

Mohammad Alkilzy, Christian H. Splieth

Self-assembling peptides for caries prevention and treatment of initial carious lesions, a review

Abstract: The shift from reparative to regenerative dentistry reflects the current trend in medicine and also mirrors the new understanding of caries as a chronic disease. Self-assembling peptide P11-4 showed promising results in the regenerative biomimetic remineralization of initial carious lesions. This approach may present safe and acceptable preventive and minimal invasive treatment for initial caries in children and adolescents. Further studies it is suggested to investigate this novel approach in treatment of molar incisor hypomineralization and cavitated carious lesions.

Keywords: P11-4; self-assembling peptide; caries prevention; minimal invasive treatment; pediatric dentistry; biomimetic remineralization; enamel regeneration; initial caries; Curodont Repair

Introduction

Dental caries is a chronic, infectious, progressive disease, starting with initial loss of tooth minerals and leading to total tooth destruction over time. Caries occurs when an imbalance between re- and de-mineralization takes place at the site of loss of minerals in hard dental tissue [23]. Therefore, the classic “treatment” of cavities by drilling and filling constitutes repair of the damage, but does not treat the disease itself. “Real” caries treatment would consist of shifting the equilibrium to re-mineralization, for instance by improving the daily oral hygiene regime, healthy diet and fluoride application. The new understanding of caries kinetics and monitoring the de-/re-mineralization equilibrium can indicate measures for correcting the mineralization balance [10]. In the early stages of caries, prior to the cavitation of the enamel surface, many non- and minimally invasive treatments have been suggested to avoid

further tooth destruction and subsequently restorations. The primary aim of non-invasive treatments is to inactivate or arrest the caries. This can be achieved by diet control and plaque removal which allows the carious lesions to remineralize naturally through saliva [9]. The presence of fluorides clearly enhances remineralization and prevents further demineralization [19, 22]. Unfortunately, most preventive approaches rely on changes in the patient's behaviour, but this is not always an easy job especially in children and adolescences. A possible alternative is sealing caries risk surfaces mechanically [2], but sealants are prone to microleakage and subsequent caries in the long run [13] (Fig. 1).

An alternative approach to the current strategies for managing initial non-cavitated carious lesions is the recently introduced self-assembling peptide P11-4 [1] for regenerating demineralized tooth tissue. This peptide forms a 3D-matrix within demineral-

ized carious lesions, which enables *in vivo* hydroxyapatite crystal formation facilitating the regeneration of the lost enamel structure [10, 15] (Fig. 2). The peptide has shown encouraging results as a scaffold for enamel regeneration [26]. This paper presents the current scientific evidence and clinical implementations for the self-assembling peptide P11-4 in a modern caries management.

Biomimetics with self-assembling peptides

The self-assembling peptide P11-4 is clinically available as Curodont Repair and it has the chemical structure: Ace-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH₂. It is synthetic and manufactured under cGMP and contains no human- or animal-derived components. Biocompatibility studies (according to ISO 10993 or equivalent) have shown that P11-4 did not raise any cytotoxic effects or any immunological response [7, 12, 18]. P11-4 has been

Conditions and indications for minimal invasive caries treatment with SAP	Limitations and contra-indications for minimal invasive caries treatment with SAP
Active initial lesions without cavitation (Fig. 4)	Cariou lesion with cavitation
Lack in the patient's compliance with toothbrushing and dental hygiene	Good patient's compliance to dental hygiene
Moderate caries risk and activity	Patients with low caries risk
Age groups with elevated caries activity such as adolescences and young adults	Elderly patients with slow caries progression or already arrested lesions
Progression of the lesion in spite of preventive approaches	Patients with allergy to one or more elements of the product

Table 1 Indications and limitations for minimal invasive caries treatment with SAP

proven safe in animals and patients in vivo. No adverse device effects have been reported so far [3].

P11-4 undergoes under specific physicochemical conditions and above a critical concentration a hierarchical self-assembly to form tapes and ribbons within seconds, and fibrils and fibers within the following 24 h [1, 6, 8] (Fig. 3). These resulting self-assembling peptide fibres forming the 3D Self-Assembling Peptide Matrix (SAPM) can grow to a significant length. If the conditions for self-assembly are given, the assembly process cannot be stopped at the intermediates unless no more monomeric peptides are available. Confocal microscopy and mass spectroscopy showed that monomeric P11-4 diffuses through the pores of demineralized enamel, where fibre formation is triggered and the 3D matrix is formed [16]. Furthermore, C-labeled P11-4 indicated that about 35 % of P11-4

remained within artificial carious lesions [16] being available for de novo hydroxyapatite crystal formation. Two studies [10, 15] used the microtomography (microCT) analysis of remineralized specimens and found a remineralization of up to 90 % of the original enamel density.

A series of in-vitro studies insured the high affinity of the P11-4 matrix for Ca^{2+} ions and its action as a nucleator for de novo hydroxyapatite formation [6, 16, 17, 25]. Furthermore, the P11-4 fibers bind to the already existing Ca^{2+} ions of the hydroxyapatite lattice of the tooth enamel [6], enabling stable bridge binding of the new regenerated enamel to the tooth hard tissue. A study of Kind et al. [16] investigated the diffusion of P11-4 into a carious lesion by time resolved confocal microscopy pictures. The results showed that the P11-4 diffuses beyond the volume of the carious

lesion into the enamel layer below as was defined as the carious lesion on a microradiograph. After the formation of fibres is complete the formed fibers seem to occupy the observed lesion.

Clinical implementation of self-assembling peptide

Feasibility of clinical application, safety and clinical effect of P11-4 were first examined in an un-controlled safety trial treating arrested carious lesions on buccal surfaces in 15 adults [6]. As no adverse events were recorded by the use of the product and the blinded evaluation of the lesions showed a significant improvement of lesions judged by colour, size and perceived progression, further clinical studies were feasible. Table 1 lists some clinical indications and limitations for minimal invasive caries treatment with self-assembling peptides (SAP).



Figure 1 Fissure sealants are prone to microleakage and subsequent caries in the long run and require continuous follow-ups and corrections when indicated.

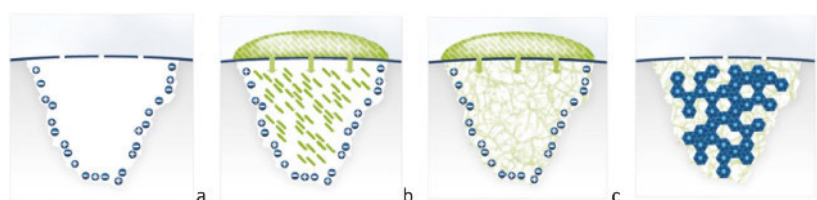


Figure 2a–d Illustration of the treatment of a carious lesion by the self-assembling peptide P11-4. **(a)** Initial carious lesion. **(b)** A drop of monomeric self-assembling peptide P11-4 is applied on the lesion surface; the monomers diffuse into the lesion. **(c)** P11-4 assembles within the carious lesion, forming a 3D scaffold. **(d)** De novo hydroxyapatite crystals form around the self-assembling peptide scaffold.

(Fig. 2 and 3: With friendly permission of credentis ag)

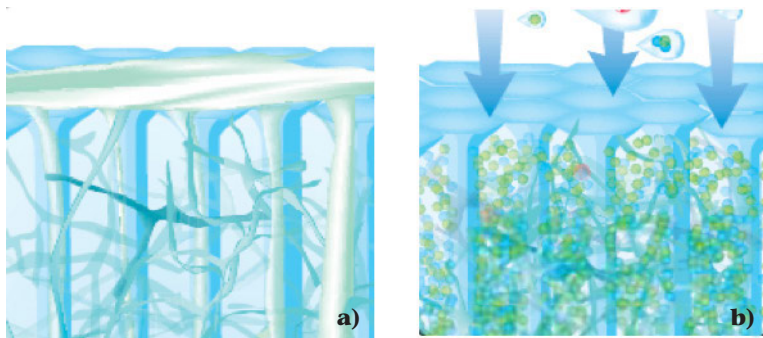


Figure 3a and b P11-4 forms within an early carious lesion an organic 3D-matrix (a), the matrix is highly affine to Ca^{2+} and PO_4^{2-} ions thereby enabling de novo formation of dental hard tissue by biomimetic mineralisation (b).

Self-assembling peptide for non- and minimal invasive treatment of occlusal initial caries

Occlusal surfaces of erupting permanent molars are highly prone to caries. Because of the infra-occlusal position of these teeth during the eruption, an effective toothbrushing is difficult. Even sealing of these teeth presents almost difficulties because of the gingiva covering the occlusal surfaces in partially erupted teeth (Fig. 4). Therefore, a randomized controlled single-blind study was conducted by Alkilzy et al. [3, 4] on children with visible active early caries on erupting permanent molars to investigate the safety and clinical efficacy of P11-4 for treatment of initial caries. Subjects were randomized to either the test group (P11-4 + fluoride varnish) or control group (fluoride varnish alone). Caries was assessed at baseline and at 3- and 6-month post treatment per laser fluorescence, a visual analog scale, the International

Caries Detection and Assessment System (ICDAS), and the Nyvad caries activity criteria [21]. Safety and clinical feasibility of the treatment approaches were also assessed. The P11-4 (Curodont Repair) was applied according to the instruction of the manufacturer (Fig. 5). Compared with the control group, the test group showed clinically and statistically significant improvement in all outcomes at 3- and 6-month follow-ups. The laser fluorescence readings (odds ratio = 3.5, $P = 0.015$) and visual analog scale scores (odds ratio = 7.9, $P < 0.0001$) were significantly lower for the test group, and they showed regression in ICDAS scores (odds ratio = 5.1, $P = 0.018$) and conversion from active to inactive lesions according to Nyvad criteria (odds ratio = 12.2, $P < 0.0001$, Fig. 6). Results suggest that Curodont Repair may present a simple, safe and effective non-invasive treatment for early occlusal carious lesions on erupting teeth in conjunction with

topical fluoride. Figure 3 shows a treatment of an initial carious lesion in the occlusal surface of first permanent molar during eruption using Curodont Repair.

Self-assembling peptide for caries prevention in patients with orthodontic brackets

Jablonski-Momeni et al. [14] investigated in a randomised, cross-over in situ trial the ability of 1,000 ppm self-assembling peptide P11-4 (Curodont Protect) to remineralize artificial initial caries lesions compared to the use of 22,600 ppm fluoride varnish. Laser fluorescence values (LF) and Micro-CT analysis was used to assess mineral changes within the specimens. The test group with P11-4 showed significantly more remineralization than the negative ($p = 0.01$) and positive control with fluoride ($p = 0.003$). The authors concluded that P11-4 showed prevention of caries and remineralization of enamel around orthodontic brackets.

The manufacturer of Curodont Protect claims that once the product is applied on the surface of the tooth the fibres can bind to the tooth via the Ca^{2+} -binding sites to the aprismatic HA crystal on the surface of the tooth. As the surface of the tooth – which undergoes constant re-, demineralisation – is not prismatic in structure the long axis of the HA crystal is available for binding, and the natural zeta-potential of the enamel surface seems to facilitate the binding. As a result, the 3D matrix will bind onto the tooth surface with multiple binding sites.



Figure 4 a-c (a and b) Active initial carious lesions in permanent molars during erupting where fissure sealing is very difficult to not possible. (c) Non-cavitated initial carious lesion on premolar. In these cases, the minimal invasive treatment with SAP P11-4 is good indicated.

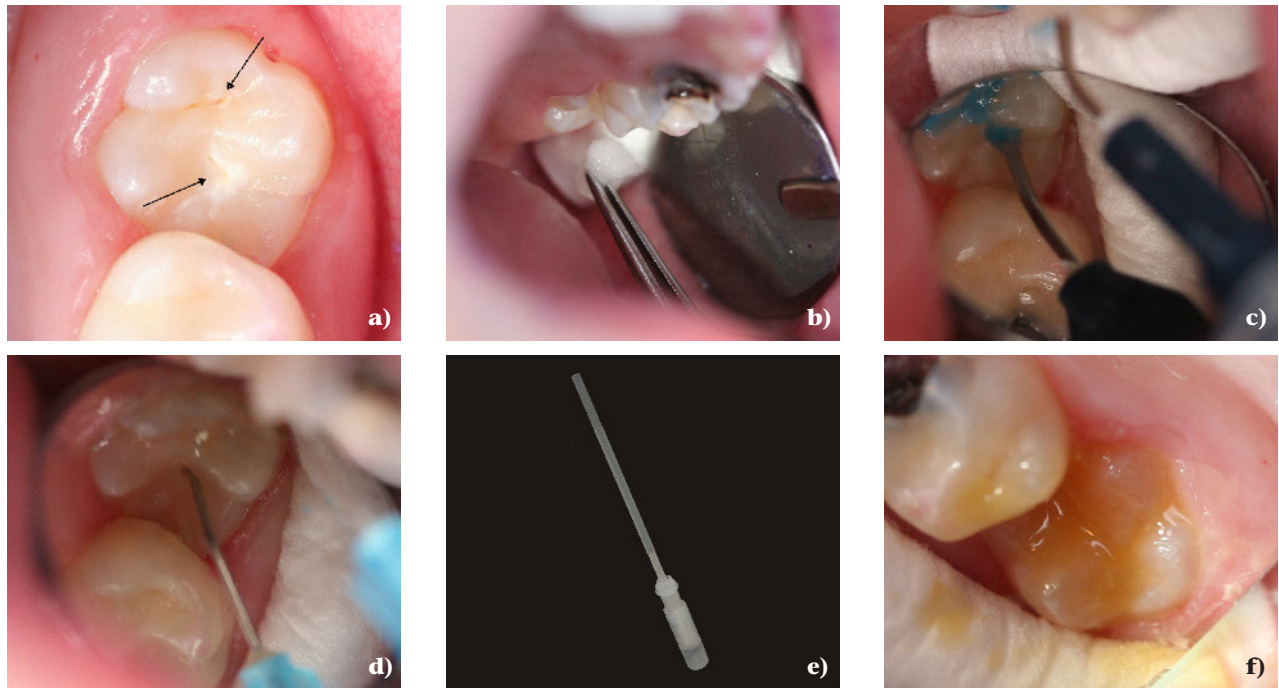


Figure 5a-f Clinical application of P11-4 (Curodont Repair). Initial active carious lesions in first permanent molar at eruption (a), elimination of organic debris with 3 % NaClO (b), etching with 35 % phosphoric acid (c), application of P11-4 and waiting 3–5 min for diffusion into the lesion and matrix formation (d), the recent applicator of P11-4 Curodont Repair (e), application of fluoride varnish (f).

Self-assembling peptide for treatment of buccal caries and white spots

Bröseler et al. [5] compared in a prospective, randomized, split-mouth, clinical trial the efficacy of the self-assembling peptide P11-4 to fluoride varnish in the treatment of early buccal carious lesions. Subjects with at least 2 clinically affected teeth were treated at day one D0 and day 90 D90 with P11-4 (test) or fluoride varnish (control). At day 180, fluoride varnish was applied on

all study lesions. Standardized photographs were taken at D0, D30, D90, D180 and D360 and the decrease in size between test and control groups was blindly morphometrically assessed. The results showed a significant difference between test and control groups, indicating a decrease in test lesions and stabilization of control lesions size ($p = 0.001$). The authors concluded that the size of early carious lesions treated with P11-4 was significantly reduced and this size reduction was

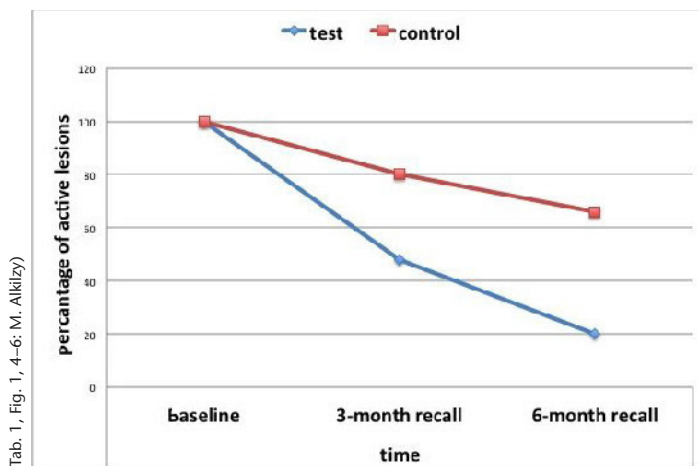
superior to that of fluoride varnish treatment.

Self-assembling peptide for treatment of proximal caries

A study by Schlee et al. [24] investigated the clinical performance of self-assembling peptide P11-4 on non-cavitated initial proximal carious lesions 12 months after treatment. Twenty-six patients with 35 carious lesions were included in a practice-based case series. The x-rays of the proximal lesions at baseline and at day 360 were evaluated pairwise in a randomized and blinded manner with respect to the time point. The one-year results showed a predominant shift toward regression of the initial lesions, with 20 of 28 lesions showing total or partial regression, 4 unchanged, and 4 progressing. The authors suggested that the initial proximal carious lesions can regress after treatment with P11-4, but additional factors might influence the overall treatment outcome.

Summary and suggestions

The shift from reparative to regenerative dentistry reflects the current trend in medicine and also mirrors the new understanding of caries as a



(Tab. 1, Fig. 1, 4–6: M. Alkilzy)

Figure 6 Significant reduction in caries activity of the lesions treated with SAP in comparison to control lesions at 3- and 6-month recalls [3].

chronic disease. Self-assembling peptide P11-4 showed promising results in the regenerative biomimetic remineralization of initial carious lesions. This approach may present safe and acceptable preventive and minimal invasive treatment for initial caries in children and adolescents. However, studies with longer observation periods are needed to confirm the consistent effect of P11-4. Further studies are suggested to investigate this approach in treatment of molar incisor hypomineralization and cavitated carious lesions.

Conflict of interest:

The authors state that they have no conflict of interest as defined by the guidelines of the International Committee of Medical Journal Editors.

References

- Aggeli A, Fytas G, Vlassopoulos D, McLeish TC, Mawer PJ, Boden N: Structure and dynamics of self-assembling beta-sheet peptide tapes by dynamic light scattering. *Biomacromolecules* 2001; 2: 378–388
- Ahovuo-Saloranta A, Forss H, Walsh et al.: Sealants for preventing dental decay in the permanent teeth. *Cochrane Database Syst Rev* 2013; March 28;(3):CD001830. doi: 10.1002/14651858.CD001830.pub4.
- Alkilzy M, Tarabaih A, Santamaria RM, Splieth CH: Self-assembling peptide P11-4 and fluoride for regenerating enamel. *J Dent Res* 2018; 97: 148–154
- Alkilzy M, Santamaria R. M, Schmoeckel J, Splieth CH: Treatment of carious lesions using self-assembling peptides. *Adv Dent Res* 2018; 29: 42–47
- Bröseler F, Tietmann C, Bommer C, Drechsel T, Heinzel-Gutenbrunner M, Jepsen S: Randomised clinical trial investigating self-assembling peptide P11-4 in the treatment of early caries. *Clin Oral Investig* 2019 Apr 29. doi: 10.1007/s00784-019-02901-4
- Brunton PA, Davies RP, Burke JL et al.: Treatment of early caries lesions using biomimetic self-assembling peptides – a clinical safety trial. *Br Dent J* 2013; 215: E6
- Burke, JL: In situ engineering of skeletal tissues using self-assembled biomimetic scaffolds, in Leeds Dental Institute. The University of Leeds, Leeds 2011, p. 1–277
- Davies RP, Aggeli A, Beevers AJ et al.: Self-assembling β -sheet tape forming peptides. *Supramolecular Chemistry* 2006; 18: 435–443
- de Almeida Pdel V, Grégio AM, Machado MA, de Lima AA, Azevedo LR: Saliva composition and functions: a comprehensive review. *J Contemp Dent Pract* 2008; 9: 72–80
- Deyhle H, Dziadowiec I, Kind L, Thalmann P, Schulz G, Müller B: Mineralization of early stage carious lesions in vitro – a quantitative approach. *Dent J* 2015; 3: 111–122
- Editorial. *Nature medicine* 2014; 20: 795
- Felton S: Self assembling b-sheet peptide networks as smart scaffolds for tissue engineering, in chemistry. University of Leeds, Leeds 2005, p. 184
- Heyduck C, Meller C, Schwahn C, Splieth CH: Effectiveness of sealants in adolescents with high and low caries experience. *Caries Res* 2006; 40: 375–381
- Jablonski-Momeni A, Korbmacher-Steiner H, Heinzel-Gutenbrunner M, Jablonski B, Jaquet W, Bottenberg P: Randomised in situ clinical trial investigating self-assembling peptide matrix P11-4 in the prevention of artificial caries lesions. *Sci Rep* 2019; 9: 269. doi: 10.1038/s41598-018-36536-4
- Kang J, Davies RP, Brookes SJ, Kirkham J: Multi-level modelling to further understanding of micro-CT data. In proceedings, enamel 9 conference in Harrogate, University of Leeds, Leeds 2016, Abstract 68
- Kind L, Stevanovic S, Wuttig S et al.: Biomimetic remineralization of carious lesions by self-assembling peptide. *J Dent Res* 2017; 96: 790–797
- Kirkham J, Firth A, Vernals D et al.: Self-assembling peptide scaffolds promote enamel remineralization. *J Dent Res* 2007; 86: 426–30
- Kyle S, Aggeli A, Ingham E, McPheron MJ: Recombinant self-assembling peptides as biomaterials for tissue engineering. *Biomaterials* 2010; 31: 9395–9405
- Marinho, VC, Worthington HV, Walsh T, Clarkson JE: Fluoride varnishes for preventing dental caries in children and adolescents. *Cochrane Database Syst Rev* 2013 Juli 11;(7):CD002279. doi: 10.1002/14651858.CD002279.pub2
- Marthaler TM: Changes in dental caries. *Caries Res* 2004; 38: 173–181
- Nyvad B, Machiulskiene V, Bælum V: The Nyvad criteria for assessment of caries lesion activity. In: Stookey GK (editor): *Clinical models workshop: remin-demin, precavitation, caries: proceedings of the 7th Indiana Conference, Indianapolis, Indiana, USA. Indiana University School of Dentistry* 2005, p. 99–116
- Petersson LG, Twetman S, Pakhomov GN: The efficiency of semiannual silane fluoride varnish applications: a two-year clinical study in preschool children. *J Public Health Dent* 1998; 58: 57–60
- Pitts NB, Zero DT, Marsh PD et al.: Dental caries. *Nat Rev Dis Primers* 2017; May 25; (3):17030. doi: 10.1038/nrdp.2017.30
- Schlee M, Schad T, Koch JH, Cattin PC, Rathe F: Clinical performance of self-assembling peptide P11-4 in the treatment of initial proximal carious lesions: A practice-based case series. *J Investig Clin Dent* 2018; 9: e12286
- Sousa J, Carvalho RG, Barbosa-Martins LF, Tersariol IL, Nascimento FD, Puppin-Rontani RM: Peptide P11-4 acts as a nucleator of hydroxyapatite crystals formation. *J Dent Res* 2017; 96(Spec. Iss. A): 3297
- Thomson BM, Hardaker L, Davies RPW et al.: P11–15 (NNRFWEFENN): a second generation biocompatible, self-assembling peptide with potential to promote enamel remineralization. *Caries Res* 2014; 48: 384–450 (Abs. Nr. 47)



(Photo: private)

PD DR. MOHAMMAD ALKILZY
 Dep. of Preventive &
 Pediatric Dentistry, University of
 Greifswald, Fleischmannstr. 42,
 17487 Greifswald, Germany
 alkilzym@uni-greifswald.de



(Photo: private)

PROF. DR. CHRISTIAN SPLIETH
 Dep. of Preventive &
 Pediatric Dentistry, University of
 Greifswald, Fleischmannstr. 42,
 17487 Greifswald, Germany
 splieth@uni-greifswald.de