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Original Paper

Effects of mechanical and thermal aging on microleakage of different fissure sealants

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The purpose of this study was to examine the microleakage of three different fissure sealants after they were aged by mechanical loading and thermocycling *in vitro*. To this end, a bonding agent (Prime & Bond® NT) and three different fissure sealants (Clinpro, Helioseal F, Teethmate F1) were used, whereby microleakage was evaluated using a dye penetration method after mechanical loading and/or thermocycling. Sealant-treated teeth were allocated into four groups: mechanical loading (50,000 times), thermocycling (10,000 times), mechanical loading (50,000 times) + thermocycling (10,000 times), and one control group. For each fissure sealant, both experimental and control groups showed statistically significant differences in average microleakage score ($p<0.05$). Further, for each fissure sealant, the highest average microleakage score was obtained in mechanical loading + thermocycling group. When comparison was done for each aging method, the average microleakage scores showed statistically significant differences among the three fissure sealants ($p<0.05$). Based on the results of this study, it was also concluded that it is necessary to develop reliable *in vitro* test methods for dental materials.

Key words: Mechanical Loading, Thermal Cycling, Fissure Sealants

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INTRODUCTION

Fissure sealants are materials applied to the tooth surface to obliterate fissures and remove the sheltered environment in which caries may thrive. This conservative technique of tackling pit and fissure caries is a minimal-intervention approach which even most children have no difficulty in accepting[1)]. Therefore, pit and fissure sealants undoubtedly play a critical role in preventing occlusal caries in both primary and permanent teeth[2,3)]. Against this background, the use of pit and fissure sealant materials has been promoted for a number of years to prevent the incidence of dental caries. Owing to the widespread adoption of pit and fissure sealants, their mechanical properties and clinical effectiveness are well documented in published literature[4)].

It has been suggested that a bonding agent be placed before the sealant was applied, although there are many detractors to this application technique[5-7)]. In some studies, it was said that application of bonding agent before fissure sealant increased the latter's effectiveness[8-10)]. The study of Koyuturk *et al.*[11)] showed that application of bonding agent prior to application of fissure sealant yielded beneficial results in terms of microleakage. On the other hand, a clinical evaluation indicated that the use of a bonding agent prior to the application of a pit and fissure sealant did not increase the retention rate[12)].

On the evaluation of dental materials, well-conducted randomized controlled clinical trials are considered to be the standard[13)]. However, considerable time and resources are needed for these trials. It must also be put into perspective that dental materials evolve rapidly. Therefore, the clinical success of these materials must be estimated in an easy, rapid, and realistic way. By simulating the oral cavity conditions (thermal changes and chewing forces) in a laboratory environment to mimic the natural aging process, results very similar to those obtained under *in vivo* conditions may be obtained under *in vitro* conditions. On this note, the use of mechanical loading and thermocycling in laboratory studies has been considered as potential methods to simulate *in vivo* challenges[14-18)].

At this juncture, it must be mentioned that these studies[14-18)] also revealed and highlighted the need to develop *in vitro* methods that are able to evaluate dental materials reliably. Riding on the usefulness of *in vitro* studies, the purpose of this study was to examine the microleakage of three different fissure sealants after they were aged by mechanical loading and thermocycling *in vitro*. In parallel, the reliability of the *in vitro* methods employed to simulate the *in vivo* challenges was reviewed and discussed in this paper.

MATERIALS AND METHODS

Tooth specimens

A total of 120 freshly extracted, sound third molar teeth deemed suitable for sealant application were chosen and stored in a saline solution with 0.1% sodium azide[16,19]. After removing the soft tissue remnants, calculus, and fissures, the teeth were cleaned with fluoride-free pumice and a rubber cup. To examine the occlusal fissure morphology, teeth were cleaned using a bristle brush and pumice slurry, washed with water for 15 seconds, and then dried with an air jet for 10 seconds.

Teeth were grouped according to fissure morphology using visual examination and illumination from a clinical light source. Visually, shallow fissures appeared to be formed by cuspal inclines which met at a wide angle. The bases of the fissures were visible when examined under the light source with no clefting evident between the cuspal inclines. Deep fissures, on the other hand, appeared slit-like with clefts between inclines forming a narrow angle. The bases of the fissures were not visible when examined under illumination. As for intermediate type fissures, they were characterized by the appearance of a uniform width of the fissures clefts. The cuspal inclines formed an angle narrower than the fissures designated shallow. Usually, the bases of the fissures were visible when examined under illumination[8]. Intermediate type fissures were used in this study.

All teeth were subsequently washed under tap water to remove fluoride-free pumice from their surfaces prior to sealant application, and then subjected to drying with an air syringe for 10 seconds. Following which, each tooth was etched with 35% phosphoric acid gel (3M ESPE, St. Paul, MN, USA) for 30 seconds, washed for 15 seconds, and

Table 1 Characteristics of bonding agent and fissure sealants used in this study

		Components	Manufacturer	Lot No.
Bonding	Prime & Bond NT	PENTA, UDMA, Resin R5-62-1, T-Resin, D-Resin, Nanofillers, Photoinitiators, Stabilizer, Cetylamine hydrofluoride, Acetone, photoinitiator	Dentsply, De Trey, Konstanz, Germany	060700088
Fissure sealants	Clinpro	Triethylene glycol dimethacrylate, Bisphenol a diglycidyl ether dimethacrylate, Tetrabutylammonium tetrafluoroborate, Silane treated silica	3M ESPE Dental Products, St. Paul, MN, USA	5FY
	Helioseal F	Bis-GMA, Urethane dimethacrylate, Triethylene dimethacrylate, High dispersed silica, Fluorsilicate glass Titanium dioxide Catalysts and Stabilizers	Ivoclar Vivadent AG, FL- 9494 Schaan, Liechtenstein	H26302
	Teethmate F1	2-hydroxyethyl methacrylate, Triethyleneglycol dimethacrylate, 10-Methacryloyloxydecyl dihydrogen phosphate, Methacryloylfluoride-methyl methacrylate copolymer, Hydrophobic aromatic dimethacrylate, dl-Camphorquinone, Initiators Accelerators Dyes Others	Kuraray Medical Inc., 1621 Sakazu, Kurashiki, Okayama 710-8622, Japan	00258D

Bis-GMA: bisphenyl-glycidyl-methacrylate; PENTA: dipentaerythritol pentaacrylate phosphoric acid ester; UDMA: urethane dimethacrylate.

Fig. 1 Electronic thermal cycling machine used in this study.

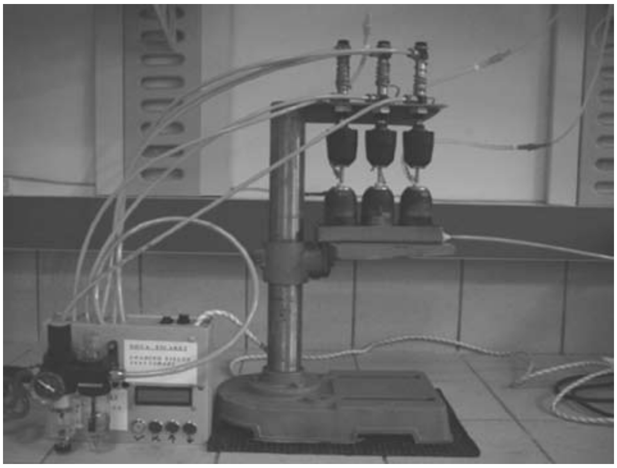

Fig. 2 Chewing simulator used in this study.

dried for 15 seconds. According to manufacturer's instructions, Prime & Bond NT dentin bonding agent was applied to the etched and dried enamel surfaces (Table 1). As for the three fissure sealants (Clinpro, Helioseal F, Teethmate F1) used for sealing the fissures (Table 1), they were polymerized using a halogen light curing unit (Monitex BlueLEX, Monitex Industrial Co. Ltd., San-Chung City, Taipei, Taiwan) for 30 seconds. The curing time unit was applied according to the manufacturer's instruction. After curing, the margins of sealants were checked for any failure of sealant retention and application under a stereomicroscope (SZ-TP, Olympus, Tokyo, Japan).

Thermocycling and mechanical loading

Sealant-treated teeth were allocated into four groups: Mechanical loading (50,000 times), thermal cycling (10,000 times), mechanical loading (50,000 times) + thermal cycling (10,000 times), and one control group. Specimens were thermocycled using an electronic thermal cycling machine (Nova Tic, Konya, Turkey) in water baths at 5±2°C, at room temperature (22±2°C), and at 55±2°C with a dwell time of 30 seconds in each bath (Fig. 1).

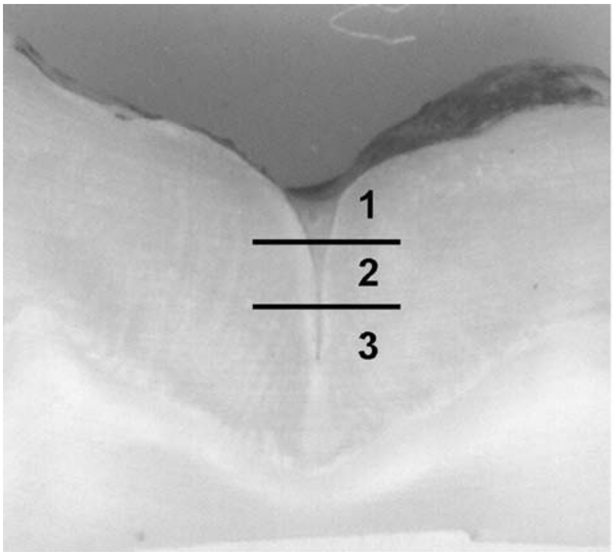

Fig. 3 The scoring system.

Mechanical loading process was performed using a chewing simulator designed to imitate the chewing forces that are produced during function (Vega chewing simulator, Nova Tic, Konya, Turkey) (Fig. 2). Samples were fixed to the chewing simulator, and the center of each tooth was occluded against a stainless steel antagonist with a rounded end (5 mm in diameter). A mechanical load of 50 N was applied at a frequency of 0.5 Hz.

Microleakage assessment

The apical foramens of teeth were covered with a sticky wax, and the surface of each specimen was covered with two layers of nail varnish leaving a 1-mm window around the sealant. All specimens were immersed in a 5% basic fuchsin dye solution for 24 hours. Following immersion in the dye solution, the teeth were washed under running tap water for 30 seconds to remove excess solution.

The mesial and distal sides of each tooth were ground using a disk mounted on a slow-speed handpiece. Each tooth was sectioned longitudinally in a buccolingual direction through the line connecting the buccal and palatal cusp tips to provide four or five sections from each tooth for microleakage evaluation.

One trained (and blinded) examiner was asked to score the dye penetration depth in each section using a stereomicroscope (×60 magnification). The scoring system (Fig. 3) used in this study was the same as that used by Grande *et al.*[20], which was as follows: 0 − No dye penetration; 1 − Dye penetration into the occlusal third of the enamel-sealant interface; 2 − Dye penetration into the middle third of the interface; and 3 − Dye penetration into the apical third of the interface. Highest score was established as the final score obtained after examining both the buccal- and palatal-inclined cuspal planes in each section.

Statistical analysis

Statistical analysis was performed using Kruskal−Wallis and Mann−Whitney U tests with Bonferroni-adjusted alpha level. Level of statistical significance was set at 0.05.

RESULTS

Tables 2 and 3 show the microleakage scores. For each fissure sealant, statistically significant differences in microleakage were observed among the mechanical loading, thermal cycling, and mechanical loading + thermal cycling groups ($p<0.05$). Further, for each fissure sealant tested, the highest average microleakage score was obtained in mechanical loading + thermal cycling group. In particular, Clinpro and Teethmate F1 yielded the highest microleakage score in mechanical loading + thermal cycling group ($p<0.05$). As for Helioseal F, similar microleakage scores were observed for mechanical loading, thermal cycling, and mechanical loading + thermal cycling groups ($p>0.05$), but were statistically higher than the control group ($p<0.05$).

When comparison was done for each aging method, statistically significant differences were observed ($p<0.05$). In the control group, Clinpro showed less microleakage than Teethmate F1 and Helioseal F ($p<0.05$). In mechanical loading group, Helioseal F showed the highest microleakage, while Teethmate F1 showed lower microleakage than Helioseal F but higher microleakage than Clinpro ($p<0.05$). In thermal cycling group, Helioseal F showed the highest microleakage, while Teethmate F1 and Clinpro showed similar microleakage ($p<0.05$). In mechanical loading + thermal cycling group, Clinpro showed significantly higher microleakage than the other fissure sealants ($p<0.05$).

DISCUSSION

In the oral cavity, daily functioning, thermal fluctuations, not to mention habitual bruxism and trauma, impose stresses and strains upon the tooth and restorative system. This may consequently affect and weaken the adhesive bond. In a bid to predict the microleakage of fissure sealants after

Table 2 Microleakage mean scores

	Control		Mechanical loading		Thermocycling		Mechanical loading + Thermocycling		
	n	Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD	p
Clinpro	62	0.00±0.00 A,a	77	0.08±0.39 A,a,b	67	0.30±0.80 A,b	63	1.92±1.15 A,c	0.00
Helioseal F	65	0.26±0.62 B,a	62	0.76±0.95 B,b	65	0.89±1.02 B,b	64	1.08±1.04 B,b	0.00
Teethmate F1	57	0.53±0.91 B,a	52	0.48±0.98 C,a	52	0.25±0.56 A,a	63	1.06±0.98 B,b	0.00
p		0.00		0.00		0.00		0.00	

Same lowercase letters in same row indicate no statistically significant differences. Same capital letters in same column indicate no statistically significant differences.

Table 3 Microleakage scores according to cycles

	Control				Mechanical loading				Thermocycling				Mechanical loading + Thermocycling			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Clinpro	62	0	0	0	73	3	0	1	57	4	2	4	10	14	10	29
Helioseal F	52	11	0	2	31	21	4	6	31	16	12	6	23	22	10	9
Teethmate F1	38	13	1	5	40	4	3	5	41	10	0	1	21	24	11	7

some time of clinical usage, we sought to simulate the effects of functional stress (fatigue) and thermal changes in the oral cavity using a laboratory test design. Therefore, in this study, the microleakage of three different fissure sealants was evaluated under simulated clinical conditions (mechanical loading and/or thermocycling).

It has been widely accepted that current adhesive resins and dental materials, as opposed to the earlier versions, have good biocompatibility with the dental tissue[21)]. These materials were developed to reduce voids and porosity in the adhesive layer, enhance fissure obturation at the enamel-resin interface, and thereby improve sealant retention rates. The spin-off benefit is reduced incidence of fissure caries, especially for deep fissures which are more sensitive to caries attack. However, for the enamel surface in deep fissures, its proper conditioning may be compromised by the inability to remove debris, dry adequately, and ensure total penetration of the resin. Therefore, bonding agents are used to enhance the adhesion and penetration of fissure sealants due to the former's ability to displace water and tolerate some degree of water contamination on the tooth surface[8,11,21)]. For this reason, a bonding agent was used in this study with the aim of increasing fissure sealant penetration and decreasing microleakage.

By virtue of the functions and characteristics of dental materials and their application techniques, it is indeed difficult to evaluate them under *in vitro* conditions. Consequently, considerable time and resources are needed for clinical trials. However, dental materials evolve so rapidly, which means that the clinical success of these materials must be estimated in an easy, rapid, and realistic way. The *in vivo* conditions of the oral cavity may be simulated *in vitro* in a laboratory environment using an appropriate and reliable simulation method. Against this backdrop of *in vitro* simulation of *in vivo* conditions, several studies have revealed and highlighted the need to develop reliable *in vitro* methods for the evaluation of dental materials[11,14-18)].

Dental materials in the oral cavity are constantly exposed to heat and pH changes[22-24)]. Formation of marginal gaps caused by thermal stress and microleakage stems from the different thermal expansion coefficient of tooth tissue[25)]. The coefficients of thermal expansion of resin materials (25-60 ppm/°C) are greater than that of enamel (11.4 ppm/°C) and dentin (8 ppm/°C)[26)]. Therefore, to assess the *in vitro* performance of resin materials, thermal cycling and mechanical loading are the common methods used to simulate the long-term stresses to which the resin restorations are exposed[27)]. In this respect, the issues about the number of cycles and immersion time used in thermal cycling are widely discussed — and accompanied with wide-ranging data support — in published literature[28,29)]. In this study, the specimens were kept in each bath for 30 seconds. For constant temperature aging, many thermal aging regimes have cited 37°C as an appropriate temperature; while for extreme temperature ageing effects, a limited temperature range of 0−67°C has been adopted[30-34)]. In this study, the temperature range was between 5 and 55°C, which was claimed by various studies to be most clinically relevant[22,35,36)].

Thermocycling allows bonded specimens to be subjected to extreme temperatures, thereby simulating the intraoral conditions. During thermocycling, repetitive contraction-expansion stresses are generated at the resin-dentin interface due to higher contraction-expansion coefficient of the restorative material than tooth. This may then eventually result in crack propagation along the resin-dentin interface[37)]. As the chief aim of this study was to predict microleakage between tooth and fissure sealant after one year of *in vivo* clinical service, specimens were subjected to 10,000 times of thermocycling — which were reported to correspond to approximately one year of *in vivo* functioning[38)].

As for the effect of thermocycling on microleakage of resin restorations, some studies claimed that microleakage was significantly increased as a result, while other studies indicated otherwise[16,39)]. In this study, a low number of thermal cycles (10,000 times) was applied to the specimens as was done in a previous study[11)], as it was shown that a low number of thermal cycles had no influence on microleakage. In this way, the effect of thermocycling on microleakage was barred and precluded in this study.

To the end of predicting the microleakage of fissure sealants after at least one year of *in vivo* clinical service, artificial aging was employed in our *in vitro* study. Subsequently, 50,000 times of mechanical occlusal loading were arrived at by proportioning the data that restorations undergo 1,000,000 active stress cycles in 20 years[40)]. The frequency of mechanical loading was adjusted to 0.5 Hz, a value close to the chewing cycle *in vivo*[41)]. While higher frequencies (1 Hz to 60 Hz) that were used in dental literature[42,43)] may minimize the laboratory working time, it may lead to internal heating of the specimens[44)]. As for the effect of the chewing forces that are produced *in vivo* during function, its simulation thereof is indeed an uphill task because of multiple factors such as type of tooth, age, sex, and tooth movements that may interfere with the chewing function. Leveraging on previous studies performed with chewing simulators[14,15,18)], a constant force of 50 N was chosen to simulate the average load during mastication[45)].

In the present study, it was found that mechanical loading and thermocycling, when applied together, significantly increased the microleakage of the three different fissure sealants tested. This result indicated that among the three aging regimes, the combined aging regime yielded more reliable results. At the same time, it also revealed that more improvements await *in vitro* test designs. In published literature currently, information is scarce on the influence of combined mechanical loading and thermal cycling on the microleakage of fissure sealants[20,46,47]. However, apart from the factor of artificial aging method, the use of different contents in fissure sealants, different force magnitudes during mechanical loading, and different numbers of cycles during thermocycling may explain the observed differences in the results.

For each aging method, the microleakage values of the three fissure sealants tested showed statistically significant differences ($p<0.05$). In the control group, Clinpro showed significantly lower microleakage than Teethmate F1 and Helioseal F ($p<0.05$). The slight difference in microleakage among the sealants in the control group was probably due to some damaged samples. In mechanical loading group, Helioseal F showed the highest microleakage, while Teethmate F1 showed lower microleakage than Helioseal F but higher microleakage than Clinpro. On these results observed in mechanical loading group, a possible explanation may lie in the different mechanical strengths of the fissure sealants to withstand the chewing load. In thermal cycling group, Helioseal F showed the highest microleakage, while Teethmate F1 and Clinpro showed similar microleakage. On these results observed in thermal cycling group, a possible explanation may lie in the different contraction-expansion coefficients of the fissure sealants during thermal changes. In mechanical loading + thermal cycling group, Clinpro showed significantly higher microleakage than the other fissure sealants. Taken together, it was thus suggested that the fissure sealants tested shows different microleakage behaviors when mechanical loading and thermal cycling were applied together or separately.

CONCLUSIONS

Within the limitations of the present study, the following conclusions were drawn:

(1) For microleakage studies that involve fissure sealants, results of this study seemed to advocate a combined aging regime of mechanical loading and thermal cycling. *In vitro* simulation of the *in vivo* oral conditions might be crucial to attaining a better evaluation and understanding of the performance of fissure sealants.

(2) Electronic thermal cycling machines, electronic chewing simulators, and a combined utilization of these devices are foreseen to be of great assistance in future studies.

(3) By virtue of the functions and characteristics of dental materials and their application techniques, it is indeed difficult to evaluate them under *in vitro* conditions. When coupled with the results of this study, a heightened need was revealed to develop reliable *in vitro* methods for evaluation of dental materials.

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