Al-Azhar Journal of Dentistry

Manuscript 1580

Restorative Dentistry Issue (Removable Prosthodontics, Fixed Prosthodontics, Endodontics, Dental Biomaterials, Operative Dentistry)

Effect of Self-Assembling Peptide P11-4 on Bonded Resin-Dentin Interface

Nafesa M. Sakr

Wael E. Jamil

Asmaa A. Mosleh

ORIGINAL STUDY

Effect of Self-assembling Peptide P_{11}-4 on Bonded Resin−dentin Interface

Nafesa M. Sakr*, Wael E. Jamil, Asmaa A. Mosleh

Operative Dentistry, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt

Abstract

Purpose: This study was designed to evaluate the influence of self-assembling peptide P_{11}-4 on microtensile bond strength (μTBS) and nanoleakage of bonded risen−dentin interface. **Patients and methods:** Fifty-six human molars were used. Dentin specimens were prepared through enamel removal, and half of them were subjected to artificial caries production protocol. Specimens were classified into two groups as follows (28 each): (A1) sound dentin (SD) and (A2) caries-affected dentin (CaD). Each group was subdivided into two subgroups as follows (each = 14): (P1) samples with peptide P_{11}-4 treatment (Curodont Repair) and (P2) samples without P_{11}-4 treatment. Then, each subgroup was subdivided into two divisions ($n = 7$) according to the assessment time: (T1) 24 h and (T2) 3 months. Samples were stored in distilled water at 37 °C. Five samples from each division were used for μTBS assessment and two samples for nanoleakage evaluation. **Results:** It revealed that the P_{11}-4 significantly increased the μTBS of sound dentin at 3 months and caries-affected dentin at both 24 h and 3 months. P_{11}-4 improved significantly the μTBS of caries-affected dentin up to the level of those recorded for sound dentin. Meanwhile, there was a significant reduction in μTBS of untreated caries-affected dentin after 3 months. **Conclusion:** Biomimetic remineralization using P_{11}-4 is believed to be a bright method for enhancing μTBS of caries-affected dentin and increasing adhesive restoration longevity.

Keywords: Biomimetic remineralization, Bond durability, Peptide P_{11}-4

1. Introduction

Dental hard tissues demineralize and remineralize as a result of dental caries; which is a sugar-driven biofilm-mediated complex dynamic illness. Due to diminished vascularization as well as regenerating cells, these tissues have a low capacity for regeneration [1]. Restorations are essential to maintain the functionality of the tooth after caries has destroyed enough dental tissues [2]. Continuing advances in adhesive dentistry are leading to practice changes in contemporary restorative dentistry. In modern dentistry practice, the demand for aesthetic tooth-colored restorations and adhesive procedures has become daily work [3,4].

Modern adhesives are unable to entirely infiltrate the collagen network with resin monomers. As a result, water-rich gaps form within the exposed collagen fibers, which cause nanoleakage and micropermeability of the hybrid layer. Then enzymatic breakdown of the partially exposed collagen fibril is caused by endogenous matrix metalloproinas (MMPs) and cysteine cathepsins [5]. When the substrate is caries-affected dentin, the penetration of monomers is significantly more challenging. Due to the preceding partial demineralization, caries-affected dentin is more vulnerable to acid etching, which creates a partially infiltrated thicker hybrid layer [6]. The long-lasting adhesion is deteriorated by collagenolytic activity, bacterial acids, resin component hydrolysis, mechanical stresses, and temperature [7].

Most recent research focus on dealing with this problem using several strategies. These strategies include anti-enzymatic and collagen modification strategies. The anti-enzymatic techniques can be applied either as pretreatments or as useful elements in dental adhesives. The collagen modification can be achieved by formulating polymers that are resistant to degradation. Understanding that these tactics only address one aspect of a complex issue is crucial. Generally, better outcomes could be achieved by these techniques. However, if the organic contents are

Received 1 November 2022; accepted 16 January 2023.
Available online 12 January 2024

* Corresponding author. Al-Azhar University, Girls-Branch, Faculty of Dental Medicine for Girls, Nasr City, Egypt.
E-mail address: novasakr2@gmail.com (N.M. Sakr).

https://doi.org/10.58675/2974-4164.1580

still there, enzymatic or hydrolytic breakdown will unavoidably take place. Consequently, a different strategy may be required [8].

MMP and cathepsin activities have been inhibited by ethylene diamin tetraacetic acid (EDTA), chlorhexidine, ethanol-wet bonding, moderate self-etching adhesives, or collagen cross-linkers, which in turn stabilize the bonded interface [9]. Using remineralizing agents is part of a creative strategy aiming to substitute nanometric-sized apatite crystals for water in the intrafibrillar and interfibrillar collagen gaps. These apatite crystals strengthen the resin−dentin bond and restore the structural integrity of dentin that has been affected by caries [10]. It is also suggested that this approach might prevent proteolytic enzymes from doing their role as MMPs as well as cathepsins mobility and access to collagen could be hindered by the formed apatite crystals [11]. Degradative moieties might also be prevented from acting by attracting calcium ions to exposed collagen spots [10].

A synthetic peptide with 11 amino acids (CH3CO-QQRFEWEFEQQ-NH3), self-assembling peptide P_{11}-4, is a member of the β-sheet-forming peptide family. In response to environmental signals, it assembles into hierarchical structures creating a three-dimensional scaffold [12]. Due to the presence of anionic groups in the side chains of peptide P_{11}-4, the nucleation of hydroxyapatite is facilitated by the prefabricated three-dimensional scaffold. These anionic groups help calcium phosphate precipitate in a hierarchical pattern onto the prepared three-dimensional scaffold [11]. The peptide could regulate the formation and the development of apatite crystals on the surface of the enamel [13,14]. Remineralization of dentin is believed to be a function of peptide P_{11}-4. Moreover, it may recognize and attach to collagen fibers because it contains amino acids that are similar to those in collagen fibers.

This binding makes collagen fibers thicker, more elastic, and more resistant to breakdown [15]. There have been earlier descriptions of the activity as well as functionality of peptide P_{11}-4 in dentin in published studies [2,16]. So, the purpose of this study was to assess the influence of self-assembling peptide P_{11}-4 on resin−dentin interface with either sound or caries-affected dentin. The null hypothesis of the study was that peptide P_{11}-4 causes no difference in resin−dentin interface integrity.

2. Materials and methods

2.1. Trial design and sample size calculation

To study the effect of P_{11}-4 on the bonding interface after 24 h and 3 months, a power analysis was designed to have sufficient power to conduct a two-sided statistical test of the research hypothesis that there is no effect for peptide P_{11}-4 on resin−dentin interface. According to research by de Sousa et al. (2019) [2], a total sample size of 40 (5 in each group) was adequate to detect an effect size of 0.75 a power (1-β error) of 0.8, using a two-sided hypothesis test, significance level (α error) of 0.05 for data. Two samples were added to each group for the evaluation of nanoleakage. G* P software version 3.1.9.7 (University of Düsseldorf, Düsseldorf, Germany) [17] was used to calculate the sample size.

2.2. Selection of teeth

Fifty-six sound molars were used in this study. Molars were extracted from patients due to periodontal diseases. After the approval of the Ethics Committee of the Faculty of Oral and Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt (approval code: REC-OP-22-03), a written informed consent was signed by each patient about using their teeth. Teeth were cleaned by a scalpel and low-speed handpiece with pumice paste to remove the attached soft tissues and the remaining debris. Teeth were examined to exclude those with defects, caries, or cracks, and then they were placed in distilled water, which is kept refrigerated for a month till use [18]. Forty teeth were used for the assessment of μTBS and 16 were used for the evaluation of nanoleakage.

2.3. Preparation of the samples

The selected teeth were mounted in a Teflon mold (15 mm diameter and 40-mm height). Enamel trimming from the occlusal surface was done 1.0 mm below the dentinoenamel junction (DEJ) using a diamond disk in a cutting machine. Then surfaces of flat dentin were ground with a 600-grit silicon carbide sandpaper [19].

2.4. Caries-affected dentin production protocol

Half of the samples ($n = 28$) were immersed in 5 Ml of 6 % carboxymethyl cellulose acid gel (0.1 M lactic acid titrated to pH 5.0 in a KOH solution) at 37 °C. For 48 h, samples were left in the gel without being replaced [20].

2.5. Sample grouping

According to dentin substrate (A), samples were classified into two main groups ($n = 28$); A1: sound dentin (SD) and A2: Caries-affected dentin (CaD). According to treatment with peptide P_{11}-4 (P), each

group was subdivided into two subgroups ($n = 14$) and P1: treated with P_{11}-4 and P2: untreated with P_{11}-4. Therefore, samples were apportioned into four subgroups ($n = 14$): [(SD + P_{11}-4), (SD), (CaD + P_{11}-4), and (CaD)]. Each subgroup was subdivided into two divisions ($n = 7$) according to the assessment time (T): T1: 24 h and T2: 3 months. Five specimens from each division were used for the assessment of μTBS and two teeth for nanoleakage evaluation.

2.6. Application of self-assembling peptide P_{11}-4

According to manufacturer's instructions, peptide P_{11}-4 in the form of Lyophilized Curodont ™ Repair (Credentis AG, Dorfstrasse, Windisch, Switzerland) was activated by water by mixing the two prepacked cylinders of the Curodont applicator. The activated applicator was then applied to the dentin surface for 5 min. Then, a super-saturated solution of Ca^{2+} and PO_4^{3-} was applied for 1 min. The surplus solution was absorbed by an absorbent paper [2,10,16,20].

2.7. Bonding procedure and build-up of composite

Bonding for all dentin surfaces was done using Single Bond Universal Adhesive (3 M Deutschland GmbH 41453 Neuss Germany) applied in the self-etch technique [21]. The adhesive was applied with a rubbing motion for 20 s with a disposable applicator according to the manufacturer's instructions. The surplus solvent was eliminated by gentle air drying for 5 s until the adhesive film did not move and maintained a consistent glossy look. LED light curing unit (Woodpecker light cure unit LED-F (LK-G38), 1200–1500 mW\cm^2, 420–480 nm, China) was used for adhesive curing for 10 s according to the manufacturer's instructions.

A nanohybrid resin composite [Filtek Z350 XT, A3 (3 M ESPE, St. Paul, MN, USA)] was used for composite block build-up (4 mm height) on dentin surface using the incremental technique (1 mm for each increment). Composite build-up was done using a specially constructed split Teflon mold. The composite applicator was used for the manual packing of the composite, and the thickness was assured using a graduated probe. Curing of each composite increment was done using an LED light curing unit for 20 s, and then finally polymerized for 60 s [20]. Then specimens were incubated in distilled water at 37 °C until the assessment time.

2.8. Preparation of beams for testing μTBS

By specially designed L-shaped gripping attachment (jig), acrylic blocks with the mounted specimens were kept firm in place parallel to the direction of sectioning. Then, a 0.3 mm thick diamond-coated disk mounted into the diamond saw machine (Isomet 4000, Buehler Ltd., Lake Bluff, IL, USA) was used for sectioning the bonded specimens under a copious water coolant. Resin–dentin beams with 1 mm^2 cross-sectional area were obtained by sectioning in a buccolingual direction followed by sectioning in a mesiodistal direction. Cutting in a horizontal direction at the cementoenamel junction level was finally done for beam separation. The central beams of each specimen were selected. Their dimensions were checked using a digital caliper [19].

2.9. Microtensile bond strength (μTBS) measurement

Beams were individually fixed and glued to a specially designed apparatus using cyanoacrylate-based glue (Akfix 705 Universal Fast Adhesive). Then, the apparatus was gripped to the universal testing machine (Instron model 3345) (Fig. 1). Tensile forces were applied to the composite dentin attachment line at a crosshead speed of 1 mm/min until failure occurred. Data were calculated and recorded using computer software (Bluehill Universal Instron, England). Values of μTBS were scored in MPa [2].

2.10. Ultramorphological analysis and nanoleakage evaluation

Two coats of nail polish were applied to the bonded specimens, leaving just 1 mm of the resin–tooth interface exposed. The specimens were submerged in 50 % (wt%) ammoniacal silver nitrates for 24 h in complete darkness [22]. Then, to reduce the amount of silver ions that had seeped into the metallic silver grains, they were rinsed under running tap water for 5 min before being exposed to a photo-developing solution for 8 h [23]. To lessen the superficial silver adsorption, the specimens were then carefully polished with 1200-grit silicon carbide paper and put in an ultrasonic cleaner for 5 min. The specimens were examined using a 1000 X scanning electron microscope (SEM) equipped with an energy dispersive X-rays (EDX) unit (Model Quanta 250 FEG, FEI Company, The Netherlands) [24]. Images of the most representative area of each specimen were then taken. Differences in silver deposition and penetration were observed [19].

2.11. Evaluation of failure mode

After μTBS testing, samples were taken out of the jig. They were examined using a stereomicroscope

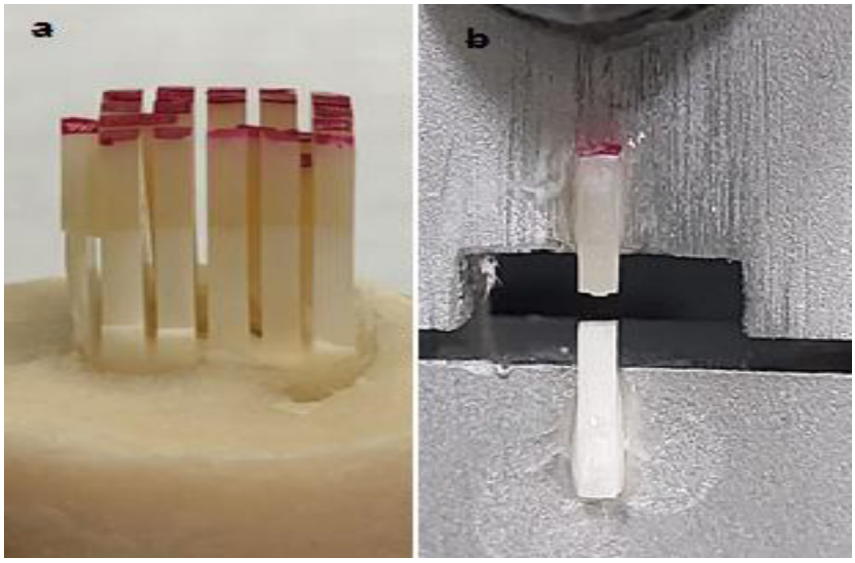

Fig. 1. (a). Longitudinal view of the prepared beams, (b) beam failure after the application of tensile forces using the universal testing machine.

(MN 100 Nikon, Japan with OmniMet image analysis software). Failure modes were evaluated and divided into three categories: cohesive, mixed, and adhesive failure. Each fracture type frequency was expressed as a percentage [25].

2.12. Statistical analysis

All quantitative data were presented as mean and standard deviation. Statistical analysis was performed using SPSS 16 (Statistical Package for the Social Sciences), GraphPad Prism and Windows Excel. Exploration of the given quantitative data was performed using the Shapiro–Wilk test and Kolmogorov–Smirnov test for normality which revealed that the significant level (P-value) was insignificant as P-value greater than 0.05, which indicated that all data originated from a normal distribution (parametric data) resembling the normal bell curve. Paired t-test was used to compare different intervals, while independent test was used to compare different groups.

3. Results

3.1. Results of microtensile bond strength (μTBS)

2The results showed that the highest mean μTBS value was recorded with the SD + P_{11}-4 group at 24 h, while the lowest mean μTBS value was recorded for untreated CaD at 3 months. Regarding the presence or absence of P_{11}-4 (P and P2), in the sound dentin group (A1), the difference in mean μTBS between P1 (with P_{11}-4 treatment) and P2 (without P_{11}-4 treatment) was insignificant at T1 (24 h) and significant at T2 (3 months), while in caries affected dentin group (A2), the difference in mean μTBS between P1 and P2 was significant at either T1 or T2.

Regarding different time intervals (24 h and 3 months) (T1 and T2) in each separate group showed that the difference in mean μTBS of all groups either treated or not treated with P_{11}-4 was insignificant between T1 and T2 except in the caries-affected dentin group without P_{11}-4 treatment, the difference in mean μTBS was significant. The interaction of variables used in the present study and the differences in mean μTBS (MPa) are shown in Table 1.

3.2. Results of evaluation of nanoleakage using SEM

The observation of the photomicrographs resulting from backscattered electron mode at a magnification of 1000X revealed that nanoleakage was manifested in all specimens. Silver penetration showed different patterns and different degrees. Groups treated with P_{11}-4 either bonded to sound or the caries-affected dentin displayed less silver nitrate infiltration along the interface compared with untreated groups at 24 h and 3 months. The untreated groups of dentin affected by caries displayed the highest levels of silver nitrate infiltration. After 3 months, it appeared that the hybrid layer had broken down (Fig. 2).

3.3. Failure mode

The percentage of different failure modes as determined by observation under a stereomicroscope for each separate group at different time

Table 1. Mean and standard deviation of microtensile bond strength of A1; (sound dentin) and A2 (caries-affected dentin) regarding the presence or absence of treatment (P1 and P2) at different time intervals (T1 and T2).

Dentin substrate	Treatment with P_{11}-4	T1 (after 24 h)	T2 (after 3 months)	Difference (paired t-test)				
				MD	SD	95 % C		*P* value
		M±SD	M±SD			L	U	
A1 (sound dentin)	P1 (with P_{11}-4 treatment)	35.8 ± 8.88	33.59 ± 8.16	−2.2	1.63	−8.1	3.6	0.43
	P2 (without treatment)	33.42 ± 10.85	28.34 ± 6.45	−5.1	12.01	−11.7	1.61	0.12
	P value (independent t-test)	0.42	0.01*					
A2 (caries-affected dentin)	*P*1 (with P_{11}-4 treatment)	33.24 ± 9.82	26.98 ± 6.85	−6.25	13.2	−13.5	1.08	0.06
	*P*2 (without treatment)	25.88 ± 7.4	19.31 ± 8.97	−6.51	11.39	−12.8	−0.26	0.04*
	P value (independent t-test)	0.001*	0.001*					

M, mean; MD, mean difference; P, probability level which is significant at $P \leq 0.05$; SD, standard deviation.
* Denote that the *P* value is statistically significant.

intervals is shown in Fig. 3. The percentage of different failure modes showed that adhesive failure was elevated with an increase in storage time without P_{11}-4 treatment of both sound and caries-affected dentin.

4. Discussion

The generated interdiffusion layer (hybrid layer), produced using the modern dentin bonding technologies is generally agreed to degenerate with time as a result of collagenolytic and hydrolytic

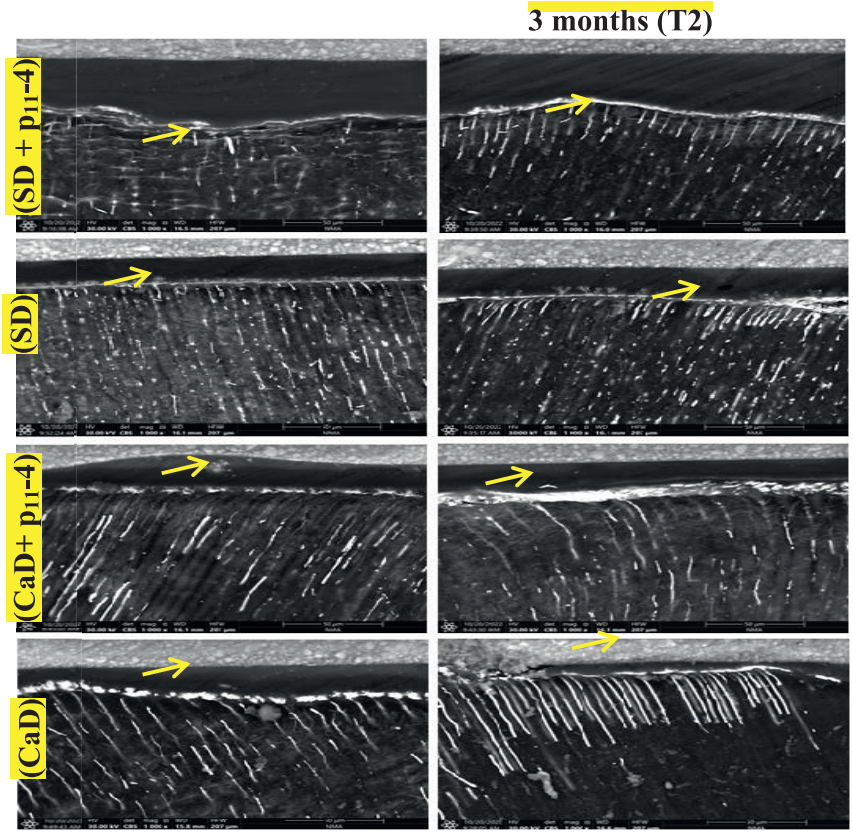

Fig. 2. SEM micrographs of nanoleakage at bonding interfaces for all experimental groups after 24 h and 3 months. Yellow arrows indicate silver deposits at the resin–dentin interfaces. SD: sound dentin, CaD: caries-affected dentin.

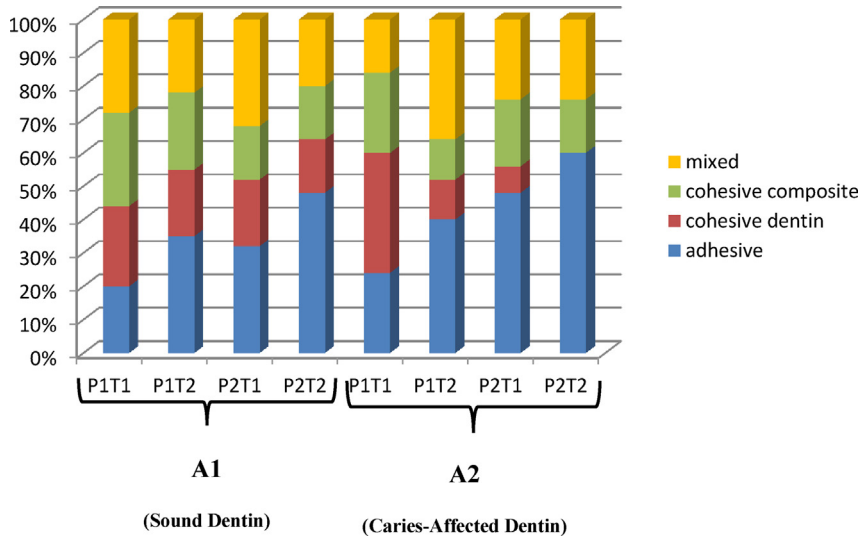

Fig. 3. Bar chart shows the percentage of failure modes in each group at different time intervals.

breakdown. Enhancing the stability of the dentin–resin interface, in particular with demineralized dentin, is one of the biggest issues in restorative dentistry [10,26]. The preservation of collagen fiber integrity in the hybrid layer is crucial for effective and long-lasting bonding. Several solutions have been suggested for this purpose and used separately or immediately combined to bond system elements (etchant, primer, or bonding) [27].

This study was designed to assess the influence of P_{11}-4 on bonded resin–dentin interface integrity. Because there was a substantial interaction between peptide P_{11}-4 pretreatment and material bonding, the null hypothesis was not accepted. The peptide P_{11}-4 is a recent method for the creation of complex molecular synthetic structures. Peptide P_{11}-4 can be used in tooth remineralization as scaffolds or templates. The scaffolds can assist calcium–phosphate in situ nucleation [28].

Fifty-six molars were used. Specimens prepared from human molars are more beneficial for testing the study hypothesis in a more clinically relevant substrate [29]. To replicate and standardize the circumstances present in naturally caries-affected dentin, 6 % carboxymethyl cellulose acid gel containing lactic acid was used to demineralize the sound dentin specimens [20]. It was established that this technique was successful in degrading the dentin's μTBS values [16]. It is believed that aging in distilled water could diminish bond strength in values comparable to aging in artificial saliva [30]. So, storage in distilled water was designated.

In this study, microtensile bond strength (μTBS) assessment of the samples for both sound and caries-affected dentin was done after 24 h and 3 months. The results of the mean of μTBS in all groups showed that pretreatment of the dentin substrate with peptide P_{11}-4 previous to the bonding procedure with universal single bond adhesive in one-step self-etch mode significantly affects the delayed μTBS of sound dentin and on immediate and delayed μTBS of caries-affected dentin. The reduction in the mean μTBS value of caries-affected dentin without P_{11}-4 treatment after 3 months of storage is statistically significant.

The effect of peptide P_{11}-4 on microtensile bond strength can be explained as follows: the immediate influence of P_{11}-4 on caries-affected dentin may be due to its ability to interact with collagen. P_{11}-4 enhances collagen fibril width and binds to it in a proportional binding way, according to several in vitro studies. The collagen-arranged structure stability might benefit from such biomodification [2]. The created fiber scaffold then binds Ca^{2+} and Po_4^{3-} that are present in dentin and strengthens its structure reducing hybrid layer deterioration. This reinforcement explains the delayed impact of P_{11}-4 on sound and dentin that has been affected by caries [5,28].

Comparing SEM images of different groups, a nanoleakage pattern was observed in all groups. This may be because the self-etch adhesive technique removes the smear layer partially, thus the presence of water in the smear layer may lead to incomplete polymerization of the adhesive followed by hydrolysis of unpolymerized monomers [19]. In untreated groups, the pattern of nanoleakage revealed a denser silver nitrate deposition along the resin–dentin interface. Moreover, caries-affected dentin groups presented the highest silver nitrate infiltration after 3 months. This may be attributable

to that the effects of P_{11}-4 on the width and strength of collagen fibers are absent.

Therefore, spaces in the hybrid layer and degradation of collagen fibers were further increased. Also, comparing the percentage of failure mode for different groups showed that adhesive failure was increased in untreated groups. The results of nanoleakage and failure mode support the results of the μTBS assessment. These results are consistent with the studies that evaluated the influence of peptide P_{11}-4 on collagen fiber thickness and resin bonding to dentin affected by caries. The studies stated that peptide P_{11}-4 can increase the stability of the hybrid layer, which may be due to the interaction with type I collagen, which in turn increases the collagen fibers' resistance to proteolysis [2,10,20].

Moreover, another study evaluated the influence of P_{11}-4 on the durability of adhesive restorations. They concluded that treatment with P_{11}-4 caused an immediate negative influence on bond strength and a delayed positive influence on bond strength. The immediate opposite results in comparison to the results of our study may be due to the use of an adhesive system in total-etch mode or due to using cavities not flat dentin surfaces [31].

Also, the results of this study are in disagreement with the study that compared the effect of P_{11}-4 and nano-hydroxyapatite compared with the effect of P_{11}-4 and nano-hydroxyapatite on bonded interface integrity. They found that P_{11}-4 does not affect bond strength and cause increase in adhesive failure. The contrary results may be attributed to the repeated application of P_{11}-4 and the delayed adhesive procedure after 7 days of storage [32].

4.1. Conclusion

Within the confines of this study, biomimetic remineralization of dentin surface with self-assembling peptide P_{11}-4 before the bonding procedure is a promising method for improving immediate bonding to caries-affected dentin, and hence it may aid in the improvement of adhesive restoration longevity.

4.2. Recommendation

Other studies are needed to evaluate the effect of P_{11}-4 on bond durability under clinical conditions.

Funding

This research didn't received any grant from funding agencies in the public, commercial, or not from profit sector.

Conflicts of interest

The authors affirm that they have no conflict of interest.

Acknowledgments

Many thanks to Prof. Dr. Badr Awad Elsayed; Professor of Inorganic Chemistry and Dr. Mohamed Sultan, Lecturer of Inorganic Chemistry, Chemistry Department, Faculty of Science, Al-Azhar University, Cairo (Boys) for their valuable participation.

References

[1] Pitts N, Zero D, Marsh P, Ekstrand K, Weintraub J, Ramos-Gomez F, et al. Dental caries. Nat Rev Dis Prim 2017;3:1–16.
[2] de Sousa J, Carvalho G, Martins L, Torquato R, Mugnol K, Nascimento F, et al. The self-assembling peptide P11-4 prevents collagen proteolysis in dentin. J Dent Res 2019;98:347–54.
[3] Bhalla V, Srivastava S. Dentin adhesion: why does it fail and methods to prevent bond degradation: an overview. IJDSIR 2019;2:259–65.
[4] Josic U, Maravica T, Mazzitelli C, Radovic I, Jacimovic J, Bianco F, et al. Is clinical behavior of composite restorations placed in non-carious cervical lesions influenced by the application mode of universal adhesives? A systematic review and meta-analysis. J Dent Mater 2021;37:503–21.
[5] Fronza B, Braga R. The use of bioactive particles and biomimetic analogues for increasing the longevity of resin-dentin interfaces: a literature review. J Dent Mater 2020;39:62–8.
[6] Costa A, Godoy F, Sobrinho L, Naves L, Raposo L, Carvalho G, et al. Influence of different dentin substrate (caries-affected, caries-infected, sound) on long-term μTBS. Braz Dent J 2017;28:16–23.
[7] Breschi L, Maravic T, Cunha SR, Comba A, Cadenaro M, Tjaderhane L, et al. Dentin bonding systems: from dentin collagen structure to bond preservation and clinical applications. J Dent Mater 2018;34:78–96.
[8] Delgado A, Da Costa M, Polido M, Azul1 A, Sauro S. Collagen-depletion strategies in dentin as alternatives to the hybrid layer concept and their effect on bond strength: a systematic review. J SciRep 2022;12:13028–40.
[9] Lobo C, Tanomaru JM, Filho M, de Souza B, Carmona J. Non-collagenous dentin protein binding sites control mineral formation during the biomineralisation process in radicular dentin. Materials 2020;13:1053–70.
[10] Moreira K, Bertassonib L, Daviesc R, Joiad F, Hoflingd J, Nascimentoe F, et al. Impact of biomineralization on resin/biomineralized dentin bond longevity in aminimally invasive approach: an 'in vitro'18-month follow-up. J Dent Mater 2021;3728:1–14.
[11] Padovano JD, Ravindran S, Snee PT, Ramachandran A, Russo AK, George A. DMP1-derived peptides promote remineralization of human dentin. J Dent Res 2015;94:608–14.
[12] Dawasaz A, Togoo R, Mahmood Z, Azlina A, Ponnuraj K. Effectiveness of self-assembling peptide (P11-4) in dental hard tissue conditions: a comprehensive review. J Polymers 2022;14:792–807.
[13] K, Abrams S, Kirkham J, Amaechi B. Remineralization of natural early caries lesions in vitro by P11-4 monitored with photothermal radiometry and luminescence. J Investig Clin Dent 2017;8:1–9.
[14] Of early caries. Clin Oral Invest 2020;24:123–32.
[15] Sekiya A, Kosugi H, Yamazaki C, Koide T. Pigment epithelium- derived factor (PEDF) shares binding sites in collagen

with heparin/heparan sulfate proteoglycans. J Biol Chem 2011;286:26364–74.
[16] Martins L, de Sousa J, de Castilho A, Rontani J, Davies R, Rontani M. Enhancing bond strength on demineralized dentin by pretreatment with selective remineralizing agents. J Mech Behav Biomed Mater 2018;81:214–21.
[17] Flexible statistical power analysis program for the social, behavioral and biomedical sciences. Behav Res Methods 2007;39:175–91.
[18] El Motayam K, Walid F, Fouad W, Randa Y. Assessment and comparison of nanoleakage and resin tag length of three different pit and fissure sealants: an In-vitro scanning electron microscope study. J Am Sci 2013;9:329–37.
[19] Tawfic H, Niazy M, El-Sharkawy D. The effect of Zn-doped adhesive alone or combined with calcium phosphate nanoparticles on the integrity of the bonded resin- dentin interface. ADJ-for Girls 2017;4(289):96.
[20] Martins L, Sousa J, Alves L, Davies R, Rontanti R. Biomimetic mineralizing agents recover the micro tensile bond strength of demineralized dentin. J Mater 2018;11:1733–47.
[21] Nour-Eldeen H, Noaman K, Wakwak M. Nanoleakage evaluation of nano-filled resin composite using different adhesive systems at different dentin depth (an in vitro study). AJDS 2021;24:33–40.
[22] Tay F, King N, Chan K, Pashiey D. How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? J Adhesive Dent 2002;4:255–69.
[23] El-Keredya D, Etmanb W, Salamab M. Nanoleakage of different composite restoration systems. Tanta Dent J 2020; 17:97–105.
[24] El-Gezawi M, Haridy R, Abo Elazm E, Al-Harbi F, Zouch M, Kaisarly M. Microtensile bond strength, 4-point bending and nanoleakage of resin dentin interfaces: effects of two matrix metalloproteinase inhibitors. J Mech Behav Biomed Mater 2018;78:206–13.
[25] Jafarnia S, Meymand J, Zandkarimi F, Saberi S, Shahabi S, Valanezhad A, et al. Comparative evaluation of microtensile bond strength of three adhesive systems. Front Dent 2022;19: 1–8.
[26] Hardan L, Devoto W, Bourgi R, Suarez C, Szymanska M, Barrera M, et al. Immediate dentin sealing for adhesive cementation of indirect restorations: a systematic review and meta-analysis. J Gels 2022;8:175–92.
[27] Josic U, Maravic T, Mazzitelli C, Bianco F, Mazzoni A, Breschi L. The effect of chlorhexidine primer application on the clinical performance of composite restorations: a literature review. J Esthetic Restor Dent 2021;33:69–77.
[28] Hojabri N, Kaisarly D, Kunzelmann K. Adhesion and whitening effects of P11-4 self-assembling peptide and HAP suspension on bovine enamel. J Clin Oral Invest 2021; 25(3237):1.
[29] Deus G, Souza E, Silva E, Belladonna F, Carvalho M, Cavalcante D, et al. A critical analysis of research methods and experimental models to study root canal fillings. Int Endod J 2022;55:384–445.
[30] Sayed A, Mubarak R. Assessment of bond performance of the current self-etch adhesive systems to dentin after aging. AJDS 2022;25:1–6. https://doi.org/10.21608/ajdsm.2021.97879.1253.
[31] Pulidindi H, Mandava J, Borugadda R, Ravi R, Angadala P, Penmatsa P. Effect of remineralizing agents on resin-dentin bond durability of adhesive restorations: an in vitro study. J Int Oral Health 2021;13:470–7.
[32] Yassen A, Abdelaal M, Haridy M. Resin composite bond integrity with desensitized dentin. Econ Dev J 2018;64: 835–45.