Self-assembling Peptide P₁₁-4 and Fluoride for Regenerating Enamel

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Abstract

Regenerative medicine-based approaches for caries treatment focus on biomimetic remineralization of initial carious lesions as a minimal invasive therapy. In vitro, self-assembling peptide P₁₁-4 enhances remineralization of early carious lesions. To investigate the safety and clinical efficacy of P₁₁-4 for treatment of initial caries, a randomized controlled single-blind study was conducted on children aged >5 y with visible active early caries on erupting permanent molars. Subjects were randomized to either the test group (P₁₁-4 + fluoride varnish) or control group (fluoride varnish alone). Caries were assessed at baseline and at 3 and 6 mo posttreatment per laser fluorescence, a visual analog scale, the International Caries Detection and Assessment System, and Nyvad caries activity criteria. Intention-to-treat analyses were performed, and safety and clinical feasibility of the treatment approaches were assessed. Compared with the control group, the test group showed clinically and statistically significant improvement in all outcomes at 3 and 6 mo. The laser fluorescence readings (odds ratio = 3.5, *P* = 0.015) and visual analog scale scores (odds ratio = 7.9, *P* < .0001) were significantly lower for the test group, and they showed regression in the International Caries Detection and Assessment System caries index (odds ratio = 5.1, *P* = 0.018) and conversion from active to inactive lesions according to Nyvad criteria (odds ratio = 12.2, *P* < 0.0001). No adverse events occurred. The biomimetic mineralization facilitated by P₁₁-4 in combination with fluoride application is a simple, safe, and effective noninvasive treatment for early carious lesions that is superior to the presently used gold standard of fluoride alone. By regenerating enamel tissue and preventing lesion progression, this novel approach could change clinical dental practice from a restorative to a therapeutic approach. This could avoid additional loss of healthy hard tissue during invasive restorative treatments, potentially enabling longer tooth life and thereby lowering long-term health costs (ClinicalTrials.gov NCT02724592).

Keywords: dental caries, peptides, tooth remineralization, dental enamel, regeneration, pediatric dentistry

Introduction

Caries occurs due to an imbalance of the re-/demineralization equilibrium, leading to a net loss of tooth minerals progressing to a cavity (Steinberg 2007). The traditional “treatment” of caries by drilling and filling constitutes a replacement of the damaged tissue by a foreign material. Such invasive, restorative treatments are associated with destruction of healthy tooth tissue, often leading to a cycle of ever larger restorations (Ismail et al. 2013), resulting in a major burden in terms of individual and public health costs (Splieth and Flessa 2008).

In an attempt to avoid or delay restorations, several noninvasive and minimally invasive treatments have been suggested for early caries, prior to cavitation of the enamel surface (Splieth et al. 2010). The noninvasive treatments are directed at inactivating or arresting caries, shifting the equilibrium toward remineralization by decreasing the solubility of the hard tissue or increasing the surface area for mineral redeposition (Weatherell et al. 1977; Reynolds 2005). In addition, diet control and plaque removal support natural remineralization of the carious lesions via Ca²⁺ from saliva (de Almeida Pdel et al. 2008). However, saliva’s regenerative potential is limited to enamel that is marginally affected by caries (Dowd 1999).

The present gold standard, treatment with fluoride ions, positively affects the re-/demineralization equilibrium by lowering the mineral’s solubility product and thereby protecting enamel from dissolution by bacterial acids (Weatherell et al. 1977). Fluoride’s preventative effect is most pronounced for sound enamel but is less effective for manifestly carious lesions (Divaris et al. 2013).

Regenerative medicine-based dental approaches, wherein damaged or diseased dental tissues are replaced with biologically similar tissues, are being adopted and represent an ongoing shift from reparative to regenerative medicine and dentistry. Recent approaches involve stimulating dentine tissue regeneration or healing via a pharmacologic pathway, but no regenerative

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A supplemental appendix to this article is available online.

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treatment of acellular enamel has yet been clinically tested and proven (Neves et al. 2017).

The subsurface body of an initial carious lesion presents a unique biological compartment, similar to a void created during guided tissue regeneration. In this secluded space, a biomimetic scaffold can facilitate natural hard tissue remineralization through saliva, resulting in guided enamel regeneration, analogous to guided tissue regeneration and guided bone regeneration (Lutz et al. 2015).

During odontogenesis, the enamel matrix proteins control the enamel formation but are almost completely degraded during the final stage of maturation (Kirkham et al. 2000; Kirkham et al. 2002). Recent studies have demonstrated the potential of a new biomimetic approach with the self-assembling peptide P₁₁-4 to form a 3-dimensional matrix within the subsurface body of the initial carious lesion, which mimics the ability of enamel matrix proteins to template and nucleate hydroxyapatite (Kirkham et al. 2007; Kind et al. 2017). In vitro studies have shown that the P₁₁-4 matrix has a high affinity for Ca²⁺, acting as a nucleator for de novo hydroxyapatite formation (Kirkham et al. 2007; Brunton et al. 2013; Kind et al. 2017). This suggests that the P₁₁-4 matrix enhances the body's own saliva-driven enamel remineralization process, enabling remineralization of more pronounced lesions but not cavitated lesions (Weatherell et al. 1977; Brunton et al. 2013; Silvertown et al. 2017). This study investigated the in vivo safety and clinical efficacy of P₁₁-4 in combination with fluoride varnish in the treatment of initial occlusal caries lesions (International Caries Detection and Assessment System [ICDAS], 1 to 3) and compared it with the current clinical gold standard fluoride varnish alone. We hypothesized that the combination treatment of P₁₁-4 and fluoride varnish would achieve superior remineralization, prevent progression, and promote regression of the initial carious lesions, as compared with treatment with fluoride varnish alone.

Methods

Study Design

A randomized controlled single-blinded study investigated the clinical effect of self-assembling peptide P₁₁-4 in addition to the effect of fluoride varnish treatment (test) in remineralizing initial occlusal caries (ICDAS, 1 to 3) on erupting permanent molars, in comparison with fluoride varnish alone (control), after 3 and 6 mo. Clinical study procedures were performed in the Department of Preventive and Pediatric Dentistry, University of Greifswald. Study approval was obtained from the ethics committee of the University of Greifswald.

Participants

Children (>5 y old) with visible and accessible, active, early occlusal caries on first or second erupting permanent molars, detected during routine dental checkup in the Department for Preventive and Pediatric Dentistry/Greifswald (February 2013 to April 2014), were eligible for the study as convenience

sample. This included recording of the dmft/DMFT index according to criteria from the World Health Organization (2013) and the caries risk assessed by the clinical judgement based on the combination of age, past caries experience, plaque, and oral hygiene practices (Anusavice 2001; American Academy of Pediatric Dentistry 2013).

Exclusion criteria were any pathology or concomitant medication affecting salivary flow, evidence of tooth erosion, fluoride varnish application <3 mo prior to study treatment, any metabolic disorders affecting bone turnover, or concurrent participation in another clinical trial. Written informed consent was obtained from the participating children and their parents prior to any study-related procedures.

Randomization and Blinding

The study was single blinded (patient blinded). A total of 70 patients were randomly assigned to either the test group—receiving self-assembling peptide P₁₁-4 (Curodont Repair; Credentis) + fluoride varnish (22,600 ppm, Duraphat; Colgate-Palmolive Co.)—or the control group, receiving fluoride varnish alone (*n* = 35 per group). One lesion per child was included into the study. A computer-generated random allocation sequence was provided by a third party and kept within sequentially numbered opaque sealed envelopes (simple randomization with a 1:1 allocation ratio). Patients who met the inclusion criteria were enrolled into the study by the investigators, and a sealed envelope was drawn to determine the assignment of the patient to the test or control group. Eligible patients received treatment until withdrawal of consent or until unacceptable safety and toxicity was identified.

Procedures

Caries lesions were assessed at baseline by 3 calibrated clinicians (Table 1). The materials were applied in compliance with the manufacturers' instructions (Appendix Fig. 1). In the test group, the self-assembling peptide P₁₁-4 was applied 1 time at the baseline visit, followed by fluoride varnish application. The control group received fluoride varnish at the baseline visit. Oral health instructions were given, and fluoride varnish was applied on all study lesions, test and control group, at the 3-mo follow-up session.

Outcomes

Primary outcome was caries regression/progression as measured by the change in the laser fluorescence readings (Diagnodent, KaVo; Diniz et al. 2015). At each visit, triplicate measurements were taken and the mean value used for statistical analysis. Laser fluorescence readings depend on the amount of fluorescent protoporphyrin released as metabolic by-products from caries-causing bacteria and the color of the carious tissue (Gurbuz et al. 2008). A decrease in the signal indicates regression of the carious lesion, whereas an increase indicates progression.

Table 1. Baseline Characteristics of the Recruited Study Cohort.

	Test Group (n = 31)	Control Group (n = 34)
Demographics		
Age, y	9 (7 to 13)	10.5 (8 to 13)
Female sex	13 (42)	14 (41)
Oral characteristics		
dft	2 (0 to 6)	1 (0 to 5)
dfs	2 (0 to 14)	1 (0 to 0)
DMFT	0 (0 to 1)	1 (0 to 3)
DMFS	0 (0 to 1)	1 (0 to 3)
Dental plaque, %	40 (30 to 50)	35 (30 to 50)
Caries risk		
High	9 (29)	7 (21)
Moderate	18 (58)	21 (62)
Low	4 (13)	6 (18)

Data are presented as *n* (%) or median (interquartile range). Missing data for primary/secondary dentition: 8 each in dft and dfs in the test group; 11 each in dft and dfs and 1 each in DMFT and DMFS in the control group. Study lesions did not count into the DMFT index, as they were initial lesions.

Secondary outcomes were as follows: 1) lesion progression assessed by visual analog scale (VAS), with values ranging from 100 to −100, where 100 indicated strong progression, 0 arrested, and −100 strong regression; 2) caries regression/progression as assessed by the ICDAS (Ismail et al. 2007); and 3) caries activity assessment according to Nyvad criteria (Nyvad et al. 2005).

According to ICDAS, initial caries was classified as 1 to 3 (1, first visual change in enamel; 2, distinct visual change in enamel; 3, localized enamel breakdown; Ismail et al. 2007). Caries regression was defined as a change into a lower ICDAS class, while progression was defined as a change to a higher ICDAS class.

Nyvad assessment of lesion activity was determined on the basis of combined visual-tactile criteria after drying of the teeth, with a focus on the surface characteristics of lesions, plaque accumulation, primary integrity, and surface texture (Nyvad et al. 2005).

Treatment safety was assessed by recording adverse events as defined in the incidence of treatment-emergent adverse events adapted from Directive 2001/20/EC and in accordance with ISO14155 and MDD93/42.

Clinical usability of the test and control products was assessed by measuring the duration of the procedure and assessing the patient's perception of comfort and smell/taste of the products through appropriate questions.

Statistical Analysis

Due to ethical consideration and because the treatment approach was new, we balanced medical and statistical considerations as recommended by the CONSORT statement (Moher et al. 2010) and chose a simple technique to determine the sample size (Senn 2007). We considered typical sample sizes of successful

trials (Altenburger et al. 2010; Paris et al. 2010; Ferrazzano et al. 2011).

As recommended by the E9 guideline (ICH E9 Expert Working Group 1999), only outcome-specific baseline data were considered. Treatment effects were also adjusted for age, sex, and plaque on subject level. With laser fluorescence as the primary outcome, gamma distribution was used to model the skewed distribution (Hardin and Hilbe 2007). Furthermore, 3- and 6-mo follow-up data were combined into a single model, and variances were adjusted for clusters among patients to increase statistical power. To obtain robust inferences on the treatment effect in terms of odds ratios, an ordinal logistic regression model was chosen, which uses only the rank ordering of outcome values and therefore is appropriate for continuous outcomes (Harrell 2015).

For VAS at either follow-up, linear regression with robust variances was chosen. For VAS at both follow-ups, random intercepts and random slopes were modeled, and fixed effects were corrected for the small sample size by using the Kenward-Roger method in mixed models. Ordinal regression was performed with the rms package of R software (version 3.3.1). All other analyses were performed with Stata/MP software (release 14.2; Stata Corporation). $P < 0.05$ was considered statistically significant. Statistical analysis was performed by an independent statistician from the Department of Mathematics and Informatics, University of Greifswald. More details on statistics are in the Appendix.

Results

Study Subjects

Seventy children (28 girls, 42 boys), with a mean age of 10.0 ± 2.7 y, were enrolled; none declined to participate (Appendix Fig. 2). Baseline characteristics of the recruited study cohort are provided in Table 1; the patient demographics and oral characteristics were similar for the test and control groups. Mean caries incidence of decayed/filled primary teeth (dft) was 2.8 ± 3.1 and decayed/missing/filled permanent teeth (DMFT), 1.3 ± 2.5 . The last recall visit for the last recruited patient was in October 2014. Four patients were lost to follow-up at all recall visits, while 1 did not fulfill the inclusion criteria; these were excluded from the analysis. Six and 3 subjects missed the 3- and 6-mo visits, respectively, due to personal travels or loss of contact. There was no withdrawal of informed consent.

Laser Fluorescence Readings

Compared with baseline (test, 45.2 ± 22.9 ; control, 33.0 ± 16.5), laser fluorescence readings decreased in the test group over time (3 mo, 29.5 ± 18.8 ; 6 mo, 27.2 ± 19.3) but remained stable in the control group (3 mo, 31.6 ± 21.0 ; 6 mo, 32.6 ± 24.9 ; Table 2). The decrease in laser fluorescence readings in the test was significantly greater than in the control group at 3 mo (difference between groups: 9.3, $P = 0.027$) and 6 mo (difference between groups: 12.4, $P = 0.011$). The group values,

Table 2. Laser Fluorescence Readings in Test and Control Groups at Baseline and 3- and 6-mo Follow-up.

	Test Group (n = 31)		Control Group (n = 34)		Treatment Effect			
	Readings	Missing Subjects	Readings	Missing Subjects	Difference (95% CI) ^a	P Value	Odds Ratio (95% CI) ^{a,b}	P Value
Baseline	45.2 (22.9)	0	33.0 (16.5)	0				
3 mo	29.5 (18.8)	2	31.6 (21.0)	4				
6 mo	27.2 (19.3)	1	32.6 (24.9)	2				
Baseline vs.								
3 mo	-14.7 (16.8)	2	-2.7 (23.1)	4	9.3 (1.0 to 17.6)	0.0274	2.9 (1.1 to 8.1)	0.0394
6 mo	-18.6 (19.8)	1	-1.1 (25.8)	2	12.4 (2.8 to 22.0)	0.0112	3.9 (1.3 to 11.6)	0.0139
3 and 6 mo	NA		NA		10.9 (2.3 to 19.4)	0.0130	3.5 (1.3 to 9.8)	0.0147

Data for groups are mean (SD).

NA, not applicable.

^aAdjusted for baseline values of laser fluorescence, age, sex, and plaque. For analyses at 3 and 6 mo, estimates were additionally adjusted for time point.

^bOdds ratios are for the control group with the test group as reference.

Table 3. Visual Analog Scale Scores of Lesion Progression in Test and Control Groups at Baseline and 3- and 6-mo Follow-up.

	Test Group (n = 31)		Control Group (n = 34)		Treatment Effect			
	Score	Missing Subjects	Score	Missing Subjects	Difference ^a (95% CI)	P Value	Odds Ratio (95% CI) ^{a,b}	P Value
Baseline	73.9 (18.4)	0	61.8 (20.4)	0				
3 mo	-0.7 (36.5)	2	24.3 (32.2)	4				
6 mo	-13.3 (39.7)	1	12.8 (39.6)	2				
Baseline vs.								
3 mo	-74.5 (37.0)	2	-39.7 (36.4)	4	31.7 (14.3 to 49.1)	0.0006	6.8 (2.2 to 21.4)	0.001
6 mo	-88.0 (40.8)	1	-49.1 (43.8)	2	38.1 (14.9 to 61.3)	0.0018	8.0 (2.3 to 27.2)	0.001
3 and 6 mo ^c	NA		NA		35.3 (17.0 to 53.6)	0.0003	7.9 (3.1 to 20.3)	<0.0001

Data for groups are mean (SD).

NA, not applicable.

^aAdjusted for baseline values of visual analog scale, age, sex, and plaque.

^bOdds ratios are for the control group with the test group as reference.

^cEstimates were additionally adjusted for time point.

adjusted for baseline imbalances, were 9.3 and 12.4 after 3 and 6 mo, respectively (Table 2). Still, the relative treatment effect on laser fluorescence readings was significantly greater in the test group than the control group at 3 mo (odds ratio = 2.9, $P = 0.039$) and 6 mo (odds ratio = 3.9, $P = 0.014$). Similarly, combining 3- and 6-mo data showed a statistically significant decrease in laser fluorescence readings for test as compared with control lesions (difference between groups: 10.9, $P = 0.013$; odds ratio = 3.5, $P = 0.015$).

VAS Scores

VAS scores in the test group decreased rapidly from baseline (73.9 ± 18.4) to near 0 at 3-mo follow-up, indicating overall arrest (-0.7 ± 36.5), and to negative values at the 6-mo follow-up (-13.3 ± 39.7), indicating lesion regression (Table 3). In comparison, VAS scores in the control group decreased gradually and by a smaller extent, remaining positive (i.e., indicating progression) throughout the study (baseline, 61.8 ± 20.4 ; 3 mo, 24.3 ± 32.2 ; 6 mo, 12.85 ± 39.6). The VAS scores in the test group were significantly greater than in the control group at 3 mo (difference between groups: 34.8, $P = 0.0006$) and 6 mo

(difference between groups: 38.9, $P = 0.0006$), adjusted for baseline values. The relative effect of treatment on VAS scores was significantly higher in the test group than in the control group at 3 mo (odds ratio = 6.8, $P = 0.001$) and 6 mo (odds ratio = 8.0, $P = 0.001$). Similarly, combining 3- and 6-mo data showed a statistically significant decrease in VAS scores in test lesions as compared with control (difference between groups: 35.3, $P = 0.0003$; odds ratio = 7.9, $P < 0.0001$).

ICDAS Caries Index

The ICDAS showed a stronger trend for regression in test lesions than in control lesions during follow-ups (Table 4). At 3 mo, 17% of test lesions and 3% of control lesions showed regression in the ICDAS ($P = 0.053$), and at 6 mo, 30% of test lesions and 6% of control lesions showed regression into a lower ICDAS class ($P = 0.066$). Compared with 0% to 7% of test lesions progressing over 3 to 6 mo, 9% to 10% of control lesions progressed to a stage requiring restorative treatment. Combining the 3- and 6-mo data showed a statistically significant improvement in the ICDAS in the test versus control group (odds ratio = 5.1, $P = 0.018$).

Table 4. ICDAS Class Scores of Lesion Progression in Test and Control Groups at Baseline and 3- and 6-mo Follow-up.

	Test Group (n = 31)		Control Group (n = 34)		Odds Ratio (95% CI) ^{a,b}	P Value
	n (%)	Missing Subjects	n (%)	Missing Subjects		
Baseline		0		0		
1	2 (6)		14 (41)			
2	26 (84)		18 (53)			
3	3 (10)		2 (6)			
3 mo		2		4		
1	4 (14)		11 (37)			
2	24 (83)		15 (50)			
3	1 (3)		4 (13)			
6 mo		1		2		
1	8 (27)		13 (41)			
2	20 (67)		16 (50)			
3	2 (7)		3 (9)			
Baseline vs. 3 mo		2		4	10.1 (1.0 to 106)	0.0531
Regression	5 (17)		1 (3)			
Progression	0 (0)		3 (10)			
Baseline vs. 6 mo		1		2	3.9 (0.9 to 16.8)	0.0665
Regression	9 (30)		2 (6)			
Progression	2 (7)		3 (9)			
Baseline vs. 3 and 6 mo		0		0	5.1 (1.3 to 19.5)	0.0182

Data are presented as the *n* (%) of subjects per group by ICDAS classification (1 to 3).

ICDAS, International Caries Detection and Assessment System.

^aAdjusted baseline values of ICDAS, age, sex, and plaque.

^bOdds ratios are for the control group with the test group as reference.

Caries Activity

The Nyvad criteria showed that, during follow-up, a significantly greater number of test lesions converted from active to inactive status as compared with control (Table 5). A total of 52% of test lesions and 20% of control lesions had become inactive by 3 mo, while 80% of test lesions and 34% of control lesions had become inactive by 6 mo. The improvement in test lesions was statistically significant, as compared with control lesions, at 3 mo (odds ratio = 18.1, $P = 0.002$) and 6 mo (odds ratio = 13.0, $P = 0.0005$). Combining 3- and 6-mo data showed a statistically significant improvement in the test lesions as compared with the control lesions (odds ratio = 12.2, $P < 0.0001$).

Adverse Events

No adverse events, medical complications, or allergic reactions related to the treatments were observed during treatment or were reported during follow-up.

Clinical Feasibility

The clinical investigators were largely satisfied with the procedure of test and control groups. In the test group, 30 applications were regarded as “satisfactory” and 5 as “acceptable.” In the control group, 33 applications were regarded as “satisfactory” and 2 as “acceptable.” None of the test or control group patients regarded the clinical application as “unsatisfactory.” The dentists found that test and control treatments were easier

to place than fillings or fissure sealants. With regard to time required and the smell and taste of the treatments, patients regarded the treatments as “comfortable” (test, 20; control, 24) or “acceptable” (test, 14; control, 10). While no complaints were reported about the procedure itself, 1 patient from each group regarded the treatment as “uncomfortable.”

Discussion

Prior to this study, the potential of the self-assembling peptide P₁₁-4 for caries remineralization had been evaluated only in vitro and in a proof-of-concept study (Kirkham et al. 2007; Brunton et al. 2013). This single-blinded randomized controlled clinical trial offers a high level of evidence for the ability of P₁₁-4 to promote biomimetic remineralization in vivo. The test group showed statistically and clinically superior results in all primary and secondary outcomes in comparison with control. The test lesions treated with the biomimetic mineralization agent P₁₁-4 and fluoride varnish exhibited significantly greater remineralization and inactivation of carious lesions than the control; thus, it is clinically superior to the current gold standard of fluoride varnish (Marinho et al. 2002; Divaris et al. 2013).

The superior outcomes achieved with the combination of fluoride and the self-assembling peptide P₁₁-4 are likely due to the complementary mechanism and location of action of the two. Fluoride is predominantly integrated in the feigned intact surface of the initial carious lesion, with little entering the sub-surface lesion body (Weatherell et al. 1977), whereas P₁₁-4

Table 5. Caries Activity according to Nyvad Criteria in Test and Control Groups at Baseline and 3- and 6-mo Follow-up.

	Test Group (n = 31)		Control Group (n = 34)		Odds Ratio (95% CI) ^a	P Value
	Active Lesions	Missing Subjects	Active Lesions	Missing Subjects		
Baseline	31 (100)	0	34 (100)	0		
3 mo	14 (48)	2	24 (80)	4		
6 mo	6 (20)	1	21 (66)	2		
Baseline vs.						
3 mo		2		4	18.1 (3.0 to 109)	0.0015
6 mo		1		2	13.0 (3.1 to 54.9)	0.0005
3 and 6 mo		0		0	12.2 (4.3 to 34.7)	<0.0001

Data are presented as the number (percentage) of subjects per group.

^aAdjusted baseline values of age, sex, and plaque. Odds ratios are for the control group with the test group as reference.

permeates into the subsurface body and forms a 3-dimensional matrix that subsequently becomes mineralized by inducing de novo hydroxyapatite nucleation (Kirkham et al. 2007; Kind et al. 2017). The presented approach identifies a novel, possibly disruptive approach, enabling a preemptive treatment of early caries disease by healing the diseased enamel tissue. While conventional experimental therapies based on natural enamel proteins, such as amelogenins, have shown some ability to penetrate the subsurface lesion (Cao et al. 2015; Ruan and Moradian-Oldak 2015; Ruan et al. 2016), the self-assembling peptide P₁₁-4 has the ability to form a biomimetic scaffold within the subsurface carious lesion and to induce mineralization around the matrix, if calcium and phosphate ions from saliva diffuse into the lesion body. Other novel enamel regeneration approaches with self-assembling molecules, such as dendrimers, gelatin, or similar, have been described in the literature but not yet tested clinically (Hannig and Hannig 2012).

The clinical safety results are in agreement with previous studies showing that P₁₁-4 does not pose any concerns (Kyle et al. 2010; Brunton et al. 2013). The clinical trial also revealed high satisfaction among patients and dentists with regard to the treatment application. All patients in this study showed interest in noninvasive caries treatment, indicating that patients' demands are shifting from restorative to more curative and tooth-preserving options. Remineralization and inactivation of early carious lesions with this combined approach could circumvent the need for later, more invasive, restorative treatments of advanced lesions, thereby preventing or significantly delaying entry into the expensive cycle of tooth destruction and restorations (Splieth and Flessa 2008; Ismail et al. 2013).

The present study has several strengths in terms of methodology and outcome analyses. Clinical assessment of caries activity requires trained investigators and is challenging and often subjective. Thus, several primary and secondary outcome measures were used to evaluate the treatment in various respects. Specifically, laser fluorescence readings, which are directly correlated to caries progression, and the ICDAS index were used, as these are well-established and validated diagnostic tools for initial caries (Ismail et al. 2007; Diniz et al. 2015). In addition, VAS for progression of the lesion and Nyvad criteria for caries activity were used (Nyvad et al. 2005). Due to limitations inherent to categorical outcomes (i.e., ICDAS or Nyvad criteria), laser fluorescence

was used as the primary outcome, as the continuous nature of laser fluorescence data permitted comparative analyses with greater statistical power. Results from all validated methods were well corroborated, indicating the reliability and robustness of the study design and results obtained.

The study was, however, not without limitations. First, the limitations inherent to single-center trials are present. For example, it is possible that despite randomization, some selection bias may have been present due to clinical practice and patient profile patterns characteristic of the center. Nonetheless, the baseline values of the caries prevalence in the study cohort (dft = 2.8 and DMFT = 1.3) were similar to those reported for children in the local community (dft = 1.8 and DMFT = 0.8; Qadri et al. 2015), indicating that the study cohort represented the general patient population well. Second, the study was based on initial occlusal caries in freshly erupted molars, and caution must be exercised when extrapolating the results for the treatment in other caries locations or patient populations. The imbalance in some baseline values is a known result of a randomized allocation. Still, the clear clinical effect is valid even after all statistical adjustments for baseline values.

In conclusion, the data demonstrated that biomimetic mineralization facilitated by P₁₁-4 in combination with fluoride is a simple, safe, and effective noninvasive treatment for early carious lesions and is superior to the present clinical gold standard of fluoride treatment alone.

Author Contributions

M. Alkilzy, C.H. Splieth, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; A. Tarabai, contributed to design and data analysis, critically revised the manuscript; R. Santamaria, contributed to data analysis, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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