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Influence of the acid etching time on retention of fissure sealants

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Yi-Fang Lo

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Publication List

The topic of my doctoral thesis was to investigate the retention rate of pit and fissure sealants in relation to different etching times. It was considered to work on the following projects:

Project 1. What is an appropriate etching time before sealant application on molars? Results from a meta-analysis.

Lo YF, Crispin A, Kessler A, Hickel R, Kühnisch J. What is an appropriate etching time before sealant application on permanent molars? Results from a meta-analysis. J Adhes Dent. 2019;21(6):487-495.

Project 2. Does etching time affect the in vitro performance of a sealant material?

Lo YF, Pitchika V, Ilie N, Hickel R, Kühnisch J. Does etching time affect the in vitro performance of a sealant material? Dent Mater J. 2020; 39(5): 862–868

1. Personal Contribution to Publications

The table below gives an overview of the personal contribution which was made to the preparation and publication of the dissertation.

	Yi-Fang Lo	Prof. Dr. Kühnisch	Other scientists
Publication 1. "What is an appropriate etching time before sealant application on molars? Results from a meta-analysis"			
Idea of project	**-**	100%	-
Study design	**20%**	75%	5% Prof. Hickel
Step 1: Literature search & identification	**60%**	20%	20% Dr. Kessler
Step 2: Hand search of the literature	**60%**	20%	20% Dr. Kessler
Step 3: Removal of duplications	**100%**	-	-
Step 4: Assessment of full-text papers	**80%**	20%	10% Dr. Kessler
Step 5: Extraction of data	**90%**	-	10% Dr. Kessler
Step 6: Processing datasets	**60%**	-	40% Dr. Crispin
Step 7: Descriptive statistical analysis	**100%**	-	-
Step 8: Explorative statistical analysis	**10%**	-	90% Dr. Crispin
Step 9: Selection of data worth publishing	**80%**	20%	-
Step 10: Creation of tables & figures	**90%**	10%	-
Step 11: Manuscript preparation & submission	**70%**	20%	10% Co-authors
Publication 2. "Does etching time affect the in vitro performance of a sealant material?"			
Idea of project	-	100%	-
Study design	**20%**	75%	5% Prof. Dr. Hickel
Step 1: Sample collection and enamel preparation	**100%**	-	-
Step 2: Specimen preparation	**100%**	-	-
Step 3: Sample ageing	**100%**	-	-
Step 4: SBS test and failure mode analysis	**90%**	-	10% Prof. Dr. Ilie
Step 5: Microleakage test	**100%**	-	-
Step 6: Processing datasets	**70%**	-	30% Dr. Pitchika
Step 7: Descriptive statistical analysis	**90%**	10%	-
Step 8: Explorative statistical analysis	**10%**	-	90% Dr. Pitchika
Step 9: Selection of data worth publishing	**80%**	20%	-
Step 10: Creation of tables & figures	**90%**	10%	-
Step 11: Manuscript preparation & submission	**70%**	20%	10% Co-authors
Drafting of the cumulative dissertation	**100%**	**-**	**-**

2. Introduction

2.1 Preface

Sealing pits and fissures is an effective caries preventive measure (1). However, the caries-protective effect is influenced by material retention, as a sealant can only be effective as long as it covers the pit and fissure completely (2). It was previously shown that sealant retention varied significantly between the available materials (3). Additionally, other clinical variables, e.g., etching time, practice set-up, and isolation technique, may also influence long-term survival.

When considering acid etching as an important pretreatment step before sealing and its influence on longevity, it is surprising that only limited data from comparative clinical studies exist until now. There is only one clinical study (4) showed that 40.0%, 50.9%, 41.8%, and 43.6% of sealants were intact after 12 months when etching the enamel for 15, 30, 45, and 60 seconds, respectively. Based on previously published meta-analysis results (3), it can be expected that approximately 80% of all sealants will be intact after a two-year observation period. Therefore, the previously published comparative clinical results (4) must be re-evaluated. Independent of clinical experience, only few in vitro studies have investigated the influence of the length of acid conditioning on the in vitro performance of sealant materials, e.g., bond strength or microleakage (5,6,7)

The shortening of acid conditioning has been discussed repeatedly since the introduction of pit and fissure sealants to simplify the treatment and reduce chair time, particularly in children (8,9). Furthermore, there seems to be also a trend towards shorter etching times in recent studies (10,11). Some clinical studies used a very short etching time for only 15 seconds (10, 12), and the documented survival rate seems to be heterogeneous. Therefore, we conducted a meta-analysis based on a systematic review of the literature, as well as a laboratory study, in an attempt to close this knowledge gap:

1. the first study (What is an appropriate etching time before sealant application on permanent molars? Results from a meta-analysis.) investigated the influence of different acid etching times on the retention rate of pit and fissure sealants based on a systematic search of the literature of clinical trials with a minimum observation time of two years.

2. the second study (Does etching time affect the in vitro performance of a sealant material?) aimed to assess the shear bond strength (SBS), failure mode, and microleakage of sealants under the inclusion of different commonly recommended acid etching times, namely, 15, 30, 45, and 60 s on permanent tooth materials on a comparative, in vitro study basis.

2.2 Materials and Methods

In the first study, the systematic review was reported according to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement (13).

PICOS model. We structured our literature search based on the five components of the "PICOS" model (14). A systematic search was carried out in the following databases: PubMed via Medline, EMBASE, Cochrane Central Register of Controlled Trials (CENTRAL), Scopus, and Google Scholar up to March 2017. A thorough manual review of the reference lists of the retrieved publications was also performed. Endnote X7.7.1 (Bld 11961, Thomson Reuters, Toronto, Ontario, Canada) was used to manage the studies.

Selection of studies. Clinical trials evaluating the longevity of resin- or methacrylate resin-based pit and fissure sealants with a phosphoric acid etching technique used before sealant application on occlusal surfaces of permanent molars, with a minimum of 2 years of follow-up were included. In the first step, two trained reviewers (Y.L. and A.K.) independently screened the titles and abstracts of all identified studies and determined the eligibility. In the case of any disagreements or uncertainties, the reviewers consulted with the supervisor (J.K.) to resolve the issue.

Data extraction and management. Regarding the evaluation criterion, the retention of the sealants was recorded as a success (total retention) or failure (partial or total loss). Missing numerical values were recorded as "not reported". All data were entered into a MS Excel worksheet (Office 2015, Microsoft Corporation, Redmond, WA, USA).

Statistical analysis. Statistical analyses of the effect of etching times were performed using negative binomial regression models. The absolute number of failures in a given study was estimated from the proportion of molars with defective sealants among all molars examined at the end of the study multiplied by the number of molars examined at baseline. The total sealant survival time at risk of failure was estimated as half the study duration in the case of defective sealants and as the whole study duration otherwise. The natural logarithm of the survival time at risk (in years) was used as an offset in the negative binomial regression models.

The second study included ninety-six healthy, caries-free, extracted human third molars to test SBS. All teeth were free of any developmental disorders, fillings, or fissure sealants, and showed complete root development. The roots were sectioned 1 mm apically to the cementoenamel junction, and the crowns were further sectioned into 3 parts (mesial, buccal, lingual). This process resulted in 288 tooth surfaces, which were randomly assigned to 8 study groups according to the randomization table. All tooth surfaces were embedded in cold-curing methyl methacrylate resin. The SBS was compared between different etching times (15, 30, 45, and 60 s) on aprismatic (unground) and prismatic (ground) enamel.

In the prismatic enamel group, a standard ground surface was prepared to produce a flat, parallel area, which was sufficient to place a sealant button to obtain a standardized horizontal plane for SBS testing. In the aprismatic group, the natural enamel surface of each specimen was maintained. An etching procedure with 37% phosphoric acid gel was performed for 15, 30, 45, or 60 s. The samples were subsequently inserted into a bonding clamp, and the sealants were further applied. The samples were aged by one-day storage in distilled water at 37°C in a thermal oven followed by thermocycling between 5°C (±2°C) and 55°C (±2°C) for 5000 cycles. The notched-edge (ISO standard) SBS test (15) was performed using a universal testing machine, the SBS was calculated in MPa. All samples were examined for failure modes under a stereomicroscope at 20-fold magnification.

Under the same laboratory setting, five human third molars were assigned to each of the eight subgroups in order to test microleakage. For the group with prismatic enamel, the superficial enamel in the areas of the central, supplemental grooves, as well as the involved area of each cusp were removed by a flame-shaped finishing diamond bur. The samples were subsequently immersed in 0.5% methylene blue solution for 24 h at 37°C. The tooth crowns were fully embedded in cold-curing methyl methacrylate resin. The resin blocks were further fixed and the crowns were sectioned in the buccolingual direction into at least 5 slices. The front and back of each slice were inspected. If dye penetration was present, each side was quantitatively measured concerning the total length of the interface between the enamel and the sealant. All measurements were performed with the imaging software ImageJ (version 1.52, Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). Descriptive and explorative data analyses were performed using Stata/SE 14.2 (StataCorp 2015, College Station, TX, USA). Pairwise comparisons of microleakage values between different etching times were performed using the Mann-Whitney U test. Linear regression analysis was also performed to compare the results from SBS testing. A significance of $\alpha = 0.05$ (two-tailed test) and a 95% confidence level were used for all analyses.

2.3. Results

In the first study, a total of 28 relevant clinical studies with 36 datasets were identified and included in the meta-analysis. Overall, the included studies were published between 1978 and 2014, and the follow-up duration ranged from 2 to 20 years. Studies with an etching time of 15 s (n=3), 20 s (n=2), 30 s (n=10), 40 s (n=1) and 60 s (n=20) were identified. The majority of the included patients (n=1891) and tested molars (n=3295) were obtained from studies with 60 s of etching, and only five reports were identified for etching times of 15 and 20 s. In the case of investigations with 30 s of etching time, ten reports were registered. On the other hand, the majority (n=33) of the included reports originated from clinical trials with two or three years of observation. Only one study followed patients for 20 years. The main finding from the negative binomial regression model showed that no significant association between the different etching times and retention rates was identified.

In the second study, the investigation of SBS on aprismatic enamel revealed that longer acid etching tended to result in slightly higher fracture resistance, but the difference was not found to be significant. The same finding was also registered for prismatic enamel. Here, a significant difference of SBS between 15 s/ 60 s and between 30 s/ 60 s etching times on prismatic enamel was detected. There was no significant difference in SBS in relation to tooth surfaces and the type of enamel preparation. Adhesive failure was the most predominant type of failure among all groups, followed by mixed failures. Cohesive failures and enamel failures were rarely observed. No serious differences existed between the aprismatic and prismatic enamel group regard to the failure mode. The results from the simple linear regression showed that only etching time had a significant influence on the SBS. The estimate from the regression analysis resulted in 13.9 MPa for the reference group (15 s etching time), whereas a 60 s etching time had a positive effect by significantly increasing the SBS by 2.3 Mpa. The mean values for microleakage were found to be low throughout all groups, and no significant difference was detected. It should be noted that a longer etching time in aprismatic enamel contributed to a decrease in microleakage. In contrast, this trend was reversed in prismatic enamel.

2.4 Discussion

In the first study, based on the results from the negative binomial regression model, there is no evidence of a significant influence of etching times on the retention rate of pit and fissure sealants in permanent molars. However, it should be noted that the data of the included studies were not equally distributed with respect to the etching times and materials used: the majority (n=30) of the studies were obtained from investigations that used 30 and 60 seconds of etching. In contrast, only four clinical trials (10,16,17,18) were identified for etching times of 15 or 20 seconds; furthermore, all of these studies had a short observation period. On the other hand, auto-polymerizing materials were documented with the longest follow-up period of up to 20 years (19). For the materials introduced more recently, namely, light-polymerizing, and fluoride-releasing and light-polymerizing sealants, the maximum observation periods were only 5 years (20). In general, the body of clinical knowledge originates from studies that used “light-polymerizing”, and “fluoride-releasing and light-polymerizing” sealants and pre-treated the outer prismless enamel layer with phosphoric acid for 30 or 60 seconds.
Based on the limited data for etching times of 15 and 20 seconds, this study could not draw a firm conclusion regarding the usage of shortened etching times. According to the data from available clinical sealant studies, which used 30 or 60 seconds of acid etching, neither of the etching times was found to be superior in the regression analysis. Therefore, a minimum of 30 seconds of acid etching seems to be sufficient prior to fissure sealing to safeguard appropriate sealant retention.

In the second study, a slight, insignificant increase in SBS was observed for both enamel groups with longer etching times. Pairwise comparative statistical analyses (Mann-Whitney U test) also revealed that a significant increase in SBS was registered between 15 s/ 60 s and 30 s/ 60 s etching times when prismatic samples were used; all other systematically performed comparisons remained insignificant.

Concerning the results from the linear regression model, it was found that only the parameter of 60 s etching time was statistically significant. All other variables, e.g., 15 s, 30 s, and 45 s etching times, type of enamel preparation, and tooth surfaces remained insignificant. When considering the information from previous available in vitro studies (21,22,23,24), it might be concluded that longer acid etching times improved the SBS on prismatic enamel. However, regarding the (frequently) low numbers of samples in each study (n=30 (21), n=10 (22), n=10 (23), n=10 (24)), the results from simple (pairwise) statistical comparisons could explain (non-)significant differences and should therefore not be overrated.

With respect to failure mode, adhesive failures were most commonly detected; cohesive and enamel failures were rare. Interestingly, there was a minor trend of more mixed failures when etching aprismatic enamel for 60 s. This finding may indicate an increase in adhesive performance; nevertheless, the results should not be overrated.

When analyzing the present data of microleakage, it can be concluded that the mean values of dye penetration were generally low. However, the present investigation recorded a non-significant descending trend in aprismatic enamel when etching time increased. In the case of prismatic enamel, an opposite trend was observed. The ascending performance of microleakage in prismatic enamel with the increase of etching time might be a sign of over-etching (7,25,26).

When considering the SBS results from this in vitro study, it should be noted that only small differences between all tested groups existed. However, the data indicated that an increasing time of acid etching resulted in slightly higher SBS in both enamel groups, and the linear regression analysis also revealed a significant advantage of 60 s acid etching. Furthermore, the trend of more mixed fracture failures and less microleakage when etching time increased in aprismatic enamel support the recommendation of 60 s of acid etching before sealant application. However, when emphasizing the small test differences between 30 s and 60 s, it can be argued that an application time of 30 s may also provide acceptable clinical results.

3. Zusammenfassung

Basierend auf den durchgeführten wissenschaftlichen Arbeiten können die folgenden Schlussfolgerungen gezogen werden:

- Die Fissuren- und Grübchenversiegelung ist eine wirksame kariespräventive Maßnahme und wird heute vor allem bei kariesaktiven Kindern eingesetzt. Die Verkürzung der Säurekonditionierung wurde wiederholt diskutiert, um die Behandlung zu vereinfachen und die Behandlungszeit insbesondere bei Kindern zu verkürzen. Demgegenüber liegen nur begrenzte Daten aus vergleichenden klinischen Studien vor.
- Daher wurde im ersten Projekt der vorliegenden Dissertationsschrift der Einfluss der Ätzzeit auf die Überlebensrate von Fissurenversiegelungen an bleibenden Molaren auf der Grundlage einer systematischen Literaturrecherche und Metaanalyse analysiert. Das zweite Projekt evaluierte die Scherhaftung (SBS), Bruchmuster und die Farbstoffpenetration der Fissurenversiegelung in Bezug auf unterschiedliche Ätzzeiten an aprismatischem und prismatischem Schmelz unter Laborbedingungen.
- Die systematische Literaturrecherche umfasste 28 klinische Studien mit 36 Testgruppen, welche aus Studien mit einer Beobachtungsdauer von mindestens zwei Jahren stammten. In den Studien wurden auto-polymerisierende, licht-polymerisierende und fluoridfreisetzende, licht-polymerisierende Versiegelungsmaterialien verwendet, sowie der Zahnschmelz für 15, 20, 30, 40 und 60 Sekunden mit Phosphorsäure vorbehandelt. Das Ergebnis der Literaturrecherche bzw. negativen binomialen Regressionsanalyse war, dass eine begrenzte Anzahl klinischer Daten für 15 und 20 Sekunden verfügbar war. Daher sollten Überlebensraten aus Studien mit kurzer Säureätzzeiten eine vorsichtige Interpretation erfahren. Die Ergebnisse aus der Regressionsanalyse signalisieren, dass vor der Fissurenversiegelung der Zahnschmelz für mindestens 30 Sekunden konditioniert werden sollte.
- Das zweite Projekt untersuchte die Scherhaftung unter Verwendung von aprismatischem (nicht präpariertem) und prismatischem (präpariertem) Schmelz, welcher vor der Versiegelung für 15, 30, 45, oder 60 Sekunden geätzt wurde. Im Ergebnis der Scherversuche wurden nur geringe Unterschiede zwischen allen getesteten Gruppen aufgefunden. Eine paarweise Analyse ergab jedoch, dass bei Verwendung von prismatischen Proben ein signifikanter Anstieg der Scherfestigkeit zwischen 15 s / 60 s und 30 s / 60 s Ätzzeiten registriert wurde. Bei aprismatischem Schmelz wurde dagegen kein signifikanter Unterschied zwischen die Äztzeiten festgestellt. Das lineare Regressionsmodell ergab, dass eine Ätzzeit von 60 Sekunden zu einem signifikanten Anstieg der Scherfestigkeit führte. Darüber hinaus wurde festgestellt, dass die Mittelwerte für die Farbstoffpenetration in allen Gruppen niedrig waren und kein signifikanter Unterschied feststellbar war. Mit zunehmender Ätzzeit zeigte sich allerdings ein nicht signifikanter absteigender Trend der Farbstoffpenetration im aprismatischen Zahnschmelz.

- Zusammenfassend kann damit eine Ätzzeit von 30 Sekunden bei aprismatischem Schmelz empfohlen werden, da dies zu einer verkürzten Behandlungszeit führt und damit potentiell die Behandlungscompliance bei Kindern erhöht werden kann.
- Im Hinblick auf die begrenzten Vergleichsdaten zu verschiedenen Säureätzzeiten scheint in Zukunft mehr klinische Forschung erforderlich zu sein.

4. Abstract

Based on the performed scientific works, the following conclusions can be drawn:

- Sealing pits and fissures is an effective caries-preventive measure and is mostly indicated for caries-active children today. The shortening of acid conditioning has been discussed repeatedly to simplify the treatment and reduce the operation time, particularly in children. However, only limited data from comparative clinical studies are available.
- Therefore, the first project analyzed the influence of etching times on the survival rate of pit and fissure sealants in permanent molars on the basis of a systematic search of the literature and meta-analysis. The second project evaluated the shear bond strength (SBS), failure mode, and microleakage of fissure sealing materials in relation to different etching times on aprismatic and prismatic enamel under laboratory conditions.
- The systematic review included 28 clinical studies with 36 datasets that originated from the trials with at least two years of observation. Studies that used auto-polymerizing, light-polymerizing, and fluoride-releasing and light-polymerizing sealants, as well as acid etching times of 15, 20, 30, 40, and 60 s were detected. The finding from descriptive analysis was that there was a limited number of clinical data for 15 and 20 s available. Thus, conclusions regarding very short acid etching times should be omitted. Negative binomial regression analysis revealed no significant difference between retention rate and different etching times. Therefore, a minimum of 30 s of acid etching might be sufficient prior to fissure sealing.
- The second project investigated the SBS using aprismatic (unprepared) and prismatic (prepared) enamel that were etched for 15, 30, 45, or 60 s before sealant placement. When considering the SBS results in detail, it should be noted that only small differences between all tested groups existed. Nevertheless, the pairwise analysis revealed a significant increase in SBS was registered between 15 s/ 60 s and 30 s/ 60 s etching times when prismatic samples were used. The linear regression model showed that 60 s of etching time led to a significant increase in SBS. The mean values for microleakage were found to be low throughout all groups, and no significant difference was detected. However, a non-significant descending trend of microleakage in aprismatic enamel exhibited when etching time increased.
- In conclusion, a minimum of 30 s of etching time could be recommended on aprismatic enamel before sealant placement for daily practice, as it reduces treatment time and might improve the treatment compliance of children.
- Concerning the limited comparative studies on the influence of different acid etching times on retention rate, more well-conducted clinical researches are needed in the future.

5. Paper I

What is an Appropriate Etching Time Before Sealant Application on Permanent Molars? Results from a Meta-analysis

Yi-Fang Lo[a] / Alexander Crispin[b] / Andreas Kessler[c] / Reinhard Hickel[d] / Jan Kühnisch[e]

Purpose: This meta-analysis investigated the influence of different acid etching times on the retention rate of pit-and-fissure sealants based on clinical trials with a minimum duration of two years.

Materials and Methods: A literature search was carried out in electronic databases along with hand searching to identify clinical trials that evaluated pit-and-fissure sealants in permanent molars. From 1280 identified abstracts, 195 studies were selected for full-text analysis, and 28 studies with 36 test groups were included in this meta-analysis. Test groups with etching times of 15 (n = 3), 20 (n = 2), 30 (n = 10), 40 (n = 1) and 60 s (n = 20) were found. Incidence rates of pit-and-fissure sealant losses were modelled using negative binomial regression.

Results: The regression analysis did not reveal a significant influence of the etching time on the survival of pit-and-fissure sealants based on the identified and included clinical trials.

Conclusions: Due to the limited number of clinical data for 15 and 20 s, conclusions regarding very short acid etching times were not possible. On the basis of the regression analysis, a minimum of 30 s of acid etching might be sufficient prior to fissure sealing.

Keywords: acid etching, caries prevention, clinical studies, enamel pretreatment, meta-analysis, pit-and-fissure sealants, systematic review.

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doi: ##.####/j.jad.a#####

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Sealing pits and fissures is an effective caries preventive measure.[1] However, the caries-protective effect is influenced by material retention, as a sealant can only be caries protective as long as it covers the pit and fissure completely.[30] It was previously shown that sealant retention varied significantly between the available materials.[20] Additionally, other clinical variables, eg, etching time, practice set-up, and isolation technique, may influence long-term survival.

When considering acid etching as an important pretreatment step before sealing and its influence on longevity, it is surprising that to date, only limited data from comparative clinical studies exist. There is only one clinical study,[9] which showed that 40.0%, 50.9%, 41.8% and 43.6% of sealants were intact after 12 months when etching the enamel for 15, 30, 45 and 60 s, respectively. Based on previously published meta-analysis results,[20] it can be expected that approximately 80% of all sealants will be intact after a two-year observation period. Therefore, the previously published comparative clinical results must be interpreted with caution. Furthermore, several working groups used reduced

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Table 1 Search strategy used in electronic databases

PICOS	MeSH Terms/ keywords
Patient	permanent molar* OR molar* OR fiss* OR children AND
Interventions	seal* OR caries prevent* OR caries-prevent* AND
Comparator	Sealant materials
Auto-polymerizing sealants	Self-cured OR self cured OR self curing OR self-curing OR self polymerized OR self-polymerized OR self polymerizing OR self-polymerizing OR chemically-cured OR chemically cured OR chemically polymerized OR chemically-polymerized OR chemically-polymerizing OR chemically polymerizing OR chemically OR auto polymerized OR autopolymerized OR auto-polymerized OR auto polymerizing OR autopolymerizing OR auto-polymerizing OR auto-polymerising OR cold-cured OR cold cured OR cold-curing OR cold curing OR compon* OR mix* OR
Light-polymerizing sealants	light-cured OR light cured OR light curing OR light-curing OR light polymeri* OR light-polymeri* OR light polymeri* OR light-polymeri* OR light-activated OR light activated OR visible light OR visible-light-cured OR
Fluoride-releasing and light-polymerizing sealants	fluoride-releasing OR fluoride releas* OR fluoride-containing OR fluoride contain* OR F releasing OR F-releasing OR fluroshield OR helioseal-F OR helioseal F OR teethmate OR fissurit OR fissurit F OR delton plus OR
Outcome measures	clinical performance OR success OR success rate* OR retention OR retention rate* OR remain* OR caries OR Influence* OR influencing* OR survival OR survival rate* OR outcome* OR effect* OR effectiveness OR efficacy AND
Study type	Meta-Analysis OR Meta Analysis OR RCT OR random* OR double blind OR double-blind OR clinical trial* OR trial* OR half mouth OR half-mouth OR split mouth OR split-mouth OR clinical result* OR clinical stud* OR vivo OR longitudinal study OR cohort OR community OR follow-up OR month* OR year* OR compare OR comparing OR comparative OR comparative study OR long-term

etching times, eg, < 30 s, in clinical trials that predominantly investigated the survival of (new) sealant materials. There seems to be a trend towards shorter etching times in recent studies.[15,16,25,26,40] Furthermore, some clinical studies used a very short etching time of only 15 s,[15,25] and the documented survival rates seem to be heterogeneous. While one study documented good or satisfactory retention rates,[26] the other four trials reported very low survival rates.[15,16,25,40] Due to the limited and heterogeneous data, the clinical consequences are disparate. Therefore, the present meta-analysis based on a systematic review of the literature was conducted in an attempt to close this knowledge gap. Consequently, this study analyzed the influence of etching time on the survival of pit-and-fissure sealants in permanent molars. It was hypothesized that there is no influence of the etching time on sealant retention.

MATERIALS AND METHODS

This systematic review was reported according to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement.[23]

PICOS Model

We structured our literature search based on the five components of the PICOS model: patients, intervention, comparator, outcome, and study type.[32] Table 1 summarizes all the keywords and possible synonyms used for the systematic search in the following databases: PubMed via Medline, EMBASE, Cochrane Central Register of Controlled Trials (CENTRAL), Scopus, and Google Scholar up to March 2017. A thorough manual review of the reference lists of the retrieved publications was performed to identify additional articles. There was no restriction placed on the language or date of publication when searching the databases. Furthermore, several known articles were added manually. Endnote X7.7.1 (Bld 11961, Thomson Reuters; Toronto, Ontario, Canada) was used to retrieve the studies from MEDLINE and EMBASE. The number of identified documents before and after the removal of duplicates is shown in Fig 1.

Selection of Studies

Clinical trials evaluating the longevity of resin- or methacrylate resin-based pit-and-fissure sealants with a phosphoric acid etching used before sealant application on occlusal surfaces on permanent molars, with a minimum of 2 years of follow-up, were included in the investigation. In addition, data from any subgroups from sealant studies, which served as control groups and fulfilled the inclusion criteria, were incorporated as well, eg, Feigal and Quelhas.[13] The majority of the included studies cleaned teeth before acid etching, eg, using a bristle brush or rubber-cup with pumice powder. In the case of studies reporting the same sample, we included those that presented the most recent information.

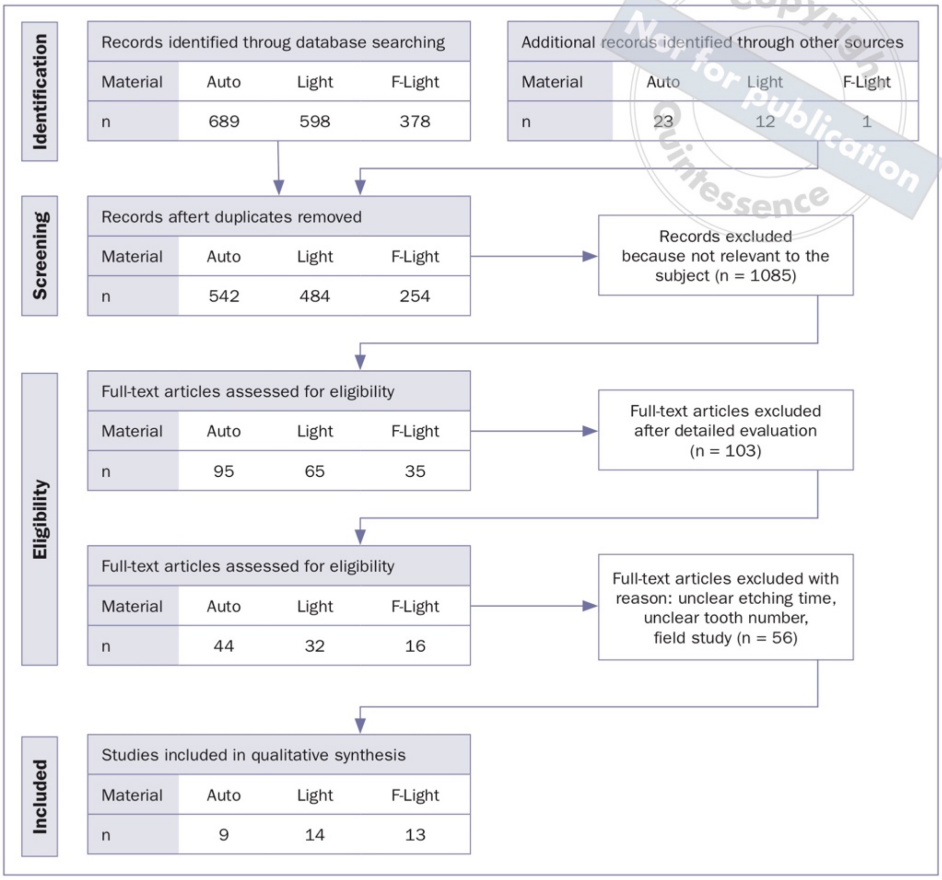

Fig 1 Flowchart of study selection according to the PRISMA statement.

Teeth sealed with glass-ionomer cement, compomer, and other products were excluded. Further, data from field trials were not considered. Studies with any invasive pretreatments, such as fissurotomy or preventive resin restorations, no etching time information, use of drying or bonding agents, or absence of clear results and retention data as the outcome were excluded.

In the first step, two trained reviewers (YL and AK) independently screened the titles and abstracts of all identified studies and determined eligibility. When no abstract was available or the summary was inconclusive, the decision was made based on the full text. In the case of any disagreements or uncertainties, the reviewers consulted with the supervisor (JK) to resolve the issue. As a result of this primary selection step, the eligible number of publications was reduced to 195 (Fig 1). Subsequently, full-text articles of all relevant and potentially relevant studies, those appearing to meet the inclusion criteria, and those with insufficient data in the title and abstract were obtained to determine study relevance. The full-text papers were assessed independently by the two reviewers (YL and AK), and in the case of disagreement, a consensus was reached together with the supervisor (JK). The final number of studies included after screening and a summary of details are listed in Fig 1 and Table 2.[2,3,5-7,10,11,13-16,18,19,21,22,26-28,31,33-41]

Data Extraction and Management

Data were extracted independently by two trained review authors (YL and AK); in the case of unclear findings, a consensus was reached (YL, AK, and JK). The included publications were assessed in a standardized manner to systematically record all relevant details. The following basic information was extracted from each study: year of publication, population characteristics and size, type and numbers of teeth, age of participants, brand and manufacturer of the sealant system, dental set-up, isolation method, etching time, study design, and follow-up period. Regarding the evaluation criterion, the sealant retention was recorded as a success (total retention) or failure (partial or total loss). If only the percentage of retention was published, each rate was calculated based on the available data. Missing numerical values were recorded as “not reported”. When etching time was provided as a range of times, the shortest etching time was recorded. All data were entered into an MS Excel worksheet (Office 2015, Microsoft; Redmond, WA, USA).

Table 2 Summary of included clinical studies with respect to etching time and retention rate in permanent molars

Reference	Follow-up (years)	Study population at baseline			Sealant material		Etching time (sec)	Outcome		
		Age (years)	N Subjects	N Molars	Type	Products		N Sealants	N Intact sealants	Survival rate (%)
Sheykholeslam and Houpt (1978)	2	6-10	205	205	AP	Delton	60	148	175	84.6
Williams et al (1986)	2	nr	64	64	AP	Concise	60	46	60	76.7
Houpt et al (1987)	3	6-8	73	144	AP	Delton	60	78	110	70.9
Rock et al (1990)	3	6-7	186	372	AP	Delton	60	245	318	77.0
Gandini et al (1991)	2	6-11	62	76	AP	Delton	60	59	70	84.3
Mills and Ball (1993)	2	5-16	53	120	AP	Delton	60	34	59	57.6
Karlzen-Reuterving and Dijken (1995)	3	6-7	47	74	AP	Delton	20	57	72	79.2
Wendt et al (2001)	20	6-7	72	288	AP	Delton	60	100	153	65.4
Barja-Fidalgo et al (2009)	5	6-8	36	46	AP	Delton	30	6	28	21.4
Stephen et al (1985)	2	5-11	72	75	LP	Prismashield	30	53	58	91.4
Williams et al (1986)	2	nr	100	100	LP	ICI Resin	60	69	86	80.2
Houpt et al (1987)	3	6-8	73	160	LP	Delton	60	78	114	68.4
Rock et al (1990)	3	6-7	186	186	LP	Delton	60	131	158	82.9
Rock et al (1990)	3	6-7	186	186	LP	Prismashield	60	115	160	71.9
Gandini et al (1991)	2	6-11	62	77	LP	Concise	60	66	71	93.0
Gandini et al (1991)	2	6-11	62	76	LP	Sealite Kerr	60	46	70	65.7
Feigal and Quelhas (2003)	2	7-13	nr	18	LP	Delton	30	11	18	61.1
Lampa et al (2004)	2	6-13	31	100	LP	Delton	60	66	100	66.0
Ganesh and Tandon (2006)	2	6-7	100	100	LP	Concise	15	4	100	4.0
Zimmer et al (2009)	3	5-15	95	177	LP	Helioseal	60	160	177	90.4
Yilmaz et al (2010)	2	7-13	80	80	LP	Admira Seal	60	17	67	25.4
Baseggio et al (2010)	3	12-16	320	640	LP	Fluorshield	30	572	628	91.1
Guler and Yilamz (2013)	2	7-13	50	100	LP	Admira Seal	60	2	66	3.0
Carlsson et al (1997)	2	6-7	121	431	FR	Helioseal F	60	330	431	76.6
Vrbic (1999)	3	10.5	96	243	FR	Helioseal F	60	210	219	95.9
Pinar et al (2005)	2	8-10	30	60	FR	Fissurit F	30	30	44	68.2
Puppin-Rontani et al (2006)	2	7-9	57	114	FR	FluoroShield	30	10	50	20.0
Yakut and Sönmez (2006)	2	6-9	60	60	FR	Fissurit F	30	60	60	100.0
Dukic and Glavina (2007)	2	7-17	41	33	FR	Teethmate F1	40	20	33	60.6
Amin (2008)	2	7-10	45	30	FR	Helioseal F	30	21	26	80.8
Yilamz (2010)	2	7-13	40	80	FR	Fissurit F	15	18	62	29.0
Yilmaz (2010)	2	7-13	40	80	FR	Fissurit FX	15	14	62	22.6
Bendinskaite et al (2010)	5	6-9	88	121	FR	Fissurit F	30	58	93	62.4
Oba et al (2012)	2	9-20	35	43	FR	Fissurit F	20	30	37	81.1
Ulusu et al (2012)	2	7-15	173	173	FR	Fissurit F	30	30	137	21.9
Erdemir et al (2014)	2	16-22	34	110	FR	Helioseal F	60	82	96	85.4

AP: auto-polymerizing sealant, LP: light-polymerizing sealant, FR: fluoride-releasing and light-polymerizing sealant, nr: no data reported.

Assessment of Risk of Bias in Included Studies

Two review authors (YL and AK) independently assessed the risk of bias of included studies. Disagreements were resolved by consensus (YL, AK, and JK). As recommended by the Cochrane Handbook for Systematic Reviews of Interventions Version 5.1,[17] we assessed the following methodological sources of bias: selection bias (random sequence generation, allocation concealment, power calculation), performance bias (blinding of study participants and personnel, previous calibration of the operators), detection bias (blinding of outcome assessors), attrition bias (proper report of missing data), and reporting bias (proper report of out-

Table 3 Characteristics of the included clinical studies with respect to sealants

Material	Design	Publication interval	Number of studies	Study duration in years	Etching time used (s) 15	20	30	40	60	Population at baseline: Number of subjects	Number of molars
Auto-polymerizing sealants[1]	CT2	1978–2007	9	2–20	–	1	1	–	7	798	1389
Light-polymerizing sealants	CT2	1985–2013	14	2–3	1	-	3	-	10	14173	2075
Fluoride-releasing and light-polymerizing sealants[1]	CT2	1997–2014	13	2–5	2	1	6	1	3	860	1578
Σ		1978–2014	36	2–20	3	2	10	1	20	3075	5042

[1]No field trial was identified according to the inclusion criteria; [2]CT: clinical controlled study; [3]study number was not reported.

Table 4 Summary of included clinical studies with respect to etching time and retention rate in permanent molars

Material	Design	Etching time	Number of studies	Study population at baseline: N patients	N molars	Intact fissure seals (N) with respect to the total number of applied fissure sealants (Σ) as a function of the length of retention in years: 2 N	2 %	2 Σ	3 N	3 %	3 Σ	5 N	5 %	5 Σ	20 N	20 %	20 Σ
Auto-polymerizing sealants[1]	CT2	20	1	47	74	–	–	–	57	79.2	72	–	–	–	–	–	–
		30	1	36	46	–	–	–	–	–	–	6	21.4	28	–	–	–
		60	7	715	1269	287	78.9	364	323	75.5	428	–	–	–	100	65.4	153
Light-polymerizing sealants	CT2	15	1	100	100	4	4	100	–	–	–	–	–	–	–	–	–
		30	3	>3923	733	64	84.2	76	572	91.1	628	–	–	–	–	–	–
		60	10	925	1242	266	57.8	460	484	79.5	609	–	–	–	–	–	–
Fluoride-releasing and light-polymerizing sealants[1]	CT2	15	2	80	160	32	25.8	124	–	–	–	–	–	–	–	–	–
		20	1	35	43	30	81.1	37	–	–	–	–	–	–	–	–	–
		30	6	453	558	151	47.6	317	–	–	–	58	62.4	93	–	–	–
		40	1	41	33	20	60.6	33	–	–	–	–	–	–	–	–	–
		60	3	251	784	412	78.2	527	210	95.9	219	–	–	–	–	–	–
Total		15	3	180	260	36	16.1	224	–	–	–	–	–	–	–	–	–
		20	2	82	117	30	81.1	37	57	79.2	72	–	–	–	–	–	–
		30	10	>8813	1337	215	54.7	393	572	91.1	628	64	52.9	121	–	–	–
		40	1	41	33	20	60.6	33	–	–	–	–	–	–	–	–	–
		60	20	1891	3295	965	71.4	1351	1017	81	1256	–	–	–	100	65.4	153
Total			36	3075	5042	1266	62.1	2038	1646	84.2	1956	64	53.9	121	100	65.4	153

[1]No field trial was identified according to the inclusion criteria; [2]CT: clinical controlled study; [3]study number was not reported.

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Table 5 Coefficients from the negative binomial regression model using the natural logarithm of the time at risk (in years) as reference

Etching time	Estimate	95% CI	Wald χ^2	p–value
Intercept	–1.5662	–2.0071; –1.1253	48.47	<0.0001
15 s	1.0001	–0.5441; 2.5443	1.61	0.2043
20 s	–0.8162	–2.4174; 0.7850	1.00	0.3178
30 s	0.2621	–0.5134; 1.0375	0.44	0.5078
40 s	0.1608	–2.0335; 2.3552	0.02	0.8858
60 s	0.0000 (reference)	–	–	–

The global likelihood ratio test yielded χ^2[df = 4] = 3.29, p = 0.5103.

comes). Within each domain, we classified each study as having "low", "high" or "unclear" risk of bias, with the latter indicating lack of information or uncertainty about the potential for bias.

Statistical Analysis

Statistical analyses of the effect of etching times were performed using negative binomial regression models. The absolute number of failures in a given study was estimated from the proportion of molars with defective sealants among all molars examined at the end of the study multiplied by the number of molars examined at baseline. The total sealant survival time at risk of a failure was estimated as half the study duration in the case of defective sealants and as the whole study duration otherwise. The natural logarithm of the survival time at risk (in years) was used as an offset in the negative binomial regression models.

RESULTS

A total of 28 relevant clinical studies with 36 test groups were identified and included in the meta-analysis. Overall, the included studies were published between 1978 and 2014, and the follow-up duration ranged from 2 to 20 years. Studies with an etching time of 15 (n = 3), 20 (n = 2), 30 (n = 10), 40 (n = 1), and 60 s (n = 20) were identified (Table 3).

In all studies, the sealants were applied in facilities equipped with complete dental units and performed with a split-mouth design on permanent molars. Sealants were applied using cotton rolls for tooth isolation, except one study reported the use of rubber-dam[2] and four studies did not provide information about the isolation procedure.[6,19,34,38] The results of the retention rates in relation to the etching time and study duration are summarized in Table 4. The majority of the included patients (n = 1891) and tested molars (n = 3295) were obtained from studies with 60 s of etching (Tables 2 and 4), and only five reports were identified with etching times of 15 or 20 s[15,19,26,40] In addition, only a limited number of teeth (n = 377) (Table 4) were included in those studies, compared with those in the 60-s group. In the case of 30-s etching time, ten reports were registered (Table 2 and 4). These studies mostly utilized fluoride-releasing and light-polymerizing sealant (n = 6)[2,6,27,28,35,39]and light-polymerizing sealant (n = 3).[5,13,34] Moreover, it should be noted that the majority (n = 33) of the reports originated from clinical trials with two or three years of observation (Table 2 and 4); only one study followed patients for 20 years.[37] In the case of studies with shorter etching times, namely 15 and 20 s, little long-term data was found.

In addition to the descriptive data analysis (Table 4), a negative binomial regression model (Table 5) was developed. The main finding from this model was that no significant association between the different etching times and retention rates was identified (likelihood ratio test: χ^2[df = 4] = 3.29, p = 0.5103).

The assessments of risk of bias for each individual study and the results by domain over all studies are presented in Table 6. Overall, heterogeneity was observed in most of the domains of the included studies.

DISCUSSION

The current systematic review addressed an important clinical question, namely, whether there is an influence of etching time on the longevity of pit-and-fissure sealants in permanent molars. Based on the results from the negative binomial regression model (Table 5), there is no evidence of a meaningful influence of etching time on the retention rate of pit-and-fissure sealants in permanent molars. Therefore, the initially formulated null hypothesis is accepted. Nevertheless, this finding needs to be discussed from a methodological point of view, which may have influenced our results. It is evident from Table 4 that the data from the included studies are not equally distributed with respect to the etching time and materials used. The majority (n = 30) of the clinical studies were obtained from investigations that used 30 and 60 s of etching (Table 4). In contrast, only four clinical trials[15,19,26,40] were identified with etching times of 15 or 20 s; furthermore, all of these studies had a short observation period. On the other hand, auto-polymer-

Table 6 Review authors' judgments about each risk of bias item for each included study, presented as percentages across all included studies

Reference		Was the sealant treatment randomly allocated? (selection bias)	Was the allocation properly concealed? (selection bias)	Is the study population sufficiently powered? (selection bias)	Proper blinding of the participants and operators? (performance bias)	Were operators trained by senior doctors before study begins? (performance bias)	Proper blinding of the operators and investigators? (detection bias)	Were drop-out rates and reasons for withdrawals reported? (attrition bias)	Were appropriate outcomes reported and were key outcomes missing? (reporting bias)
Sheykholeslam and Houpt (1978)		?	−	−	−	−	−	?	+
Stephen et al (1985)		−	−	−	+	−	+	+	+
Williams et al (1986)		+	−	−	−	−	−	+	+
Houpt et al (1987)		?	−	−	−	−	−	?	+
Rock et al (1990)		?	−	−	−	?	+	−	+
Gandini et al (1991)		−	−	−	−	?	−	+	+
Mills and Ball (1993)		−	−	−	−	?	−	−	+
Karlzen-Reuterving and Dijken (1995)		?	−	−	−	?	−	+	+
Carlsson et al (1997)		?	−	−	−	−	?	+	+
Vrbic (1999)		−	−	−	−	+	?	?	+
Wendt et al (2001)		−	−	−	−	−	+	+	+
Feigal and Quelhas (2003)		?	−	−	−	−	+	+	+
Lampa et al (2004)		?	−	−	−	−	−	+	+
Pinar et al (2005)		−	−	−	−	−	+	?	+
Ganesh and Tandon (2006)		?	−	−	−	?	−	+	+
Puppin-Rontani et al (2006)		−	−	−	−	?	−	−	+
Yakut and Sönmez (2006)		−	−	−	−	?	−	+	+
Barja-Fidalgo et al (2007)		+	−	−	−	−	+	+	+
Dukic and Glavina (2007)		−	−	−	−	−	−	+	+
Amin (2008)		?	−	−	−	?	−	?	+
Zimmer et al (2009)		−	−	−	−	?	+	+	+
Baseggio et al (2010)		−	−	−	−	+	+	+	+
Bendinskaite et al (2010)		?	−	+	−	−	+	+	+
Yilamz (2010)		+	+	+	−	−	+	?	+
Oba et al (2012)		?	−	−	−	−	−	?	+
Ulusu et al (2012)		?	−	−	−	−	−	?	+
Guler and Yilamz (2013)		+	−	+	−	?	+	−	+
Erdemir et al (2014)		+	−	−	−	?	+	+	+
∑	Low risk of bias	5 (17.9)	1 (3.6)	3 (10.7)	1 (3.6)	2 (7.1)	12 (42.9)	16 (57.1)	28 (100.0)
	Unclear risk of bias	12 (42.9)	0 (0)	0 (0)	0 (0)	11 (39.3)	2 (7.1)	8 (28.6)	0(0)
	High risk of bias	11 (39.9)	27 (96.4)	25 (89.3)	27 (96.4)	15 (53.6)	14 (50.0)	4 (14.3)	0(0)

Red dots: absence of reporting of that domain; green dots: complete reporting of that domain; yellow dots: unclear or incomplete reporting of the domain.

izing materials were documented with the longest follow-up period of up to 20 years.[37] For the materials introduced more recently, namely, light-polymerizing, fluoride-releasing and light-polymerizing sealants, the maximum observation periods were only 5 years[6] (Table 4). In general, the body of clinical knowledge originates from studies that used the latter groups of materials and pre-treated the outer prismless enamel layer with phosphoric acid for 30 or 60 s. Accordingly, it is obvious that materials or procedures that are less extensively described in clinical studies should be viewed with caution by clinicians. This notion is further supported by data from short-term clinical investigations which used a reduced etching time. These in vivo studies made heterogeneous and/or below-average clinical performance obvious,[4,15,16,25,26,29,40] and should therefore be interpreted with caution.

The main finding from the negative binomial regression analysis (Table 5) was that there was no significant difference in the retention rate of sealants according to etching time (30 or 60 s). Based on this result, it can be concluded that the shorter etching time should be preferred in daily clinical practice and that the conventional recommendation seems to be outdated. When discussing and interpreting the finding that 30 and 60 s of acid etching time do not influence the longevity of sealants, the advantages of a shorter etching time should first be mentioned. The time advantage seems to be the most important issue for young patients and (pediatric) dentists. While patients will benefit from shorter operative times, which are accompanied by reduced risks associated with phosphoric acid in the oral cavity, a shorter application time also makes the clinical procedure more convenient and efficient for patients and dentists. However, a shorter etching time could make controlling the effect more difficult, as the whitish etching pattern after forced air drying may not appear as white as that following the conventional etching time of 60 s. This incomplete etching pattern could lead to a reduction of retention. This possibility should be taken into consideration, as the frosty etching pattern has been frequently mentioned as a clinical quality marker for successful enamel etching.

The present systematic review and meta-analysis has strengths and limitations. The unique aspect of this work is the analysis of the influence of etching time on the basis of a systematic literature search, which may close an existing knowledge gap. When considering the identification, screening, and selection process for all studies, it should be stated that we applied strict inclusion/exclusion criteria. Most importantly, all of the included studies provided substantial information regarding etching time and retention on permanent molars only, with a minimum observation time of two years. The most frequent reasons for the exclusion of studies were duration of less than two years, sealing of primary teeth, and missing or incomplete reported data (Fig 1). Furthermore, field trials were excluded due to the divergent study setting compared with the typical clinical study setting. Also of note is that differences in the clinical workflow exist between studies and may limit the comparability of the clinical sealant application and/or the recording of the outcome, which may also cause some risk of bias. This risk was largely neglected in reports and could not be fully resolved in meta-analytical studies. Considering the heterogeneity of the included studies (Table 6), it should be mentioned that only some of the studies performed random allocation,[3,11,16,38,40] allocation concealment[40] and sample size calculation,[6,16,40] blinding of the participants and operators,[34] calibration of the operators,[5,36] and blinding of the outcome assessors.[3,5,6,11,13,16,27,31,34,37,40,41] Several studies did not provide complete provide information about attrition rates.[16,22,28,31] Therefore, the results obtained should be carefully considered.

Besides this, the structural differences between permanent and primary teeth should be taken into consideration. The outer aprismatic enamel layer, which is thicker in primary than in permanent teeth[12,24] and more resistant to acid etching,[8] needs to be considered. Therefore, the result of the present study is only valid for permanent molars – and perhaps premolars – and are not transferable to the primary dentition. Future sealant trials should take these important methodological parameters into consideration in order to reduce the risk of bias.

CONCLUSIONS

Due to the heterogeneity of the included study, the results should be interpreted with caution. Based on the limited data for etching times of 15 and 20 s, the present study could not draw a firm conclusion regarding the use of shortened acid etching times before the application of pit-and-fissure sealants. Based on the data from clinical sealant studies, which used 30 or 60 s of acid etching, neither of the etching times was found to be superior in the regression analysis. Finally, a minimum of 30 s of acid etching seems to be sufficient prior to fissure sealing to ensure adequate sealant retention. Considering the limited number of clinical studies that compared different etching times, there is an urgent need for well-conducted investigations on the influence of acid etching.

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Clinical relevance: This meta-analysis investigated the possible influence of the time of acid etching on sealant retention on basis of existing data from clinical studies which were identified due to a systematic search of the literature. With respect to the descriptive and explorative statistical analysis, enamel should be etched for at least 30 s before applying pit and fissure sealant.

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6. Paper II

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Does etching time affect the *in vitro* performance of a sealant material?

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This *in vitro* study evaluated the shear bond strength (SBS), failure mode and microleakage of fissure sealing materials in relation to different etching times on aprismatic and prismatic enamel. Ninety-six healthy human third molars were randomly allocated to the following groups: 1) prismatic or aprismatic enamel; 2) etching: 15, 30, 45, 60 s. After 5,000-fold thermocycling SBS, failure mode and microleakage were measured. Statistical evaluation included Mann-Whitney-*U*-test and linear regression analysis. In the aprismatic enamel group, an increasing etching time resulted in higher SBS. The linear regression model revealed that 60 s of etching time led to a significant increase in SBS. Microleakage was found to be low in all test groups. This study indicated that 60 s of etching time showed to a significantly better SBS. When considering the small differences of SBS, failure modes and microleakage between 30 and 60 s etching time, 30 s acid etching seems to also be justifiable.

Keywords: Pit and fissure sealing, Enamel pretreatment, Acid etching, Shear bond strength, Microleakage testing

INTRODUCTION

Sealing pits and fissures is an effective caries-preventive measure[1)] and is mostly indicated for caries-active children today. When considering the clinical workflow, it is evident that acid etching is an important pretreatment step to guarantee long-lasting bonding of the sealant material on the enamel. The shortening of acid conditioning has been discussed repeatedly since the introduction of pit and fissure sealants to simplify the treatment and reduce the chair time, particularly in children[2-4)]. It is surprising that to the best of our knowledge, only limited data from comparative clinical studies[5,6)] exist regarding the influence on longevity. Only one study was conducted, in which Duggal *et al.*[5)] found 40.0, 50.9, 41.8 and 43.6% intact sealants after 12 months when etching the enamel for 15, 30, 45 and 60 s, respectively. When comparing these data with longevity results from the overwhelming majority of clinical studies, retention rates of approximately 80% can be expected after two years when using a minimum etching time of 30 s[7)]. The documented results seem not to be fully plausible and should therefore be re-evaluated. Independent of clinical experience, few *in vitro* studies have investigated the influence of the length of acid conditioning on the *in vitro* performance of sealant materials, *e.g.*, bond strength or microleakage[8-10)]. With respect to the existing knowledge gaps, it seems difficult to reach final conclusions for daily dental practice. Therefore, this comparative *in vitro* study aimed to contribute to this discussion and assess the shear bond strength (SBS) and microleakage of a sealant material under the inclusion of different commonly recommended acid etching times, namely, 15, 30, 45, and 60 s with permanent tooth material. The null hypothesis of this study was that there would be no difference in SBS and microleakage performance between the different etching times and enamel preparation.

MATERIALS AND METHODS

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee.

Sample preparation

Ninety-six healthy, caries-free, extracted human third molars were used in this investigation for testing SBS. All teeth were free of any developmental disorders, fillings, and fissure sealants and showed complete root development. Sclerotic teeth from elderlies were excluded as well. After extraction, the teeth were stored in sodium azide solution (0.2%). Prior to use, the teeth were washed thoroughly under running water to eliminate the remaining traces of storage solution. The roots were sectioned 1 mm apically to the cementoenamel junction, and the crowns were further sectioned into 3 parts (mesial, buccal, lingual) with a diamond disc (Dental Diamond Disc, H 340-F-300, Horico, Berlin, Germany). The distal surfaces were excluded from the sample collection due to the findings from a previously published article[11)]. This process resulted in 288 tooth surfaces, which were randomly assigned to 8 study groups (n=36; 12 samples from the mesial, buccal, and lingual surfaces). Randomization was coded such that only one surface from each tooth was assigned to a group (Fig. 1). All samples were numbered according to the randomization table.

All tooth surfaces were embedded in cold-curing methyl methacrylate resin (Technovit 4004, Heraeus

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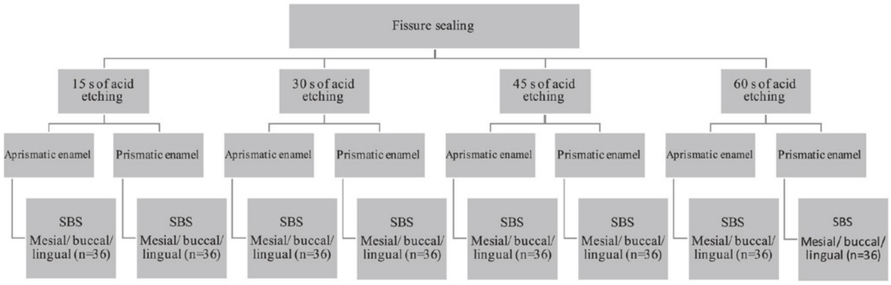

Fig. 1 An overview of the investigated sealant procedures and the applied methods.

Kulzer, Wehrheim, Germany) by means of a 15-hole mould (Ultradent Products, South Jordan, UT, USA). Each flat, superficial enamel surface was situated horizontally in the embedding material, producing cylinders measuring 1 inch in diameter by 1 inch in length. To ensure that the bottom surface of the specimen was parallel to the top surface, a mounted grinding mandrel (Ultradent Products) on a model trimmer's working table (HSS88, Wassermann, Hamburg, Germany) was used. After embedding, all tooth surfaces were carefully cleaned and rinsed with water spray. The SBS was compared between different etching times (15, 30, 45 and 60 s) on aprismatic (unground) and prismatic (ground) enamel.

Enamel preparation
In the group with prismatic enamel, standard surfaces were prepared as follows: To ensure that the ground enamel surface of the tooth was parallel to the bottom surface, a mounted grinding mandrel on a model trimmer's working table was used initially to produce a flat, parallel grinding area, which was sufficient to place a sealant button to obtain a standardized horizontal plane for SBS testing. Next, 120-grit silicon carbide abrasive paper (Leco, St. Joseph, MI, USA) under running water on a grinding machine (VP100, Leco) and 400-grit paper (Leco) was applied until the surface was even and smooth. In the aprismatic group, which simulated the real clinical situation of fissure sealing, the natural enamel surface of each specimen was not ground.

Placing the fissure sealant material
In vitro application of the sealant was strictly performed according to the manufacturer's clinical recommendations. The tooth surface was initially cleaned with a fluoride-free polishing paste (Zircate Prophy Paste, Dentsply De Trey, York, PA, USA) and rinsed with a water spray and dried with water- and oil-free air. After checking the cleanliness of each enamel surface under a stereomicroscope (Stemi SV11, Carl Zeiss, Jena, Germany), an etching procedure with 37% phosphoric acid gel (Total Etch, Lot W98952, Ivoclar Vivadent, Schaan, Liechtenstein) was performed for 15, 30, 45 or 60 s. The tooth surface was then rinsed with a water spray for 20 s and dried with pressurized air for 5 s until a chalky-white enamel surface was visible. The teeth were then inserted into the bonding clamp, and the cylindrical plastic mould (Button Mold Insert, ISO 29022, Ultradent Products) was adapted to achieve a gap-free fit on the enamel surface. The sealant (Helioseal F, Lot X08122, Ivoclar Vivadent) was then applied in two steps. Each layer (~2 mm) was light cured for 20 s with a light-curing unit (Bluephase Style, 1,200 mW/cm^2, wavelength 385 to 515 nm, Ivoclar Vivadent). To prevent uncontrolled leakage of the fissure sealant onto the enamel, a light-cured resin barrier (OpalDam, Ultradent Products) was applied around the perimeter of the bonding mould before applying the fissure sealant. After light curing, the resin barrier and bonding mould were carefully removed. The procedure rendered a sealant cylinder 2.37 mm in diameter perpendicular to the enamel surface, as required by ISO 29022:2013[12].

Sample ageing
The samples were aged according to our previous protocols: First, one-day storage in distilled water at 37°C in a thermal oven (Modell 400, Memmert, Schwabach, Germany) followed by thermocycling (Haake W15, Thermo Haake, Karlsruhe, Germany) between 5°C (±2°C) and 55°C (±2°C) for 5,000 cycles, with a dwell time of 30 s and a transfer time of 5 s.

Notched-edge SBS testing and failure mode analysis
The notched-edge (ISO standard) SBS test[12] was performed using a universal testing machine (MCE 2000ST, Quicktest Prüfpartner, Langenfeld, Germany). First, the samples were placed in a metal sample holder (Test Base Clamp, ISO 29022, Ultradent Products) with the occlusal surface facing down. A notched-edge shear fixture with a semicircular moulded shear blade (Notched-edge Shear Blade, ISO 29022, Ultradent Products) was mounted on the universal testing machine and placed over the sealant cylinder on the aligned specimen. The notched-edge shear blade had to be positioned exactly over the cylinder and force fitted without premature contact to ensure that the load was applied directly to

the cylinder. A constant crosshead speed of 0.5 mm/min was applied until the material failed. The maximum force (N) at failure was recorded. Considering the bonded area of the fissure sealant on the tooth surface, the SBS was calculated in MPa.

All samples were examined for failure modes under a stereomicroscope at 20-fold magnification. The failure mode was described as follows: 1. adhesive, 2. cohesive within the material, 3. mixed (adhesive and cohesive within the material), and 4. enamel failure[11].

Microleakage testing

Five human third molars were assigned to each of the eight groups. All teeth were stored and cleaned as previously described. Each tooth was taken as a whole, and fissure sealing on the prismless enamel of the occlusal fissure pattern was performed in strict accordance with the manufacturer's instructions (see above). For the group with prismatic enamel, the superficial enamel in the areas of the central groove and supplemental groove as well as the involved area of each cusp were removed by a flame-shaped finishing diamond bur (FG 5236, Intensive, Montagnola, Switzerland). All samples were stored in distilled water at 37°C for 24 h in a thermal oven and aged in a thermocycling bath as described above. After thermocycling, the root surfaces were isolated with tacky wax (Boxing Wax Sticks, Kerr, Romulus, MI, USA). Afterwards, the entire tooth surface was covered with two layers of nail varnish, except the area within 1 mm of the fissure seal. The varnish was applied to avoid dye penetration to other parts of the tooth. The samples were then immersed in 0.5% methylene blue solution (Methylene Blue Extra Pure, Merck, Darmstadt, Germany) for 24 h at 37°C. All samples were rinsed with water, and the roots were sectioned off 1 mm below the cementoenamel junction with a diamond disc. The tooth crowns were then fully embedded in cold-curing methyl methacrylate resin. This treatment resulted in a rectangular block of approximately 2.5×1.2×0.8 cm for each tooth. The blocks were fixed in a sectioning saw (Isomet Low Speed Saw, Buehler, Lake Bluff, IL, USA) with a diamond blade (Diamond Blade, Leco), and the crowns were sectioned in the buccolingual direction into at least 5 slices, each with a thickness of 1 mm. The front and back of each slice were inspected, resulting in at least 10 available section sides per tooth. The side analysis was performed using a stereomicroscope with a 20-fold magnification. Every side was photographed with a digital single lens reflex camera. The following picture analysis methodically separated all sides without dye penetration and then collected all section sides with dye penetration. Additionally, quality losses, such as defects in sealant materials, were recorded. If dye penetration was present, each side was quantitatively measured in relation to the total length of the interface between the enamel and the sealant. All measurements were performed with the imaging software ImageJ (version 1.52, Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). The percentage of microleakage was calculated. Microleakage was ruled out for dye penetration through enamel, dentine or fissure sealant cracks or along the cementoenamel junction.

Statistical analysis

Descriptive and explorative data analysis was performed using Stata/SE 14.2 (StataCorp 2015, College Station, TX, USA). Descriptive statistics for SBS and microleakage for each group were calculated. Pairwise comparisons of microleakage values between different etching times (*e.g.*, 15 s aprismatic enamel *vs.* 30 s aprismatic enamel, 15 s prismatic enamel *vs.* 30 s prismatic enamel, *etc.*) were performed using the Mann-Whitney *U* test. Linear regression analysis was performed to compare the results from SBS testing. The model included etching time (15, 30, 45 and 60 s), type of enamel preparation (aprismatic and prismatic) and tooth surface (mesial, buccal and lingual), individually. Although the SBS data were not normally distributed, linear regression was performed as the residuals were normally distributed. For the sake of consistency with the results from the linear regression, all descriptive values are presented as the mean and standard deviation or as percentages. A significance of α=0.05 (two-tailed test) and a 95% confidence level were used for all analyses.

RESULTS

The investigation of the SBS on aprismatic enamel revealed that longer acid etching tended to result in slightly higher fracture resistance, but the difference was not found to be significant (Table 1). When comparing the results for 15 and 60 s etching times, there was an increase from 14.0 to 16.1 MPa. In principle, the same finding was also registered for prismatic enamel. Here, a significant difference between 15 and 60 s as well as between 30 and 60 s acid etching times in the prismatic group was detected (Table 1). There was no significant difference between the tooth surfaces and the type of enamel preparation.

The results for the failure mode analysis (Table 2) revealed that adhesive failure was the most predominant type of failure among all groups, ranging from 75.0 to 94.4%, followed by mixed failures, ranging between 5.6 and 22.2%. Cohesive failures and enamel failures were rarely observed among the groups (Table 2). In general, no serious differences existed between the aprismatic enamel group and prismatic enamel group.

The results from the simple linear regression (Table 3) show that only etching time had a significant influence on the SBS, while the type of enamel preparation or tooth surface played an insignificant role. The estimate from the regression analysis resulted in 13.9 MPa for the reference group (15 s etching time), whereas a 60 s etching time had a positive effect by significantly increasing the SBS by 2.3 MPa. On the other hand, 30 or 45 s etching times did not have any influence on the SBS (Table 3).

The mean values for microleakage were found to be low throughout all groups (Table 4), and no significant difference was detected. However, it should be noted that

Table 1 SBS relative to the 15, 30, 45, and 60 s etching times, type of enamel preparation and tooth surface. Comparative statistical comparisons were made between all etching times in the groups with aprismatic and prismatic enamel, as well as between the corresponding etching times in aprismatic and prismatic enamel

SBS in MPa mean (SD)	Aprismatic enamel				Prismatic enamel			
	15 s	30 s	45 s	60 s	15 s	30 s	45 s	60 s
Tested tooth surface (*n*=36)	14.0 (5.6)	15.4 (6.1)	14.9 (5.0)	16.1 (5.4)	13.8 (4.7)[a]	13.5 (5.6)[b]	15.5 (5.7)	16.3 (5.9)[a,b]
Mesial (*n*=12)	14.8 (6.4)	14.7 (5.2)	14.8 (4.7)	15.3 (6.3)	13.5 (3.6)	11.9 (5.8)	15.4 (6.9)	16.9 (4.1)
Buccal (*n*=12)	12.9 (4.8)	13.1 (6.3)	15.7 (4.4)	18.0 (4.9)	15.2 (5.4)	14.4 (5.2)	15.8 (5.6)	17.1 (6.4)
Lingual (*n*=12)	14.2 (5.7)	18.5 (5.7)	14.0 (6.3)	15.0 (4.9)	12.8 (5.0)	14.1 (6.0)	15.2 (4.7)	14.9 (7.1)

[a] Pairwise comparison between 15 *vs.* 60 s acid etching within the prismatic enamel group was found to be statistically significant using a Mann-Whitney *U* test (*p*-value: 0.04).
[b] Pairwise comparison between 30 *vs.* 60 s acid etching within the prismatic enamel group was found to be statistically significant using a Mann-Whitney *U* test (*p*-value: 0.04).

Table 2 Failure mode analysis relative to the chosen type of enamel preparation and used etching times

Failure mode analysis N (%)	Aprismatic enamel				Prismatic enamel			
	15 s	30 s	45 s	60 s	15 s	30 s	45 s	60 s
Notched-edge method (*n*=36)	36 (100.0)	36 (100.0)	36 (100.0)	36 (100.0)	36 (100.0)	36 (100.0)	36 (100.0)	36 (100.0)
Adhesive failure	33 (91.7)	32 (88.9)	31 (86.1)	27 (75.0)	32 (88.9)	32 (88.9)	34 (94.4)	34 (94.4)
Cohesive failure	—	—	—	—	—	—	—	—
Mixed failure	3 (8.3)	3 (8.3)	5 (13.9)	8 (22.2)	4 (11.1)	4 (11.1)	2 (5.6)	2 (5.6)
Enamel failure	—	1 (2.8)	—	1 (2.8)	—	—	—	—

Table 3 The linear logistic regression analysis included etching time (15, 30, 45 and 60 s), type of enamel preparation (aprismatic and prismatic) and tooth surface (mesial, buccal and lingual), individually

Variable		Simple linear regression analysis	
		Estimate (95% CI) for the SBS in MPa	*p*-value
Etching time	15 s (reference)	13.90 (12.63; 15.18)	<0.001
	30 s	0.53 (−1.27; 2.34)	0.562
	45 s	1.26 (−0.55; 3.06)	0.171
	60 s	2.32 (0.51; 4.12)	0.012
Type of enamel preparation	Prismatic (reference)	14.76 (13.85; 15.67)	<0.001
	Aprismatic	0.32 (−0.96; 1.61)	0.619
Tooth surface	Mesial (reference)	14.65 (13.54; 15.76)	<0.001
	Buccal	0.64 (−0.93; 2.21)	0.423
	Lingual	0.20 (−1.38; 1.78)	0.804

*Taken as a reference in the simple linear regression analysis, indicating a significant influence at $p<0.001$

a longer etching time in aprismatic enamel contributed to a decrease in microleakage. In contrast, this trend was reversed in prismatic enamel (Table 4).

DISCUSSION

This comparative *in vitro* study investigated the influence of the etching time prior to fissure sealing in

Table 4 Microleakage of the tested sealants following 5,000 cycles of thermocycling

Microleakage	Aprismatic enamel				Prismatic enamel			
	15 s	30 s	45 s	60 s	15 s	30 s	45 s	60 s
Number of teeth (N)	5	5	5	5	5	5	5	5
Number of all available tooth slides with fissure sealants N (%)	52 (100.0)	49 (100.0)	50 (100.0)	51 (100.0)	51 (100.0)	49 (100.0)	55 (100.0)	53 (100.0)
Slices with no dye penetration N (%)	44 (84.6)	45 (91.8)	42 (84.0)	46 (90.2)	49 (96.1)	45 (91.8)	50 (90.9)	48 (90.6)
Slices with dye penetration along the sealant margin N (%)	3 (5.8)	—	1 (2.0)	2 (3.9)	—	2 (4.1)	3 (5.5)	3 (5.7)
Slices with dye penetration into the sealant material N (%)	—	2 (4.0)	2 (4.0)	1 (2.0)	1 (2.0)	2 (4.1)	2 (3.6)	2 (3.8)
Bubble, with dye penetration N (%)	1 (1.9)	—	2 (4.0)	—	1 (2.0)	—	—	—
Bubble, without dye penetration N (%)	4 (7.6)	2 (4.0)	3 (6.0)	2 (3.9)	—	—	—	—
Sides with any quality loss N (%)	8 (15.4)	4 (8.2)	8 (16.0)	5 (9.8)	2 (3.9)	4 (8.2)	5 (9.1)	5 (9.4)
Mean microleakage in % (SD)	3 (13.1)	0 (0)	1.3 (6.4)	0.6 (3.5)	0 (0)	0.7 (3.2)	2 (9.4)	2.6 (11.2)
Minimum	0	0	0	0	0	0	0	0
Maximum	72.8	0	33.6	23.5	0	17.8	61.4	60.6

Microleakage (in%) was compared within aprismatic and prismatic enamel groups individually based on different etching times (*e.g.*, 15 s on aprismatic enamel *vs.* 30 s on aprismatic enamel, 15 s on prismatic enamel *vs.* 30 s on prismatic enamel, and so on), but none of the comparisons showed a statistically significant difference.

terms of SBS, failure mode and microleakage. First, when considering the mean values of SBS in relation to the etching time, it must be noted that the differences were found to be small throughout all test groups for aprismatic and prismatic enamel simultaneously. Second, a slight, mostly insignificant increase in SBS was observed for groups with longer etching times. Pairwise comparative statistical analyses (Mann-Whitney *U* tests) also revealed that a significant increase in SBS was registered between 15 s/ 60 s and 30 s/ 60 s etching times when samples of prismatic enamel were used; all other systematically performed comparisons remained insignificant. Next, when considering the available SBS data between aprismatic and prismatic enamel, it can be concluded that there were no differences between those groups under the same etching time in the use laboratory set-up. This fact describes the conflict between the situation in clinical practice (aprismatic enamel) and compliance with the latest recommendations for laboratory testing[12] (prismatic enamel), thereby justifying the inclusion of both conditions in one study.

With respect to the results from the linear regression model —which aimed to consider all available variables in one statistical model to describe the possible influence on SBS— it was found that only the parameter of 60 s etching time was statistically significant. All other variables, *e.g.*, 15, 30 and 45 s etching times, type of enamel preparation, and tooth surfaces remained insignificant. These explorative statistical data are basically in line with the previously discussed descriptive data and led to the conclusion that the initially formulated hypothesis —that there is no difference between the different etching times before fissure sealing in terms of SBS— has to be rejected. This finding is, however, basically in line with earlier studies and needs to be further discussed. Here, the study by Holtan *et al.*[13] supports our findings for prismatic enamel, showing that compared with 15 s etching time, an etching time of 60 s significantly improved the SBS. On the other hand, the results of the present study do not fully concur with findings from other previously published publications[14-16], where it was documented that compared with a shorter acid etching time (15 s[14,15], 20 s[16]) on prismatic enamel, a longer etching time (60 s[14-16]) produced a non-significant but still higher SBS. In the case of aprismatic enamel, only one study by Tandon *et al.*[9] investigated 15, 30, 60 and 120 s etching times in relation to SBS and observed an increasing SBS with increasing etching time (which was not statistically investigated). When considering the information from available *in vitro* studies, it might be concluded that longer acid etching times improved the SBS in comparison to that with shorter acid etching times on prismatic enamel, independently of the preparation of the enamel. With respect to the low numbers of samples in each group (n=30[13], n=10[14], n=10[15], n=10[16], n=6[9]), the results from simple (pairwise) statistical comparisons could explain (non-) significant differences and should therefore not be overrated.

When interpreting the results from the failure mode analysis, it can be pointed out that the data are also in line with the previous investigations[11,17-20]. In general, adhesive failures are the most common, and cohesive and mixed failures are rare (Table 2) which is basically in line to previous investigations[11,17-20]. Interestingly, there was a minor trend of more mixed failures when etching aprismatic enamel for 60 s. On the one hand, this finding may indicate an increase in adhesive performance; on the other hand, the results should not be overrated. In the case of prismatic enamel, a reversed trend was observed.

The dye penetration test/microleakage seems to be the preferable method to evaluate material variants in the laboratory and to gain safety before clinical trials begin[21]. While there were many studies assessing microleakage of pit and fissure sealants[22-24], to our knowledge no study was available regarding the microleakage of sealants in relation to different etching times. When analyzing the present data, it can be concluded that the mean values obtained were generally low, which is also consistent with most recently published data on resin-based fissure sealants[25]. Nevertheless, the present investigation recorded another non-significant trend in aprismatic enamel: In our study, it was observed that 60 s etching resulted in a lower dye penetration compared to that with 15 s etching (0.6% *vs.* 3.0%). Again, in the case of prismatic enamel, an opposite trend was observed. The descending performance of microleakage in prismatic enamel might be a sign of over-etching in the case of the 60 s etching time[10,26,27].

The methodological strengths of the present study should be seen in the usage of an equal number of 36 samples in each group, which was randomly allocated to avoid potential sampling bias. To simulate the clinical situation of fissure sealing, we used aprismatic enamel in addition to prismatic enamel because the ground prismatic surface would create a standardized, flat enamel surface[12], which is ideal for bonding but does not reflect the clinical situation. In this present study, the distal surfaces from tooth samples were not included due to the findings from a previous study, in which it was revealed that distal surfaces significantly influenced the SBS[11]. All tested groups were subjected to 5,000 cycles of thermocycling as an alteration process, which was recommended as a standard ageing method[11]. Due to the simulation of the effect of thermal stress, which is usually encountered in the oral cavity, thermocycling might provide a long-term perspective on the longevity of the investigated sealant material. Furthermore, the notch-edge shearing technique was used in this study. This is a force-fitting technique and was recommended as a standardized method according to the International Organization for Standardization[12]. A limitation of this study might be the analysis of the SBS alone without considering the (micro)tensile bond strength test or other mechanical test methods[28,29] and that we did not (re)investigate morphological changes on enamel after acid etching[10,30]. Another limitation would be that all microleakage tests were performed on prismatic enamel only. This was justified due to the clinical relevance and that the placement of sealants on aprismatic enamel is commonly not indicated.

CONCLUSION

When considering the SBS results from this *in vitro* study, it should be noted that only small differences between all tested groups existed. However, the descriptive data indicate that an increasing time of acid etching resulted in slightly higher SBS, and the linear regression analysis also revealed a significant advantage of 60 s acid etching. Furthermore, the trend of more mixed fracture failures and less microleakage on aprismatic enamel support the recommendation of 60 s acid etching before sealant application. Otherwise, when emphasizing the small test differences between 30 and 60 s acid etching, it can be argued that an application time of 30 s may provide acceptable clinical results. With respect to the limited clinical data[5], more research seems to be needed in the future.

CONFLICTS OF INTEREST

All authors have no conflict of interest to report.

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