished for adequate viewing of WSL in the QLF and digital oral photographs.
The participants’ dentists were informed of their patients’ participation and were asked not to administer additional fluoride during this investigation. They were further asked to contact the study investigator if restorations were made on the buccal surfaces.

Subjects were informed that they would receive either the MPP paste or the control paste with a different form of calcium delivery. The patients and the observer were blinded with respect to the content of tube A or B. Examiners MWB, FB, and MHV were also blinded.

**Study procedure and outcomes**

Plaque for microbial composition and acidogenicity was sampled before debond (T0) and 6 weeks (T2), 3 months (T3), 6 and 12 months (T4, T5) thereafter. Next QLF photographs were taken post-debond (T1) and further QLF photographs were taken at 6 weeks (T2), 3 and 6 months (T3, T4), and 1 year post-debonding (T5). Finally, clinical oral photographs were taken at T1 and T5. WSL severity as assessed by QLF was the primary outcome measure. Microbial composition, as determined by conventional plating and acidogenicity of plaque, was secondary outcome measures. Additionally, WSL changes were visually assessed on digital oral photographs.

**Quantitative light-induced fluorescence**

QLF images were captured using an intra-oral fluorescence camera (QLF/Clin; Inspektor Research Systems, Amsterdam, The Netherlands) with a dedicated software (Inspektor pro version 3.0.0.42; Inspektor Research Systems) as described by Beerens et al. (22). Images were assessed for fluorescence loss \([\Delta F \text{ (per cent)}, \text{ lesion area } [A \text{ (mm}^2 \text{)]}, \text{ and integrated fluorescence loss (IFL) } [\Delta F \times A \text{ (per cent } \times \text{ mm}^2 \text{)]}\].

**Plaque processing**

Plaque was sampled from the buccal surface of the lower right first or second premolar for microbial composition. Also, plaque was sampled from the buccal surface of the upper right and left first or second premolar for acidity of plaque, before and after sucrose pulse, respectively. Plaque samples were analysed blind with respect to subject number, visit, and group allocation. Microbial composition was determined by the total numbers of CFUs (counts/sample), and the proportions of aciduric bacteria \([\text{per cent bacteria count/total count}], S. \text{ mutans} \text{ [per cent bacteria count/total count]}, \text{Lactobacillus spp. [per cent bacteria count/total count]}, \text{ and the fungus C. albicans [per cent fungal count/total count]}\] as described by Beerens et al. (22). The acidogenicity of plaque was analysed by means of capillary ion electrophoresis (Waters’ trade name: Capillary Ion Analysis, CIA [μmol acid/mg protein]) (23). Calibration curves were made for each component separately. As internal standard, oxalate was included in all samples. To normalize the samples, the protein concentration of all samples was determined (24).

**Clinical oral photographs**

For each subject and time point, pictures were collated in a photography gallery comprising four pictures per patient (Figure 1) and printed at high quality. These photographs were assessed by two calibrated examiners (FB) and (MWB).

Photographs were analysed in random order for subject and time using the ICDAS criteria (25). The ICDAS code 1 (First Visual

**Sample size**

To assess the influence of casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACFP) on the reduction of WSL, a power analysis was conducted as described by Beerens et al. (22). Based on a previous observational study at the Orthodontic Department at ACTA (26), we found a statistically significant, but clinically irrelevant, natural reduction in fluorescence loss, of 0.9 per cent (SD = 0.9 per cent), during a 24-week time period. A clinically relevant change in fluorescence loss was considered to be an average reduction of 2 per cent, implying an effect size of 0.55. The sample size was calculated for a more conservative effect size of 0.35. For an effect size of 0.35 with a power of 0.9 to be measured between the two groups, a group size of 27 was needed (G*–power 3.1.0, ANOVA for repeated measures, between factors).

Although orthodontic patients, in general, are seen at 4- to 6-week intervals during the active phase of treatment, during the retention phase, subjects often do not show up for their scheduled appointments. At the department of orthodontics at ACTA, this level of no shows is relatively high. To compensate for subject withdrawal, we aimed to include 30 subjects in each group. Subjects who dropped out before T2 were replaced to meet the required minimum group size of 27.

**Interim analysis**

The 3-month data from this study were reported in December 2010 (22). The trial was not stopped earlier than planned.

**Data analysis**

Statistical analysis was performed with SPSS (PASW statistics 21.0; SPSS Inc., Chicago, IL, USA). Change of enamel lesions, assessed by QLF, was the primary outcome measure. The average fluorescence loss for all WSL, total lesion area, and IFL were calculated for each subject and then normalized to 20 surfaces corrected for the number of missing and filled surfaces during the trial. Student’s (two-tailed)
Results

Eligible participants were recruited from January 2008 to August 2009. From the 184 screened participants, 65 were enrolled in the study and randomly assigned into two groups: the MPP group (group A; n = 35) and the control group (group B; n = 30). All participants received intended treatment. Inclusion of participants stopped when at least 30 subjects were enrolled in each groups and seen at the 6-week visit.

A flow diagram, from enrolment and group allocation to study conclusion, is shown in Figure 2. Fourteen patients dropped out by the average of the previous and following data point.

Lesion changes assessed by QLF

Arresting or reduction of extent of enamel lesions, by QLF, was the primary outcome regarding efficacy of MPP (Table 2). No significant differences between the groups were found at baseline (T1) for lesion area (A), lesion depth (ΔA), and IFL (t-test independent measurements were followed up throughout the investigation. The affected elements were distributed as follows: 14.3 per cent central incisors, 22.8 per cent lateral incisors, 29.1 per cent cusps, and 33.8 per cent premolars. This distribution was similar for the two groups. Overall compliance in the study was moderate. Questions regarding frequency of brushing and product use revealed that, during the first 6 weeks of the study, the subjects generally brushed twice a day and used the product at nighttime. Between 6 weeks and 12 months, the subjects forgot to brush and to use the product on average once a week, and this always occurred at nighttime. Assessment of product use via returned product failed because none of the subjects returned their product tubes at recall visits, despite our request.

Table 1. Baseline data, showing no statistical differences between the groups.

| Groups | MPP (A) | Placebo (B) |
|--------|---------|-------------|
| Allocated to intervention group | 35 | 30 |
| Gender ratio M:F (% male) | 16:19 (45.7%) | 12:18 (40.0%) |
| Participant age | 15 years 8 months | 15 years 3 months |
| Multi-bracket treatment duration | 2 years 5 months | 2 years 3 months |
| DMFS | 2.09 | 2.07 |
| Bleeding on probing (%) | 36 | 33 |
Table 3. Microbial composition, determined by total bacterial counts, the proportions of aciduric bacteria, Streptococcus mutans spp., Lactobacillus spp. and Candida Albicans, at five different time points (T0, T2, T3, T4, and T5) in the MPP group and the control group.

| MI Paste Plus, n = 25 | T0 | T2 | T3 | T4 | T5 |
|-----------------------|----------------|----------------|----------------|----------------|----------------|
| Colony-forming units (CFUs) (10^7) counts/sample | 5.4 ± 6.3 | 4.3 ± 4.8 | 4.3 ± 4.4 | 4.2 ± 5.2 | 5.5 ± 4.3 |
| Aciduric bacteria [bacteria count/total count (%)] | 53.2 ± 33.5 | 47.2 ± 31.7 | 46.4 ± 27.8 | 32.9 ± 27.1 | 29.3 ± 19.2* |
| Streptococcus mutans [bacteria count/total count (%)] | 9.8 ± 14.1 | 3.9 ± 7.4 | 8.6 ± 10.5 | 4.7 ± 9.6 | 8.6 ± 13.6 |
| Lactobacillus spp. [bacteria count/total count (%)] | 0.2 ± 0.5 | 0.0 ± 0.1 | 0.1 ± 0.2 | 0.0 ± 0.1 | 0.1 ± 0.2 |
| Candida Albicans [fungi count/total count (%)] | 1.0 ± 2.2 | 0.5 ± 1.9 | 0.7 ± 1.8 | 0.2 ± 0.9 | 1.0 ± 4.4 |

Control, n = 26

| T0 | T2 | T3 | T4 | T5 |
|----------------|----------------|----------------|----------------|----------------|
| Colony-forming units (CFUs) (10^7) counts/sample | 3.4 ± 3.2 | 3.6 ± 3.6 | 5.1 ± 4.6 | 8.8 ± 2.7 | 6.0 ± 9.5 |
| Aciduric bacteria [bacteria count/total count (%)] | 49.2 ± 49.4 | 48.0 ± 38.4 | 32.2 ± 25.3 | 34.4 ± 27.6 | 31.3 ± 21.2 |
| Streptococcus mutans [bacteria count/total count (%)] | 12.2 ± 19.5 | 4.3 ± 7.9 | 4.2 ± 9.2 | 10.8 ± 16.5 | 5.9 ± 9.0 |
| Lactobacillus spp. [bacteria count/total count (%)] | 0.1 ± 0.2 | 0.4 ± 1.8 | 0.3 ± 0.9 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Candida Albicans [fungi count/total count (%)] | 5.7 ± 15.2 | 11.5 ± 57.2 | 2.0 ± 7.6 | 0.9 ± 2.0 | 0.2 ± 1.0 |

Bacterial counts are expressed as a percentage of the total counts per sample obtained at each time point. Data are given as mean ± SD.

*Data significantly different from baseline.
No significant differences in acid and phosphate composition of resting plaque or after sucrose pulse were seen in time or between the groups (repeated-measures ANOVA, \( P > 0.05 \)).

### Lesion changes assessed by clinical oral photographs

Changes in enamel lesions between T1 and T5 (secondary outcome) assessed on the clinical photographs (Figure 1) are given in Table 4.

Three participants were excluded, one in the MPP and two in the control group. These participants had an incomplete photograph gallery at T5. No significant differences were found between the groups at baseline (Mann-Whitney \( U \); mean \( U = 79980, z = -4.54, P = 0.001 \)), showing less visible lesions in the MPP group than in the control group. Most of the surfaces were scored 0 for both the MPP and the control group at T1. All three participants had an incomplete photograph gallery at time point T5.

There was no significant difference between the groups over time. Lesions that scored 2 essentially did not change over time. One lesion was given an ICDAS score of 3 on the photograph gallery. This lesion was assessed as ICDAS score 2 clinically. The lesion in the control group that scored 3 at T1 and 0 at T5 has been restored with a filling and appeared undetectable.

The lesions that scored 0 at T1 and 2 at T5 are presumably lesions that appeared after gingiva reduction.

### Adverse effect

There were no harms experienced by the participants influencing general health of the participants, for either group. However, in the MPP group, a total of five patients had the assumption that their teeth gradually discoloured to a more yellow tone. These findings were considered incidental. No objective measures were used to test this possibly adverse effect; however, it was observed on the digital photographs of several patients in the MPP group.

### Overall conclusion

The use of MPP in orthodontic patients with subsurface enamel lesions did not improve these lesions over the 1-year period of the study, as evaluated by QLF imaging, microbiological composition and acidogenicity, as well as by digital oral photographs.

The lesion depth in both groups showed an overall improvement assessed by QLF (primary outcome), while the secondary optical assessment by ICDAS showed the lesions to be unchanged in both groups. No significant additional improvement was measured for patients receiving MPP. The plaque composition, regarding bacterial counts, the proportions of aciduric bacteria, \( S. \) mutans spp., \( Lactobacillus \) spp., and \( C. \) albicans showed no change compared with a more healthier composition, observed for both groups.

MPP did not have an effect on the visual changes of WSLs on the long term, when assessed on photographs. Lesions remained visible over time.

### Discussion

#### Key findings

This study is the first to address the efficacy of MMP for the treatment of post-orthodontic WSL in vivo during 1 year following debonding, that is, long term. We found a lack of positive evidence to support the effectiveness of MMP as a remineralizing agent, to be effective for the treatment of post-orthodontic WSLs. This outcome was confirmed by several independent detection methods, which strengthens this conclusion.

#### Explanation

MPP does not have a positive effect on WSL improvement seen by QLF imaging or optical assessment nor does it have a neutralizing effect on the bacterial oral flora. Regardless the application of the product or control, lesions tended to improve after removing orthodontic fixed appliance. Similarly, removing the orthodontic fixed appliance had a positive effect on the composition and acidity of the bacteria on the long term, which was not affected by either product.

#### Comparing these findings with other studies

Although the efficacy of CPP-ACP for the prevention and regression of incipient lesions has been demonstrated in vitro (13, 14), there is a lack of reliable evidence for the treatment of post-orthodontic WSL in vivo (15, 16) and the long-term effect of this remineralizing agent is unclear (17). This study is the first to address these aspects.

*In vitro* (11, 13) and *in situ* studies (14, 25, 27) have demonstrated that CPP-ACP may promote the remineralization of subsurface enamel lesions. These findings are summarized in a meta-analysis for *in vitro* and *in situ* studies regarding the effect of CPP-ACP as a caries-preventive agent (28). When evaluating *in vivo* studies, Chen et al. (15) reported a lack of reliable evidence to support remineralizing agents for the treatment of post-orthodontic WSLs. A systematic research described by Li et al. (17) reported the same conclusion, although, in this systematic research, the effect of CPP-ACP was assessed for orthodontic and non-orthodontic subsurface lesions. Our findings contradict with the findings of Bailey et al. (28) and Brochner et al. (29) who reported a positive effect of casein supplements after only 12 and 4 weeks, respectively. Bailey et al. concluded a positive effect within 12 weeks although no statistical differences were found using ICDAS code 2. Their conclusion was based on visual assessment of lesion activity of inactivity (28). Brochner et al. (29) reported a reduction in lesion area of 58

### Table 4. Enamel change, determined by International Caries Detection and Assessment System (ICDAS) at the time points of debond, blinded assessed at baseline (T1) and 12 months (T5) thereafter, in the MPP group and the control group.

| MI Paste Plus n = 24 | ICDAS score at T5 |
|---------------------|------------------|
| ICDAS score at T1   | 0 1 2 3 5 Total T1 |
| 0                   | 229 21 3 5 250 |
| 2                   | 87 102 3 1 1 192 |
| 3                   | 1 1 1 2 |
| Total T5            | 316 123 4 1 444 |

| Control n = 24 | ICDAS score at T5 |
|----------------|------------------|
| ICDAS score at T1 | 0 1 2 3 5 Total T1 |
| 0                  | 199 29 3 228 |
| 2                  | 66 108 21 195 |
| 3                  | 1 1 1 3 |
| Total T5           | 266 138 22 426 |

Missing data \( n = 3 \). Data are given as amount counted at the two different time points.

*This lesion has been restored and was scored 0 at T5.*
per cent after 4 weeks. However, the lesions investigated were very small (0.19 mm²). One may debate clinical relevancy. Andersson (30) compared the effects of CPP-ACP with fluoride mouthwashes on the regression of WSL and concluded that both regimens could promote regression of WSL after debonding of fixed orthodontic appliances, though the visual evaluation suggested an aesthetically more favourable outcome of the ACP.

Strength and limitations of this study
The study was performed in a diverse population of teenagers in Amsterdam, The Netherlands. The Netherlands is part of Western Europe and has no water fluoridation. Therefore, water fluoridation did not affect the outcome of this study. WSL developed during orthodontic treatment appear more rapidly and are more porous than WSL in non-orthodontic patients. As a result, the findings of this study are only applicable to WSL developed during orthodontic treatment. The efficacy of this remineralization agent on WSLs after orthodontic treatment with full fixed appliances was not influenced by background levels of fluoride. As for all randomized clinical trials, non-compliance of the subject could have influenced the result. The assessment of product use via returned product failed entirely because none of the subjects returned their product tubes at recall visits. Also, we did not use an application tray, for example, a removable clear retainer to improve the cream to stay in place. Though by not using an application tray, saliva could now also influence possible remineralization.

One of the possible limitations influencing the results of the study is the preservation of CPP-ACP in MPP. This could be the explanation of for the positive results found in vitro and in situ. This contradicts the findings of in vivo study results.

The question can also be raised if there was a similarity of intervention. As there might be a taste difference between the two products. Cross contamination is not to be expected as no siblings were included. We aimed to have 27 participants per group as was assessed as the effect size. Unfortunately, due to drop out, it became lower with 23 to 26 per group. The used power was 0.9 if using the power of 0.8, at least 20 participants should be included. So, we can state that a power of 0.8 is still acceptable to draw conclusions. Even so the effect found is so small that even if statistical significant it is still not clinically relevant.

Implications
The use of MPP in patients with subsurface enamel lesions after orthodontic fixed appliance treatment does not show an additional superior improvement of these lesions on the long term as measured by means of QLF imaging, microbiological composition and its acidity, as well as by digital oral photographs. This suggests that there is no clinical evidence to support that MPP is a remineralization agent as it is not effective to improve post-orthodontic subsurface lesions.

Registration
This trial is registered in The Netherlands at Amsterdam Free University of the University Medical Centre medical ethical committee under number NL.199226.029.07.

GC Benelux, Leuven, Belgium provided free supplies of MPP used in the study. None of these authors or study received personnel or consulting payments or any other form of personal benefit from GC Benelux.

Trial protocol
Full details of the trial protocol NL.199226.029.07 are available on request.

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Supplementary material
Supplementary material is available at European Journal of Orthodontics online.

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Conflict of interest
MHvdV is a co-inventor on several patents relating to quantitative light-induced fluorescence. The authors declare that otherwise there is no conflict of interest pertaining to the data presented in this article.

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