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Review article

Efficacy of a self-assembling peptide to remineralize initial caries lesions - A systematic review and meta-analysis

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ABSTRACT

Introduction/Objectives: The present review systematically analyzed clinical studies investigating the efficacy of self-assembling peptides (SAP) to reduce initiation of or to remineralize initial caries lesions.

Data: Prospective controlled (non-)randomized clinical trials investigating the efficacy of a self-assembling peptide compared to any other (placebo) treatment or untreated/standard control. Outcomes were visual analog scale (VAS), laser fluorescence, ICDAS score or morphometric measurements.

Sources: Three electronic databases (Central, PubMed, Ovid EMBASE) were screened. No language or time restrictions were applied.. Risk of Bias and level of evidence was graded using Risk of Bias 2.0 tool and Grade Profiler 3.6.

Study selection/Results: Seven studies with 508 teeth being affected in 294 patients were included. All studies were randomized controlled trials (RCT), five with a split-mouth and two with a parallel-arm design. Meta-analysis could be performed for SAP (plus fluoride varnish (FV)) *vs.* no treatment (plus FV) (control treatment). Depending on the outcome after up to 12 months SAP showed a significantly higher optical improvement than the control treatment (laser fluorescence: Standardized Mean Difference (SMD)[95 %CI] = −0.87 [−1.39,−0.34; VAS: Mean Difference (MD)[95 %CI] = −35.38[−43.13,−27.64]) or no significant difference could be observed (ICDAS/activity score; Relative Risk (RR)[95 %CI] = 0.6[0.21,1.74]; morphometric measurements: SMD[95 %CI] = −1.95[−4.54,0.65]). Level of evidence was very low for all 4 outcomes. Furthermore, six studies showed a high risk of bias and six studies were (partially) funded by the manufactures of the tested products.

Conclusion: Based on a low number of clinical trials with relatively short follow up-periods and high risks of bias, self-assembling peptides may be a viable option to remineralize enamel caries.

Clinical significance: Self-assembling peptides may be a viable option to remineralize enamel caries. However, results should be interpretated with caution due the low number of clinical trials, the short follow-up periods and the limiting grade of evidence.

1. Introduction

Although efforts in caries prevention have resulted in a trend towards a lower prevalence [1], initial caries can still be frequently observed. Within two weeks of plaque formation and the beginning of demineralization, rather shallow initial lesions can be detected on the enamel surface [2]. These lesions have higher pore volume and decreased hydroxyapatite volume, and they are filled with air when the surface is dried. As light is scattered differently within sound enamel and the demineralized areas filled with air, these initial caries lesions have an opaque appearance and are thus often called white spots.

To enhance the remineralization of white spots, application of fluoride-containing agents [3], casein phosphopeptides-amorphous calcium phosphate (CPP-APP) containing pastes [4] or bioactive glasses [5] can be used. Micro-abrasion with HCl pumice [6] or infiltration using a low viscosity resin [7] have also been suggested. Nonetheless, bioactive glasses have shown promising results *in vitro* only but not *in vivo* and the clinically visible success of the other methods varies widely. Fluoride and CPP-ACP did not reveal a distinct positive clinically visible effect [4,8]. Micro-abrasion, even though it reduced the

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unfavorable appearance of white spot lesions, resulted in a substantial loss of enamel [9].

A relative new approach also proposing to reverse initial caries lesions is the application of the self-assembling peptide (SAP) P11-4 [10]. Once the SAP-containing solution has been applied, the peptide diffuses into the lesion, where it is supposed to spontaneously self-assemble and produce three-dimensional gels comprising the so called β-sheet aggregates. Hereby, attachment of Ca^{2+}- and PO_4^{3-}- from saliva is supposed to be enhanced [11]. In a previous non-controlled clinical trial [12] the clinical appearance of class V white spot lesions was improved after a single application of an SAP-containing solution. Although these results were stable for 6 months, only 11 lesions were included in the study, and the study design contained neither a positive (*e.g.* fluoridation) nor a negative control (placebo / no treatment). In a second uncontrolled case series, 35 initial proximal caries lesions being treated with SAP were followed up for one year [13]. The radiographic and digital subtraction analyses indicated that the lesions regressed. A significant increase in the subsurface microhardness could also be observed *in vitro* after the application of SAP [14]. Furthermore, another *in vitro* study showed that quantitative light induced fluorescence values were significantly improved after SAP application, but this result was only observed when two control groups were merged in the analysis [15]. Nonetheless, in both *in vitro* studies, enamel specimens were solely stored in remineralizing solutions for four or twelve weeks, and no demineralization solution was used intermittently to simulate oral pH fluctuations. So, the remineralization potential in these experiments might be overestimated. Still, a recent systematic review on *in vitro* studies revealed that SAP seems to be a feasible option to remineralize enamel caries [16], but highlighted the lack of (randomized controlled) trials to assess the efficacy of this technique.

In the last two years several randomized controlled trials on SAP have been published. Therefore, the aim of this systematic review and meta-analysis was to critically summarize the literature and evaluate the efficacy of a self-assembling peptide to reduce the initiation of initial caries lesions or to remineralize them.

2. Materials and methods

2.1. Review design

No study registration is necessary for this review. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) were adopted throughout the process of the present systematic review [17]. The PICOS model was used to structure the clinical research question [18] (Table 1). Thus, the present review aimed at systematically retrieving and analyzing clinical studies investigating the efficacy of a self-assembling peptide to reduce the initiation of new initial caries lesions or to remineralize initial caries lesions in patients of any age. For this, control treatment could be any other (placebo) treatment or untreated control or standard control (*e.g.* fluoride varnish) and no restrictions with regard to the outcome were defined.

Table 1
PICOS schema: Population (P), Intervention (I), Comparison (C), Outcomes (O) and Study Design (S).

P	–	Participants: Patients of any age with initial caries lesions
I	–	Intervention: Self-assembling peptide
C	–	Control: Any other (placebo) treatment or untreated control or standard control (e.g. fluoride varnish)
O	–	Outcome: Primary: Development (initiation and pregression/regression) of demineralization e.g. Visual Analogue Scale, laser fluorescence or morphometric measurements
S	–	Studies: Randomized controlled clinical trials (RCTs), prospective controlled clinical trials (CCTs), prospective and retrospective cohort studies, studies with split-mouth and parallel-arm designs

2.2. Search strategy

Detailed search strategies were developed and appropriately revised for each database, considering the differences in controlled vocabulary and syntax rules by two authors (R.J.W, T.G.W.). The search strategies for Medline/PubMed is shown in Table 2. The following electronic databases were searched to find reports of relevant published studies:

- The Cochrane Central Register of Controlled Trials (CENTRAL) (up to February 28, 2021);
- MEDLINE (PubMed) (1946 to February 28, 2021);
- Ovid EMBASE (1947 to February 28, 2021)

Two authors (R.J.W, T.G.W.) independently reviewed titles and abstracts using these search strategies. The reviewers were not blinded to the identity of the journal names or article authors, their institutions, or the results of their research. No language or time restrictions were applied. A detailed sequence of filtering search results to include relevant articles can be found in the supplementary material. In order to further identify potential articles for inclusion, grey literature was searched in the register of clinical studies hosted by the US National Institutes of Health (www.clinicaltrials.gov), the multidisciplinary European database (www.opengrey.eu), the National Research Register, and Pro-Quest Dissertation Abstracts and Thesis databases. Agreement concerning study inclusion or data extraction was achieved by consultation and discussion with a third author (T.S.C). Selected articles were screened full-text. Cross-referencing was performed to identify further articles to be assessed.

2.3. Data collection

Two authors performed data extraction independently and in duplicate (R.J.W, T.G.W.). The following data was collected in predefined excel sheets: author/title/year of study, study affiliation data, study type and setting, design of the study, number/age/gender of patients, intervention applied, inclusion criteria and outcome definitions, outcome assessed with all relevant clinical variables (*e.g.* visual-analog scale, laser fluorescence or morphometric measurements), drop-outs, follow-up (maximum follow-up over all groups was used), sources of funding, trial registration, and publishing of the trial's protocol.

For longitudinal studies and clinical trials presented in different journals or in different publication years, results were presented within one column. Studies without enough data for meta-analyses were kept in the systematic review, but they were excluded from the meta-analyses.

2.4. Data synthesis and grading

Meta-analyses were conducted if studies with similar comparisons

Table 2
Search strategy as used for Pubmed.

Search	Query	Results
#1	(((assemb*) OR (self-assemb*)) AND (((peptid*) OR (SAP)) OR (P11-4))) AND ((((caries) OR (carious)) OR (decay)) OR (cavit*))	379
#2	(((caries) OR (carious)) OR (decay)) OR (cavit*)	381,911
#3	((peptid*) OR (SAP)) OR (P11-4)	907,610
#4	(assemb*) OR (self-assemb*)	318,519
#5	cavit*	236,923
#6	decay	94,499
#7	carious	7085
#8	caries	62,784
#9	P11-4	78
#10	SAP	14,845
#11	peptid*	893,812
#12	self-assemb*	69,493
#13	assemb*	318,519

reported the same outcomes. For continuous variables, the primary measures of effect between treatment and control groups were the mean differences (MD) for studies using the same outcome, and for studies using the same construct but different scales, the standardized mean differences (SMD) was used. For dichotomous outcome data (*e.g.* surface texture), the primary measures of effect was risk ratios (RR) and 95 % confidence intervals (95 %CI).

Statistical heterogeneity was assessed using a Chi^2 test and the I^2 statistic [19]. Fixed or random-effects meta-analyses were performed depending on the heterogeneity (I^2<35 %: fixed-effects; I^2>35 %: random-effect) [20]. Risk of bias for interventional, randomized controlled trials (RCTs) was performed independently and in duplicate (R.J.W, T.S.C.) using the Risk of Bias 2.0. tool [21] and for interventional, non-randomized controlled trials using the ROBINS-I-tool [22]. Grading of evidence was performed according to the GRADE network levels using Grade Profiler 3.6 [23]. Publication bias was assessed by funnel plots [24].

To avoid unit-of-analysis errors the guidelines outlined by the Cochrane collaboration (chapter 9.3.4.) were followed [25]. Therefore, baseline data were compared with data of a single time point (mostly longest follow-up period).

2.5. Sensitivity analysis

We explored whether or not the analysis of studies stratified by (i) risk of bias or (ii) study design yielded similar or different results. For this [1] studies at high risk of bias or [2] studies using a parallel-arm designs were eliminated in a second/third analysis.

3. Results

A total of 911 studies were initially identified, and after title and abstract screening, 10 studies were assessed for eligibility. After full-text screening 3 studies were excluded (Fig. 1, appendix Table 1). Eventually, 7 studies with 508 teeth with initial caries lesions in 294 patients, 7–39 years of age, were included. All studies were randomized controlled trials, five with a split-mouth [26–29] and two with a parallel-arm design [30,31] all of which investigated the efficacy of SAP on buccal [26–29,32] or occlusal [30,31] caries lesions. The outcomes were described using laser fluorescence (mostly DIAGNOdent values) [27–32], Visual Analogue Scale (VAS) [26,28,30,31], ICDAS (including Nyvad caries activity criteria and/or lesion activity assessment) [27,28, 30,28–32] and morphometric measurement [26,28,29]. Two studies used non-cavitated lesions (ICDAS score 2: [33]; non-cavitated ICDAS score 2−3: (32)), three studies used (micro-)cavitated lesions (ICDAS score 3 [30,31]: or reporting microcavities (26)), in one study 90 % of the included caries lesions presented baseline laser fluorescence values of healthy enamel when using the manufacture's specification [28], whereas no information on the lesion surface was given in one study [29]. In three studies, at least one author was identical [26,28,30] and two studies were performed in the same department [29,31]. An overview of the main characteristics of the included studies is presented in the appendix Table 2.

Meta-analyses could be performed for the following comparisons: SAP plus fluoride application *vs.* fluoride varnish application [28, 30–32], as well as for SAP *vs.* no treatment [27,29]. However, for some outcomes, one or two studies had to be excluded, since not all information required for recalculation was reported [28,30] or the studies

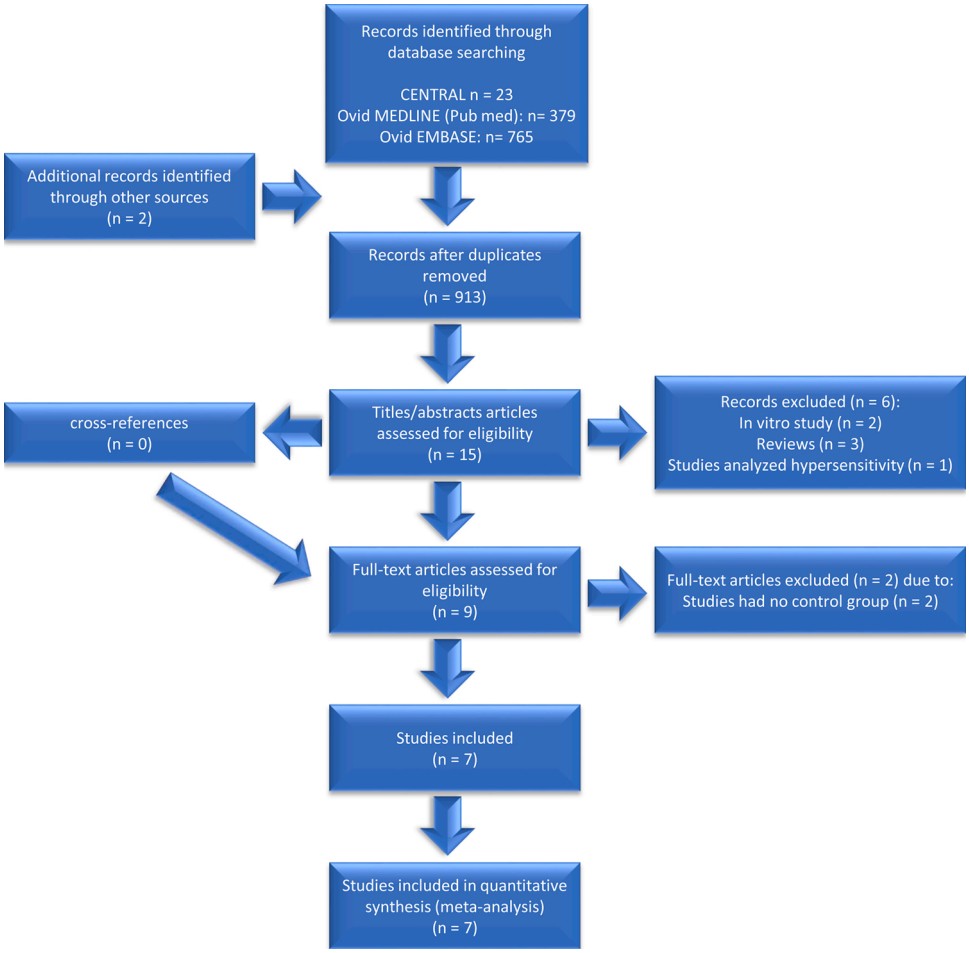

Fig. 1. Study flow.

presented no numeric results of the respective outcome [27,28,30]. Furthermore, for one study, the number of patients, but not the number of teeth being analyzed, was presented [29]. For this study, it was assumed that the number of patients and the number of teeth per group was identical.

Depending on the outcome, the treatment with SAP showed a significantly higher optical improvement than the control treatment (laser fluorescence: SMD [95 %CI] = −0.87 [−1.39, −0.34], very low level of evidence; VAS: MD [95 %CI] = −35.38 [−43.13, −27.64], very low level of evidence) or no significant difference could be observed (ICDAS/activity score; RR [95 %CI] = 0.60 [0.21, 1.74], very low level of evidence; morphometric measurements: SMD [95 %CI] = −1.95 [−4.54, 0.65], very low level of evidence) (Fig. 2). Only one study compared the optical improvement from SAP not only with fluoride varnish or no treatment, but also with infiltration using a low viscosity resin [27]. In this study, the optical improvement after infiltration was significantly higher than after the use of SAP, fluoride varnish and no treatment ($p \leq 0.007$).

Adverse events possibly attributed to one of the used products were not mentioned in 3 studies and no (serious) adverse events were observed in 4 studies [28,30–32].

3.1. Quality assessment

Of the 7 trials, quality of 1 was assessed as low [27] and 6 as high risk of bias [26,28–32] (Fig. 3). Although most of the studies reported that blinding of personnel was not feasible, a blinded outcome assessor was recruited in most of the studies.

Grading of evidence for meta-analyses showed very low level of evidence for all 4 outcomes (Appendix table 3).

3.2. Sensitivity analysis

When excluding studies at high risk of bias (or studies using a parallel-arm group design) no meta-analysis was possible. Thus, no sensitivity analysis could be performed.

4. Discussion

In this systematic review the efficacy of SAP on buccal or occlusal initial caries lesions have been critically summarized. Studies using a variety of outcomes have been extracted. The outcomes were described using laser fluorescence, visual analog scale, morphometric measurement as well as ICDAS / LAA-ICDAS scores. The median follow-up period was only 6 months with a range between 6 and 12 months. However, the present meta-analysis also showed that depending on the outcome, the treatment with SAP may be effective in the remineralization of enamel caries.

The 6 months follow-up period observed in many of the studies seems rather short. Although it can be expected that natural remineralization from saliva can be enhanced with a study design lasting at least 3 weeks, a 6-month follow-up is short when compared to the advised 3-year follow-up period for direct restauration and the advised 5-year follow-up for indirect restauration [34]. However, since the clinical application of SAP is a relatively new technique the short follow-up periods may be explained. Nonetheless, studies with long-term follow-ups are still required to confirm the stability of the observed effects.

In the present review, six studies used a split-mouth design [26–29,32] and two a parallel-arm design [30,31]. By using the split-mouth design, each patient is his own control. Thus, the risk of confounding factors is decreased and individual differences - such as the naturally predominant side of patients' brushing and chewing habits - are eliminated [35,36]. Nonetheless, when using split-mouth designs, site- and carry-over effects have to be considered [37]. For instance, fluoride being released from the fluoride-containing agent may be carried across from one side to another showing a falsely higher preventive effect for the non-fluoride materials [32]. Although most of the clinical trials in the present review have used fluoride varnish, and there is some evidence that the carry-over effect of fluoride released from varnishes might be limited to the close vicinity of the fluoridation site [38], this effect should not be overlooked. This is especially important in case of the present meta-analysis, since none of the split-mouth studies used (or at least did not describe the use of) statistical models adjusting for such designs (*e.g.* no site-effect evaluation). So, the evidence of each comparison in the meta-analysis was downgraded.

The available evidence is additionally limited by the sponsorship of the studies. Six of the seven studies were (partially) funded by the manufactures of the tested products. Furthermore, in three studies an employee of the manufacturer was listed as co-author. Only one of the studies highlighted that the manufacturer had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All these factors were reflected in the risk of bias analysis and evidence grading.

It has previously been shown that SAP has a high affinity for Ca^{2+}, acting as a nucleator for *de novo* hydroxyapatite formation *in vitro* [11,39]. Thus, attachment of Ca^{2+}- and PO_4^{3-}- from the body's own saliva is supposed to be also enhanced *in vivo* [11,39], enabling enhanced remineralization of initial non-cavitated lesions [12,31,40]. It was, therefore, interesting to see that in three studies the included lesions showed signs of cavitation (ICDAS score 3 [30,31]: or microcavities (26)) and that only two studies explicitly stated that only non-cavitated caries lesions were included [32,33].

Laser fluorescence systems, especially the use of the DIAGDOdent, have been accepted for its reproducibility and sensitivity over conventional radiography in primary occlusal caries *in vitro* [41,42] and *in vivo* [43]. They have also been evaluated for secondary caries adjacent to amalgam restorations or composite restorations [44]. A previous cost-effectiveness analysis could demonstrate that laser fluorescence in combination to visual inspection increases specificity and avoids false-positive diagnoses [45], thus avoiding costly and invasive overtreatment. However, regarding the effectiveness of fluorescence-based methods in monitoring the progression of non-cavitated caries lesions, the results are inconsistent. Using a bacterial *in vitro* model fluorescence values showed significant moderate correlation with surface microhardness values, allowing differentiation between sound and demineralized enamel over time [46]. In contrast, *in vivo* periods longer than one year were assumed to be necessary in order to observe clinically significant changes of initial carious lesions when using laser fluorescence devices [47]. Furthermore, large variation were observed within different time points [48]. Consequently, even if several studies used laser fluorescence / DIAGNOdent measurements to measure the remineralizing efficacy, the results of the respective studies should be interpreted with caution.

In conclusion, self-assembling peptides may be a viable option to remineralize enamel caries. However, results should be interpretated with caution due to the low number of clinical trials, the short follow-up periods and the limiting grade of evidence.

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CRediT authorship contribution statement

R.J. Wierichs: Conceptualization, Formal analysis, Methodology, Writing - original draft, Visualization. **T.S. Carvalho:** Formal analysis, Methodology, Writing - review & editing. **T.G. Wolf:** Conceptualization, Formal analysis, Methodology, Writing - original draft.

Declaration of Competing Interest

The authors declare no conflicts of interest.

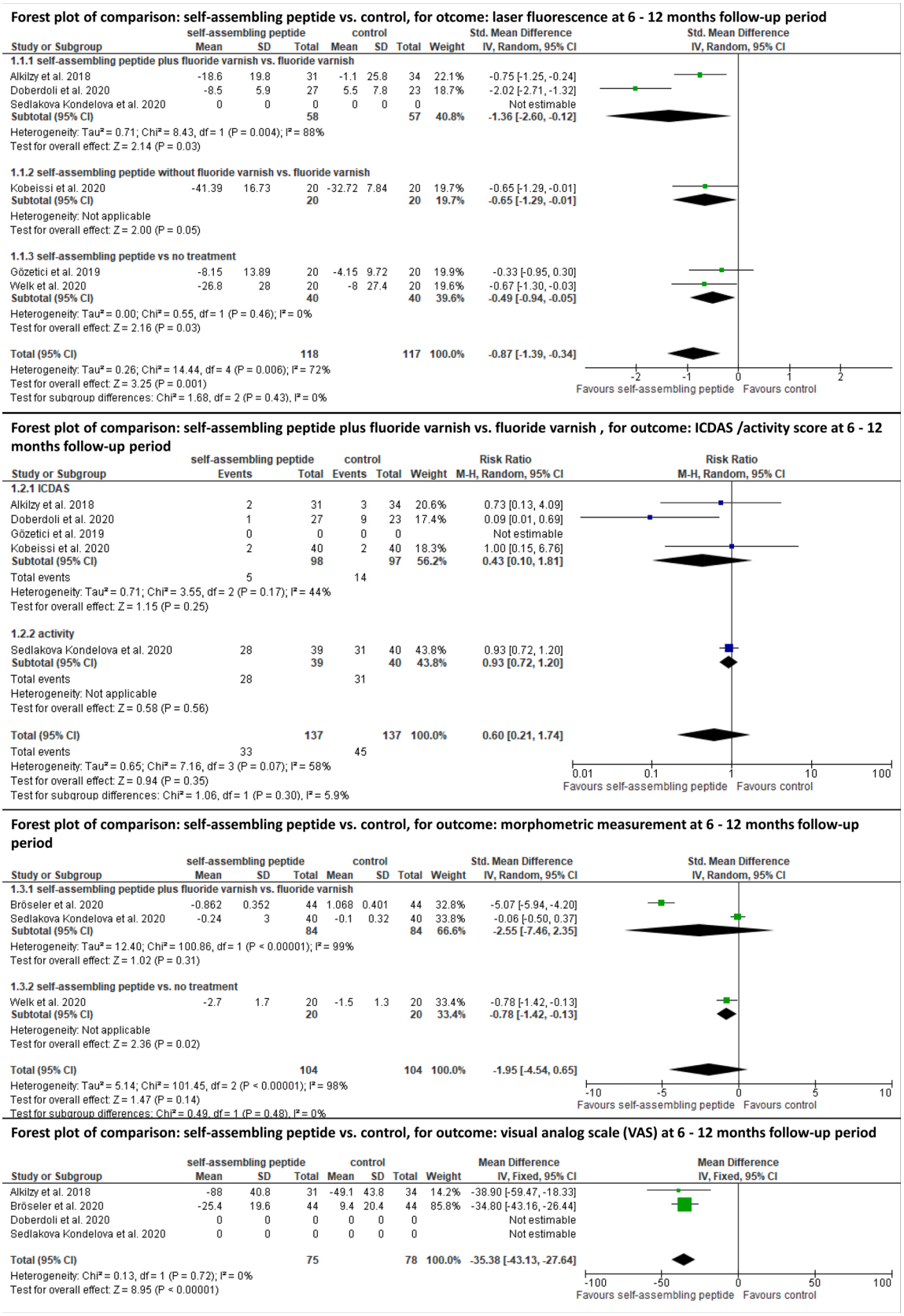

Fig. 2. Quantitative meta-analyses for outcomes laser fluorescence, ICDAS /activity score, morphometric measurement and Visual Analogue Scale (VAS). For continuous variables, the primary measures of effect between treatment and control groups were the mean differences (MD) for studies using the same outcome and standardized mean differences (SMD) for studies using the same construct but different scales. For dichotomous outcome data (*e.g.* surface texture) the primary measures of effect were risk ratios (RR) and 95 % confidence intervals (95 %CI). Forest plots, heterogeneity parameter (I2) as well as overall statistics (Z, P) are given. "not estimable": studies using the respective outcome, but could not be included in the meta-analysis for reasons mentioned in the text and appendix Table 2.

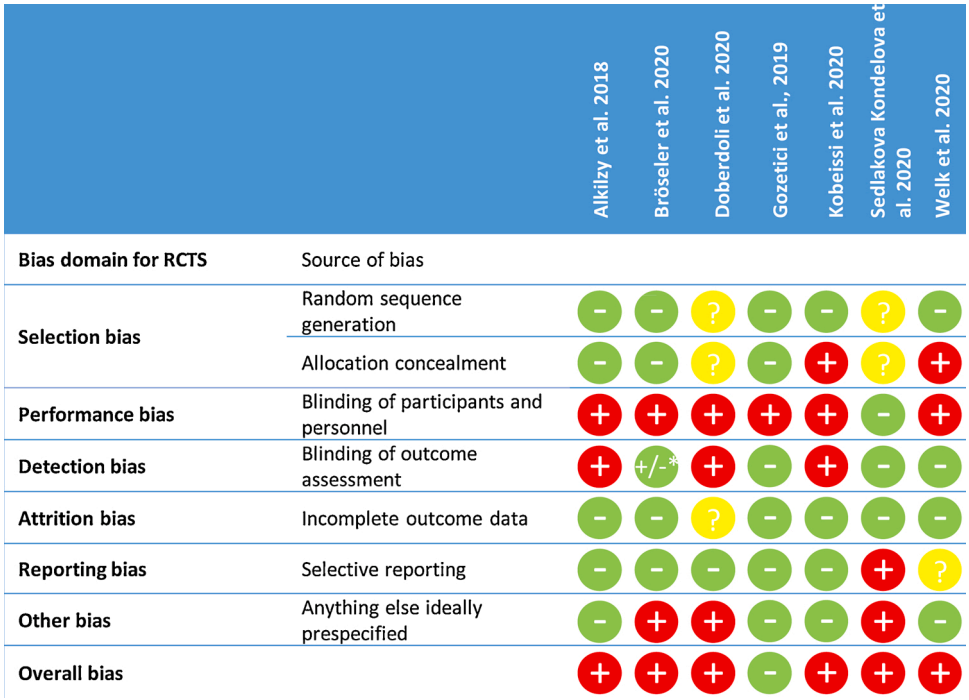

Fig. 3. Risk of bias assessment. For interventional, randomized controlled trials (RCTs) Risk of Bias 2.0 tool was used. * For one outcome (morphometric measurements) the assessor was blinded, for one outcome (VAS) the assessor was not blinded.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jdent.2021.103652.

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