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**MICRO-TENSILE BOND STRENGTH OF FOUR LUTING RESINS TO HUMAN
ENAMEL AND DENTIN**

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I

Content

Abbreviations.....	1
Introduction	1
1.1. Research background.....	1
1.2. Luting resin systems.....	2
1.3. Characteristics of dentin and dentin adhesives and enamel bonding.....	3
1.4. Non-trimming micro-tensile bonding strength (μ TBS)	4
1.5. Simulating pulpal pressure in vitro in human dentin	4
1.6 .Thermal cycling.....	5
1.7 .Purpose of the project.....	5
1. Materials and Methods	6
2.1. Luting resin systems.....	6
2.2. Tooth preparation.....	8
2.3 .Composite preparation.....	8
2.4 .Experimental subgroups.....	8
2.5 .Dentin and enamel tooth preparations.....	11
2.6. Bonding procedures.....	12
2.7. Device for pulpal pressure simulation.....	14
2.8 .Preparation of μ TBS specimens.....	14
2.9. Non-trimming micro-TBS testing.....	15
2.10. Statistical analysis.....	16
2.11 .SEM examination and fractography analysis.....	17
3. Results	18
3.1. Micro-tensile bond strength (μ TBS) to human dentin.....	18
3.2. Micro-tensile bond strength (μ TBS) to human enamel	21
3.3. Fractographic analysis.....	23
4. Discussion	29
5. Summaries	35
6. References	38
7. Appendixes	42
8. Acknowledgements	62
9. Curriculum Vitae	62

Abbreviations

Bis –GMA	Bisphenol glycidyl methacrylate
HEMA	2-Hydroxyethylmethacrylate
TEGDMA	Triethylene glycol dimethacrylate
MDP	10-Methacryloyloxydecyl dihydrogen phosphate
CE	Clearfil Esthetic cement
RA	RelyX ARC
MS	Multilink Sprint
MA	Multilink Automix
μTBS	Microtensile bond strength
DMA-PP	Dentin Multilink Automix with simulated pulp pressure
DCE-PP	Dentin Clearfil Esthetic cement with simulated pulp pressure
DMS-PP	Dentin Multilink Sprint with simulated pulp pressure
DRA-PP	Dentin RelyX ARC with simulated pulp pressure
DCE-N	Dentin Clearfil Esthetic cement without simulated pulp pressure
DMA- N	Dentin Multilink Automix without simulated pulp pressure
DMS- N	Dentin Multilink Sprint without simulated pulp pressure
DRA- N	Dentin RelyX ARC without simulated pulp pressure
ECE	Enamel Clearfil Esthetic cement
EMA	Enamel Multilink Automix
EMS	Enamel Multilink Sprint
ERA	Enamel RelyX ARC
SEM	Scanning electron microscope
TC	Thermal cycling
HL	Hybrid layer
Dt	Dentinal tubule
Rt	Resin tag
Sp	Smear plug
PP	Pulpal pressure

1. Introduction

1.1. Research background

Prosthetic preparations often require the removal of a large amount of enamel resulting in exposed dentinal surfaces. Successful bonding of luting resins to dentin has to provide high bond strength, a minimum solubility and a stable and durable tooth-resin-restoration bond. Luting resins have been improved with regard to bond strength, wear resistance, marginal quality and esthetics (1). However, although different generations of luting resins are available to dentists, bonding to dentin is still a challenge.

The bond strength of the luting resins to dentin is compromised by two major factors: adverse chemical interactions (2) and an outward flow from dentinal tubules (3). The influence of the fluid on the dentin surface to the different luting resins may relate to their adhesive permeability (4). Because of dentinal tubules containing fluid, it is difficult to bond to the dentin, but the dentinal pores are the only available area for micromechanical retention (5, 6).

Some in-vitro and in-vivo investigations have attempted to evaluate the durability and the influence of pulpal pressure (PP) on resin-dentin bonding (7-11). The general consensus seems to be, that the application of PP reduces μ TBS significantly (8, 9, 11), although antagonistic data exist (10). Another finding in these studies was that dentin surfaces to which the adhesive and the primer were applied were more permeable than smear layer-covered dentin (8, 9). The fluid permeation because of applied PP during the initial setting period seemed to deteriorate the bonding quality of luting resins (8-10). A mutual agreement in all studies was that simulated PP influences the adhesive performance of luting resins, especially of that which used HEMA (2-Hydroxyethylmethacrylate)-based primers, and that the application of constant intrapulpal perfusion should be considered when simulating luting procedures in vitro (8-11).

Little information is available about the bond strength of contemporary self-etching luting resins under pulpal pressure when combined with artificial aging, i.e. thermal cycling (TC). Recently, two-step self-etching primer systems and one-step self-etching adhesive systems have been developed in order to simplify the clinical application procedures and prevent the collapse of the collagen fibril network of the demineralised dentin. However, the literature

reports conflicting results with regard to the bonding strengths of self-etching adhesive systems to dentin.

1.2. Luting resin systems:

Self-etching luting resin systems can be classified into one-step adhesive systems and two-step adhesive systems. One-step self-etching systems combine the three functions etching, priming and bonding into one step in order to simplify the clinical procedure. They contain ionic resin monomers with acidic phosphates or carboxylic functional groups, hydrophobic monomers, water and an organic solvent (6).

Two-step self-etching systems combine etching and priming steps, and also aiming to eliminate the risk of collagen collapse. Both systems also have, what is regarded as a disadvantage, a residual smear layer remaining between the adhesive resin and the dentin (12).

Three-step etching systems are the gold standard in bonding. These systems allow the etching of the enamel and dentin simultaneously using phosphoric acid for 15 to 20 seconds. However, the surface must be left wet in order to avoid collagen collapse. The etching removes the smear layer and after etching, the exposed intact collagen fibrils are infiltrated by the adhesive resin and polymerize in situ to form a hybrid layer (HL) (7). Bond strength is more depended on the quality of the HL than to the depth of dentin etching (13).

The workflow of a three-step adhesive system to establish a bond between dentin and restorations contains the following steps: Etching of the dentin to remove the smear layer and to surface the collagen network, which is used to link the later resin to the restoration. The etching is followed by priming. A primer is a high viscous liquid, which can penetrate the dentinal collagen network because of its hydrophilic nature and paves the way for the bonding. The priming is followed by a hydrophobic adhesive. The adhesive follows the primer into the collagen network for a depth of 2 to 4 micrometers, hybridization takes place, and resin tags can seal the tubule orifices firmly. The luting resin as the next step links to the adhesive and the restoration. Two-step adhesive systems aim to simplify the procedure by not etching the dentin with an extra etchant, but to combine etching, priming and bonding in one step. The application of the luting resin to link the restoration follows as normal after the

application of the combined etchant/primer/bonder. One step adhesive luting resins are named self-adhesive as they simplify the procedure even further by uniting all the steps (etching, priming, bonding, and luting).

1.3. Characteristics of dentin and enamel bonding

Bond strength depends on the specific composition of the dentin adhesive (14) and decreases in deep dentin (15), as there is regional variability of dentin wetness. Tubules permeate more water in occlusal dentin than in proximal, lingual or buccal dentin areas (16). Bond strengths to dry and to moist dentin showed statistically significant differences. In general bonding systems are highly sensitive to extrinsic humidity and they exhibited very low adhesion values, when exposed to more humid conditions (17). In addition, the resin-impregnated layer quality, rather than thickness, is believed to be the most important factor for obtaining high tensile bond strengths (18) .

Dentin contains about 17 vol% collagen where hydroxyapatite crystals surround collagen fibrils. The small crystallite size, structure and higher carbonate content enlarge the active dentinal surface area. The amount of intertubular dentin also varies with the location. The wetting ability and the extent of adhesive systems penetrating the dentin plays major role in determining the quality of the bonding (19). Moreover, the dentinal tubules contain fluid, which is an impediment to bonding. Additionally the remaining smear layer can potentially block the dentinal tubules and acts as a diffusion barrier. The smear layer is the organic debris that remains on the dentin surface after the preparation of the dentin during the preparation of a tooth. This was originally thought of being an advantage as to protect the pulp.

Enamel contains little protein (<1wt %). Therefore, resin bond strengths to enamel might be related to the degree of etching of the enamel surface. The demineralization of the enamel depends on the PH value of the etchant and the etching time, which must be sufficient to provide adequate enamel retention (6). Acid conditioning alters the enamel surface and leads to a loss of enamel prisms and prism peripheries resulting in a characteristic etch pattern (20). Resin monomers are able to infiltrate etched enamel and form a new layer between the enamel and the restoration, similar to the HL in dentin bonding

(21).

1.4 Non-trimming micro-tensile bonding strength (μ TBS)

The methodology of the micro-tensile testing is based on the idea that a better understanding of the strength of the adhesive interface can be obtained with smaller specimens. The micro-tensile testing method allows multiple specimens to be prepared from one single tooth (22). One advantage of this technique is that the bonded interface of small specimens has a better stress distribution during loading and is ideal for evaluating the long-term durability of resin-dentin bonds. The use of such small specimens requires special test jigs that ensure application of pure tensile force and avoidance of any torque forces (23).

It is interesting to note that different bonding materials produce different modes of fracture. A large variability exists with regards to stress distributions at the dentin interface that can lead to various fracture modes between the different bonding materials, even though their tensile bond strengths were not different statistically. For some systems bonding to dentin by creating a hybrid layer (HL), the bonding failure may happen adhesively at the collagen layer on the top of HL or at the dentin layer on the bottom of HL (22) .

A modified micro-tensile method of bond strength testing has recently been developed by Sano (24). A number of potential advantages for this methodology are: 1. more adhesive failures, fewer cohesive failures; 2. higher interfacial bond strengths can be measured; 3. the ability to measure regional bond strengths; 4. means and variances can be calculated for single teeth; 5. allows testing of bonds to irregular surfaces; 6. permits testing of very small areas; and 7. facilitates examination of the failed bonds by scanning electron microscopy (25).

A natural progress of slab reduction into smaller components was called non-trimming microtensile bond strength testing. In this technique, a single tooth can provide up to 20-25 specimens with areas of 0.7 to 1.2 mm². The non-trimming method apparently exerted less stress onto the adhesive bond, which enables the researcher to measure the bond strengths of materials that produce bond strength less than 5MPa (23).

1.5. Simulating pulpal pressure in vitro in human dentin

Dentin is hydrated in the vital state via an outward flow of dentinal fluid through the dentinal tubules due to a positive pulpal pressure, estimated to be approximately 15 cm H₂O (26). Normal pulpal pressures in intact pulps are about 12 cm H₂O in cats (27) and 15 cm H₂O in humans (26). There is no general consensus in the literature how high the pulpal pressure might be exactly, but a PP range of 15 cm H₂O (9, 10) to 20 cm H₂O (8, 11) can be found in comparative studies.

1.6. Thermal cycling

A widely used aging technique is thermo-cycling. The ISO TR 11450 standard (28) states, that a thermo-cycling (TC) comprised of 550 cycles in water between 5 and 55°C is an appropriate artificial aging test.

1.7. Purpose of the project

Contradicting results have been reported in the literature with regards to the influence of applied PP (8-11). None of the above mentioned studies combined the application of PP with a stressing the bonded specimens by mechanical loading or artificial aging using thermal cycling. Therefore, the first purpose of this study was to assess the bond strength of one-step, two two-step and three-step bonding systems to dentin under the condition of 15 cm H₂O pulpal pressure and water storage of 3 days. The second purpose was, to evaluate the durability of the bonding systems when bonded to dentin or to enamel by microtensile testing after of water storage for 3 days, 30 days with 5,000 thermal cycles (TC) and 90 days with 15,000 TC. In addition the failure modes should be examined using scanning electron microscopy (SEM).

The null hypotheses to be tested were:

1. Pulpal pressure does not influence the bond strength of the luting resins to dentin under 15 cm H₂O pulp pressure after water storage of 3 days, 30 days or 90 days with thermal cycling.
2. Water storage with thermal cycling does not influence the bond strength of the luting resins to dentin.

3. Thermal cycling does not influence the bond strength of the four luting resins bonded to enamel after 3 days, 30 days (5,000 TC) and 90 days (15,000 TC) water storage.

2. Materials and Methods

2.1. Luting resin systems

Four luting resins were tested in this study: A one-step self-etching system (Multilink Sprint), two two-step self-etching systems (Clearfil esthetic cement and Multilink Automix) and a three-step etching system (RelyX ARC).

All materials were used in this study according to the manufacturers' instructions. Additionally, composite blocks made of Multicore Flow were used in this study. Chemical composition and the manufacturers of the materials are shown in Table 1.

Table 1

Composition of the adhesive luting agents and the composite used in this study

Material(manufacturer)	Composition	Batch No
<u>Luting resins</u>		
Clearfil Esthetic cement (CE) (Kuraray Medical Inc., Tokyo, Japan)	ED primer A: HEMA, MDP (10-Methacryloyloxydecyl dihydrogen phosphate), water, accelerator. ED primer B: Methacrylate monomer, water, initiator accelerator. Paste A: Bis-GMA (Bisphenol glycidyl methacrylate), TEGDMA (Triethylene glycol dimethacrylate), other methacrylate monomers, silanated glass fillers, colloidal silica. Paste B: Bis-GMA, TEGDMA, other methacrylate monomers silanated glass filler, colloidal silica, benzoyl peroxide, di-camphorquinone, pigments.	243AA 121AA
Multilink Automix (MA) (Ivoclar Vivadent, Schaan, Liechtenstein)	Primer A: an aqueous solution of initiators. Primer B: HEMA and phosphoric acid and acrylic, acidic monomers. Resin: Dimethacrylate and HEMA, barium glass, ytterbium trifluorid, initiators, stabilisators, spheroid dioxides.	K17908
Multilink Sprint (MS) (Ivoclar Vivadent, Schaan, Liechtenstein)	Dimethacrylates and acidic monomers, barium, glass ytterbium trifluorid and silicon dioxide.	K08951
RelyX ARC (RA) (3M ESPE, Seefeld, Germany)	Adper Scotchbond 1 XT primer: acrylates, HEMA, Bis-GMA, metacrylated modified polycarboxylic acid. Resin: Bis-GMA, TEGDMA, Dimethacrylate polymer	20070530
<u>Composite material</u>		K07368
Multicore Flow (Ivoclar Vivadent, Schaan, Liechtenstein)	The monomer matrix consists of Bis-GMA, urethane dimethacrylate and triethylene glycol dimethacrylate (28.5wt%). The inorganic fillers are barium glass, ytterbiumtrifluoride. Ba-Al-fluorosilicate glass and highly dispersed silicon dioxide (71.0wt%). Additional contents are catalysts, stabilizers and pigments (0.5wt %)	
Bis-GMA = bisphenol glycidyl methacrylate; TEGDMA = triethylene glycol dimethacrylate HEMA=2-hydroxyethyl methacrylate; MDP= 10-methacryloyloxydecyl dihydrogen phosphate		

2.2. Tooth preparation

Seventy-two extracted caries-free human third molars and 36 premolars were collected after patients were informed about the future scientific purpose of their teeth and signed a consent form. The study has been approved by the faculty of dentistry ethics committee of the University of Zhejiang. The teeth were stored in 1% thymol-saturated isotonic saline solution at 4°C. The teeth were randomly divided into 36 experimental subgroups.

2.3. Composite preparation

Composite blocks were fabricated by filling rectangular silicone molds (10×10×20mm) with flowable composite (Multicore Flow, Ivoclar Vivadent, Table 1) which was then light-cured for 80s (550mW/cm², Optilux 500, Kerr, Danbury, USA). Prior to luting procedures, 10×10×4mm parallel-sided composite blocks were cut with a low-speed diamond saw (Isomet, Buehler). The bonding surface of each composite block was ground with 600-grit SiC paper, cleaned in an ultrasonic bath for 20min, stored in distilled water at 4°C and used within 48h. In order to remove the contaminations on the bonding surface of composite blocks, the bonding surface was cleaned with silica-free 32% phosphoric acid and dried with water- and oil-free air.

2.4 Experimental subgroups

The teeth were cut flat to expose enamel and dentinal surfaces. The testing was conducted according to the micro-tensile bond strength (μ TBS) testing technique (24). The teeth were randomly assigned to the following 12 experimental groups (see Fig. 1 and Table 2)

Table 2.

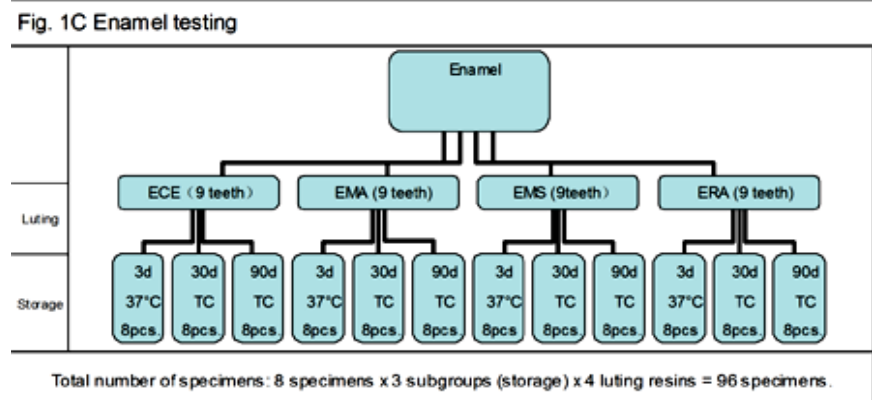
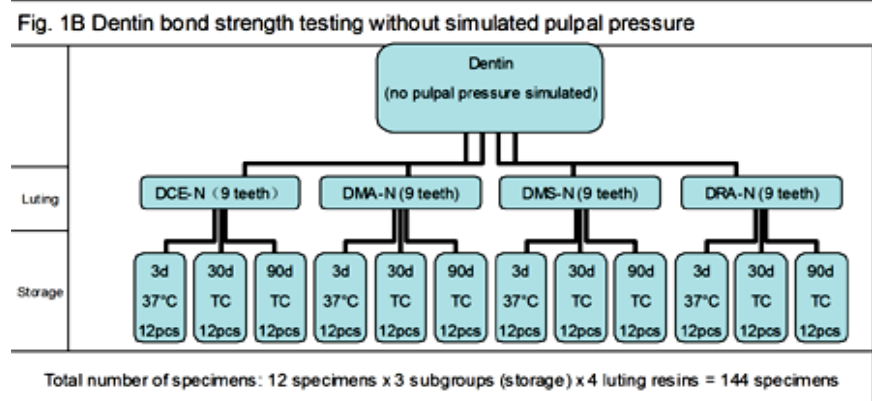
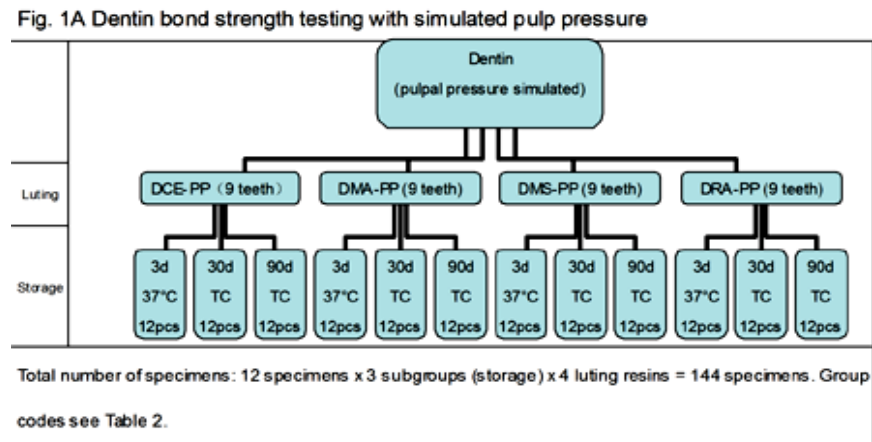
Test groups and of materials used in this study

Material	Enamel group	Dentin groups without simulated pulpal pressure	Dentin groups with simulated pulpal pressure
Clearfil Esthetic cement	ECE: 9 premolars divided into 3 subgroups. ①3d 37 °C water ②30d in 37 °C water with 5,000TC. ③90d in 37 °C water with 15,000TC	DCE-N: 9 molars divided into 3 subgroups. ①3d 37 °C water ②30d in 37 °C water with 5,000TC. ③90d in 37 °C water with 15,000TC	DCE-PP: 9 molars divided into 3 subgroups. ①3d 37 °C water ②30d in 37 °C water with 5,000TC. ③90d in 37 °C water with 15,000TC
Mutilink Automix	EMA: 9 premolars divided into 3 subgroups. ①3d 37 °C water ②30d in 37 °C water with 5,000TC. ③90d in 37 °C water with 15,000TC	DMA-N: 9 molars divided into 3 subgroups. ①3d 37 °C water ②30d in 37 °C water with 5,000TC. ③90d in 37 °C water with 15,000TC	DMA-PP: 9 molars divided into 3 subgroups. ①3d 37 °C water ②30d in 37 °C water with 5,000TC. ③90d in 37 °C water with 15,000TC
Mutilink Sprint	EMS: 9 premolars divided into 3 subgroups. ①3d 37 °C water ②30d in 37 °C water with 5,000TC. ③90d in 37 °C water with 15,000TC	DMS-N: 9 molars divided into 3 subgroups. ①3d 37 °C water ②30d in 37 °C water with 5,000TC. ③90d in 37 °C water with 15,000TC	DMS-PP: 9 molars divided into 3 subgroups. ①3d 37 °C water ②30d in 37 °C water with 5,000TC. ③90d in 37 °C water with 15,000TC
RelyX ARC	ERA: 9 premolars divided into 3 subgroups. ①3d 37 °C water ②30d in 37 °C water with 5,000TC. ③90d in 37 °C water with 15,000TC	DRA-N: 9 molars divided into 3 subgroups. ①3d 37 °C water ②30d in 37 °C water with 5,000TC. ③90d in 37 °C water with 15,000TC	DRA-PP: 9 molars divided into 3 subgroups. ①3d 37 °C water ②30d in 37 °C water with 5,000TC. ③90d in 37 °C water with 15,000TC

In each bonding group for dentin testing with simulated pulpal pressure and three storage conditions, 9 molars were divided into 3 subgroups. The same procedure was applied for dentin testing without pulpal pressure. In each bonding group for enamel testing and three storage conditions, 9 premolars were used and divided into 3 subgroups. The three storage conditions were 3 days of water storage, 30 days of water storage with additional 5,000 thermal cycles and 90 days of water storage with

15,000 thermal cycles. Eight specimens were used for each enamel subgroup and 12 specimens from each dentin subgroup were selected for tests.

Fig. 1 Design of experimental groups



2.5. Dentin and enamel specimen preparation

Surfaces for dentin bonding were prepared by cutting away the occlusal enamel and dentin perpendicular to each tooth's long axis about 1 mm below the dentin-enamel junction using a low speed diamond saw (Isomet, Buehler, Lake Bluff, IL,

USA, Fig. 2A, B). Dentin surfaces were then wet polished with 600 grit SiC paper to create a standard surface roughness and smear layer. All specimens were cleaned in distilled water in an ultrasonic bath for 20 min and then stored in distilled water at 4°C.

All teeth had their pulp chambers exposed by removing the roots below the cemento-dentinal junction using the diamond saw mentioned above. Pulp tissue was removed carefully leaving the predentinal surfaces and the dentinal tubules intact. The occlusal and pulpal dentin surfaces of all specimens were checked with a stereomicroscope (10x Magnification, Wild Makroskop M420, Heerbrugg, Switzerland) for the absence of enamel and pulp tissue. The resulting dentin surface for adhesion was defined as superficial dentin. For all specimens the dentin was kept wet during all preparations by placing a moist cotton pallet in the pulp cavity, storing them in distilled water or placing them on a wet dish. A group size of 18 teeth per material was made.

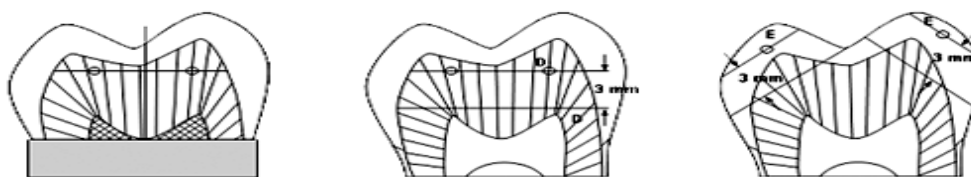


Fig. 2A

Fig. 2B

Fig. 2C

Fig. 2 Illustration of the tested dentin (Fig. 2A and B) and enamel (Fig. 2C) location. (A) Dentin group with simulated pulp pressure (B) Dentin group without simulated pulp pressure (C) Enamel group

Enamel specimens (Fig. 2C): All enamel bonding surfaces were fixed and prepared by grinding and polishing enamel surface areas of 5×6mm on the buccal and lingual side with 600 grit SiC paper under water cooling. The surfaces were then polished with pumice for 1 min, and cleaned in an ultrasonic bath for two times 10 minutes. The teeth of the enamel groups were stored in distilled water at 4°C and were used within 48h.

2.6. Bonding procedures

In the dentin groups with simulated pulpal pressure the PP was applied during the following surface treatment (Table 3): RA: Adper Scotchbond was applied on the etched dentin surface and light-cured; MA: AB primer was applied on the surface of the composite with the automix syringe, light cured 40s from each side and Oxyguard applied for 3min. CE: ED primer 2.0 was applied on the smear layer-covered dentin for 30 s and gently air dried. MS: the original smear layer was retained. A light-curing unit (Optilux 550, Kerr, Danbury, USA) with an output power intensity of 550 mW/cm² was used.

Clearfil esthetic cement (ED primer): Application of ED primer 2.0 was conducted dispensing one drop of ED primer A and B and mixed for 3-5 s. Afterwards the dentinal surface was left visibly moist and afterwards the mixed primer was applied to the specimen surface (air-dried enamel or moist dentin) using a disposable brush for 30s. The specimens' surfaces were dried thoroughly with oil-free air. CE paste A and B were mixed and the mixture applied on the surface of the composite blocks. The composite blocks were bonded to the specimens' surfaces under a load of 7.5 N. Oxyguard II was applied to the edge of each bonded specimens and light-cured for 40s with a light-curing unit.

Multilink Automix: Primer A and B primer were mixed in a 1:1 mixing ratio. The mixed primer was applied to the specimen surface (air-dried enamel or moist dentin) using a disposable brush for 30 s. Conditioning time for enamel was 30 s and for dentin 15 s. The specimens were dried thoroughly with oil-free air to form a slightly

Table 3

List of luting resins and their respective adhesive application procedures

Product name (Code)	Enamel/dentin Pretreatment	Luting agent mixing
Clearfil Esthetic cement (CE)	Dispense an equal amount of ED primer A and B, apply on abutment 30s, mild air drying.	Squeeze paste A and B from the dispenser syringe apply on surface, lute resin block light 40s from each side, apply Oxyguard for 3 minutes.
Multilink Automix (MA)	Mix two MA primer A and B in 1:1 mixing ratio, apply on enamel for 30s, on dentin 15s, air dry.	Apply the desired quantity directly on surface lute resin block with the Automix syringe, light cure 40s from each side, apply Oxyguard for 3min.
Multilink Sprint (MS)	No pretreatment	Apply the desire quantity directly on the surface, from the Automix syringe, light cure for 40s from each side. apply Oxyguard for 3 minutes.
RelyX ARC(RA)	Apply Scotchbond etchant on dentin surface for 15s, rinse, gently air dry, apply Adper™ Scotchbond bond adhesive for 15s, light cure 10s.	mix base and catalyst paste for 10s, light cure for 40s from each side.

shiny adhesive film. MA was dispensed from the double-push Automix syringe and the two pastes mixed in a 1:1 ratio. A thin film was applied to the bonding surface of each composite block. The composite blocks were bonded to the specimens' surfaces under a load of 7.5 N. Liquid strip (Glycerine gel) was applied to the edge of the bonded specimens and afterwards light-cured from each side for 40s with a light-curing unit.

Multilink Sprint: The specimen surface was dried and MS dispensed from the double-push Automix syringe and the two pastes mixed in a 1:1 ratio. A thin, even film of mixed MS paste was applied to the bonding surface of each composite block. The

composite blocks were bonded to the specimens' surfaces under a load of 7.5 N and the excess material removed in time. Afterwards the specimens were light-cured for 40s with light-curing unit.

RelyX ARC: 3M Scotchbond XT etchant was applied to enamel and dentin for 15 s and afterwards rinsed away for 10 seconds. The following drying left the tooth moist. Two consecutive coats of 3M Single bond adhesive were applied to enamel and dentin and dried for 5 seconds. Excess adhesive was avoided on all prepared surfaces. Light-curing followed for 10 seconds per bonding surface. The resin was dispensed onto a mixing pad and mixed for 10s. A thin layer of resin was applied to the bonding surface of enamel and dentin. The bonding resin was applied to the surface of each composite block. The composite blocks were bonded to the specimens' surfaces under a load of 7.5 N. Light-curing followed for 40s per side.

2.7 Device for pulp pressure simulation

The simulation of pulpal pressure was conducted by the help of the pulpal pressure simulation device (see Fig. 3). In the pulp pressure groups, the pulpal chamber of all crowns was connected with the pulpal pressure device as to be seen in Fig. 3. Each of the crown segments was luted with Parapress (Kulzer, Germany) to an 18-inch gauge stainless steel tube which had been inserted through a circular cylinder made from polyoxymethylen. This tube permitted communication with the pulp chamber and was attached to an empty 30 ml plastic syringe barrel. The barrel was filled with distilled water in order to produce a pressure of 15 cm H₂O at the dentin surface to be bonded (see Fig.3). Pulpal pressure simulation of 15cm H₂O was submitted during bonding procedures of the resin. The ready mixed luting resins were quickly applied on the surface of the composites blocks and the dentin specimens under a load of 7.5 N while connected to the 15 cm H₂O simulated pulpal pressure device.

2.8. Preparation μ TBS specimens of enamel and dentin group

After the specimens of ECE, EMA, EMS, ERA, DCE-N, DMA-N, DMS-N, DRA-N,

dCE-PP, DMA-PP, DMS-PP and DRA-PP (E=enamel, D= dentin, N=no pulpal pressure, PP=pulpal pressure) were subjected to 3d of water storage, 30d with 5,000 thermal cycles or 90d with 15,000 thermal cycles, the bonded teeth were sectioned perpendicular to the adhesive-tooth interface into serial slabs using the low-speed diamond saw (Isomet, Buehler, USA), and further sectioned into rectangular composite-dentin beams (1.0 mm × 1.0 mm wide; 8-9mm long, see Fig. 3). The μ TBS testing was conducted randomly with 8 beams in each enamel group and 12 beams in each dentin group. All the specimens were prepared in superficial dentin, so different bond strengths due to different dentin depth could be excluded.

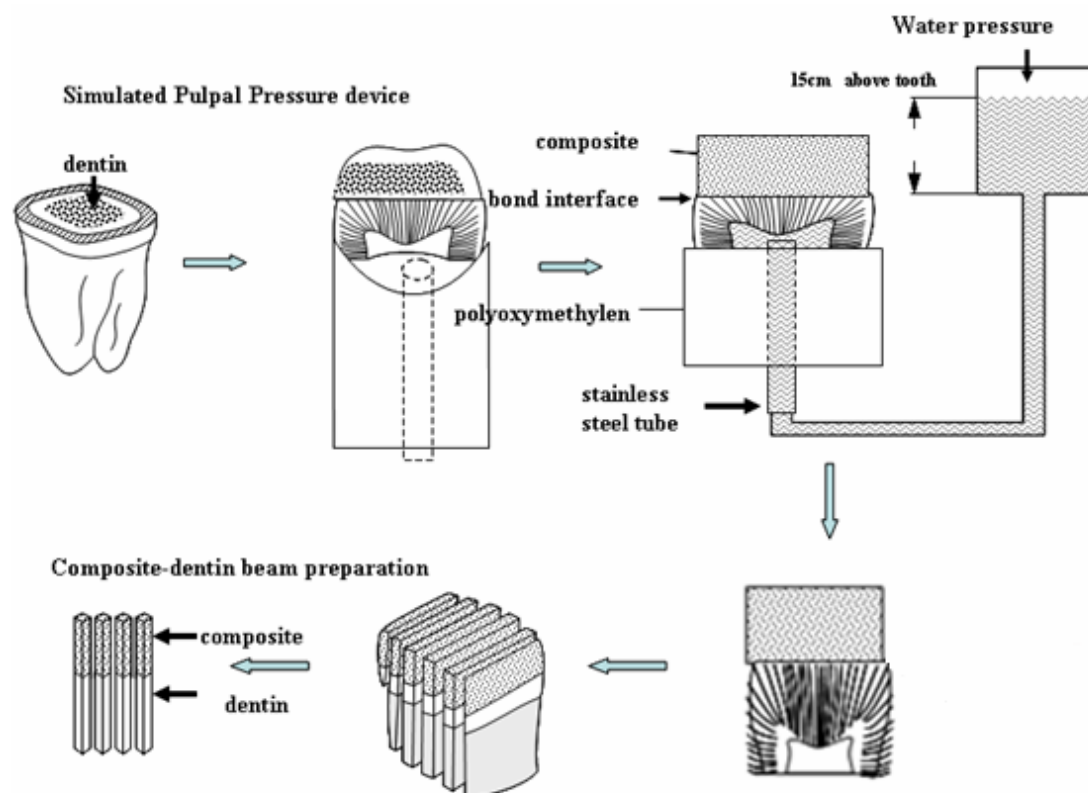


Fig. 3 Chart of the device for simulating pulpal pressure and preparing the specimens for micro tensile bonding strength testing.

2.9. Non-trimming micro-TBS testing

The μ TBS was calculated in MPa (n/mm^2), as derived from dividing the imposed force (n) by the surface area (in mm^2). Each beam was fixed to the jig of the test apparatus with a cyanoacrylate glue (Finocoll V40, Germany) and μ TBS testing was

conducted at a crosshead speed of 0.5 mm/min using a universal testing machine (Zwick Z010/024, Zwick, Germany, see Fig. 4). When a specimen failed during processing (pre-testing failure), TBS was recorded as 0 MPa and included in the statistical analysis. The failure mode was determined at a magnification of 50x using a stereomicroscope (Wild Stereomicroscope) and SEM (Philips XL 30 CP, Philips, Germany).

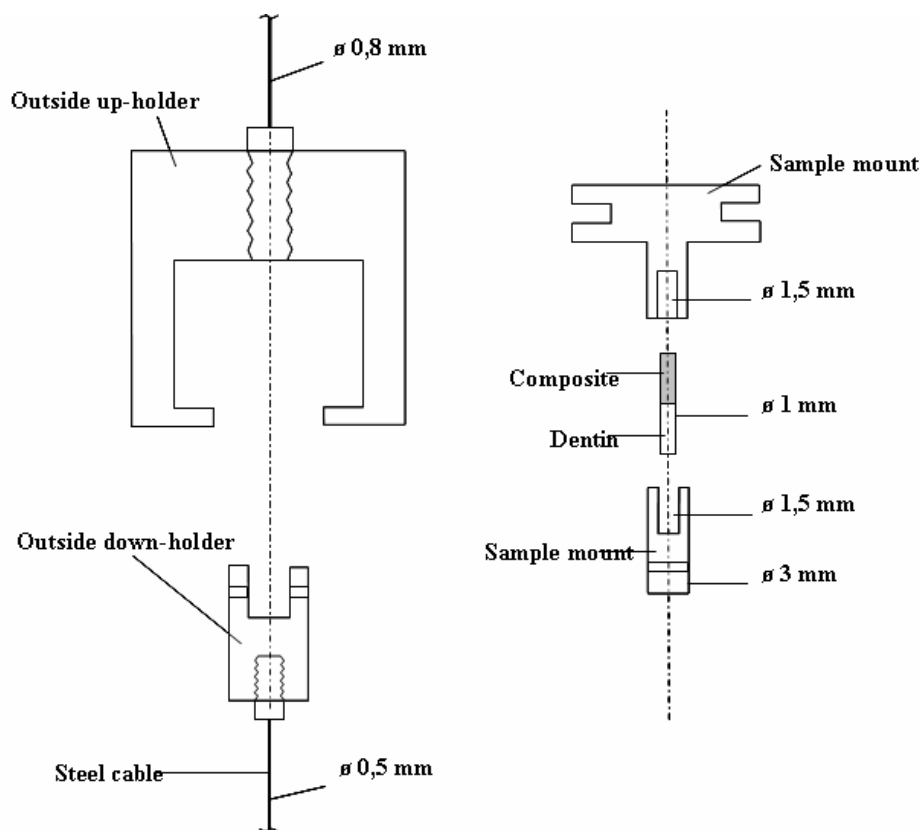


Fig. 4 The schematic drawing of the universal testing machine for μ TBS testing

2.10. Statistical analysis

Since the data of the bond strengths for the four different luting resins to dentin and enamel were not distributed normally (Kolmogorov-Smirnov and Shapiro-Wilks test), the μ TBS of the four luting resins were statistically analyzed using Kruskal Wallis H multiple comparison tests followed by Mann-Whitney-U tests for pairwise comparisons. Significance levels were adjusted with the Bonferroni Holm correction

for multiple testing (29) at a confidence level of 95%. All specimens that failed prematurely during artificial aging were included in the statistical calculation as “zero bond strength” values.

2.11 SEM examination and fractographic analysis

After μ TBS testing, the debonded dentin specimens were air-dried for 24h, gold-sputtered and observed with a scanning electron microscope (SEM, Philips XL 30 CP, Philips, Eindhoven, the Netherlands) operated at 10-25 kV to evaluate the failure modes. The failure modes were classified into one of the following modes (Fig. 3): A: Cohesive failure located in the dentin; B: Adhesive failure at the resin-dentin interface; C. Mixed adhesive and cohesive failure; D: Cohesive failure in the luting resin and E: Adhesive failure at the resin-composite interface. The portion of each failure mode on the debonded dentin surfaces was determined from the SEM micrographs with scale paper and expressed as a percentage of the total bonded surface area for each test group.

3. Results

3.1 Micro-tensile bond strength (μ TBS) of tested groups to human dentin with pulpal pressure after different storage conditions

Mean μ TBS values and standard deviations of the eight experimental dentinal groups are listed in Table 4. Examples of SEM photos can be found the appendix 1 section. Statistical testing showed that the mean values of μ TBS of DCE-PP, DMS-PP, DRA-PP were significantly lower than those of in DRA-N, DCE-N, DMS-N, ($p \leq 0.05$), whereas no significant difference was detected in DMA-N and DMA-PP ($p > 0.01$). There was no significant difference for the group DRA-PP after 3-d water storage to after 90-d water storage with 15,000TC ($p > 0.05$). Premature bond failures occurred for DMS-PP during the μ TBS testing. The μ TBS of dentin specimens of the DRA-N were significantly higher than that of DRA-PP ($p \leq 0.01$). The μ TBS of dentin specimen of DCE-PP with 3-d water storage were significant higher than that in 90-d water storage with 15,000TC ($p \leq 0.05$).

There were no significant differences in μ TBS between the DRA-N and the DMA-N ($p > 0.05$) specimens after 3-d water storage and 90-d water storage with 15,000TC. The μ TBS of dentin specimens of the DRA-N and the DMA-N were significantly higher than those of DCE-N and DMS-N ($p \leq 0.05$). The bond strength of DCE-N group between 3-d water storage, 30d and 90-d water storage with 5,000/15,000 TC was not significantly different. The lowest overall μ TBS for all water storage conditions was produced by the DMS-PP groups with or without PP.

Box plots of μ TBS results of all dentin groups are shown in Fig. 6 and 7. The box represents the spreading of the data between the first and third quartile. The whiskers extend to the minimum and maximum value measured.

Table 4

Micro-tensile bond strength (μ TBS) of tested groups to human dentin with and without the appliance of pulpal pressure after different storage conditions (n=12)

Groups	Pulpal pressure dentin (n=12)			No pulpal pressure dentin (n=12)		
	3d	30d	90d	3d	30d	90d
RA	15(6) ^{B_βa}	26(14) ^{B_βa}	27(12) ^{A_αa}	45(11) ^{A_αb}	43(16) ^{A_αb}	46(18) ^{A_αb}
MA	38(18) ^{A_αa}	41(13) ^{A_αa}	33(11) ^{A_αa}	44(10) ^{A_αa}	42(15) ^{A_αa}	35(12) ^{A_αa}
CE	21(10) ^{B_βa}	30(14) ^{B_βa}	2(2) ^{B_βb}	28(13) ^{B_βa}	27(12) ^{B_βa}	25(10) ^{B_βa}
MS	0(0) ^{C_γa}	0(0) ^{C_γa}	0(0) ^{B_βa}	22.0(11.0) ^{B_βa}	11(6) ^{C_γb}	4(5) ^{C_γc}

Means (standard deviations) in MPa.

Within the same vertical column, means with the same superscript upper-case letter are not statistically different ($p>0.05$). For each luting resin within the same horizontal row means with the same Greek subscript letter (for the same test group PP or non-PP), or means with the same superscript lower-case letter (comparing 3, 30 and 90 days of storage within the same test group) are not statistically different ($p>0.05$). Kruskal-Wallis H multiple comparison tests followed by Mann-Whitney-U tests for pairwise comparisons of groups at a confidence level of 95%.

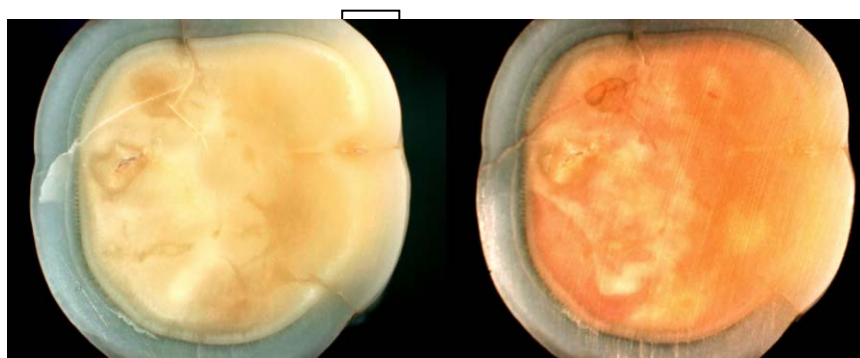


Fig. 5 Dentin surface under the stereomicroscope (A) before connection with the pulpal pressure device and (B) after being connected to the pulpal pressure device with red colored water for 2 h.

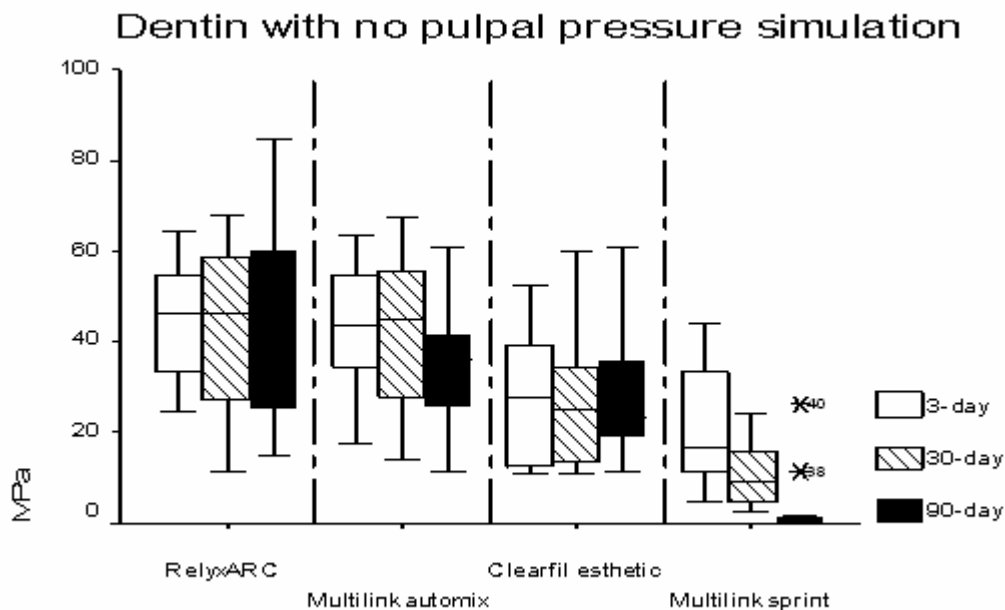


Fig. 6 Box plots of μ TBS results of dentin groups of the four luting resins without the simulation of pulpal pressure for 3-days, 30-days with 5,000TC and 90-days of water storage with 15,000TC. The box represents the spreading of the data between the first and third quartile (n=12 per group).

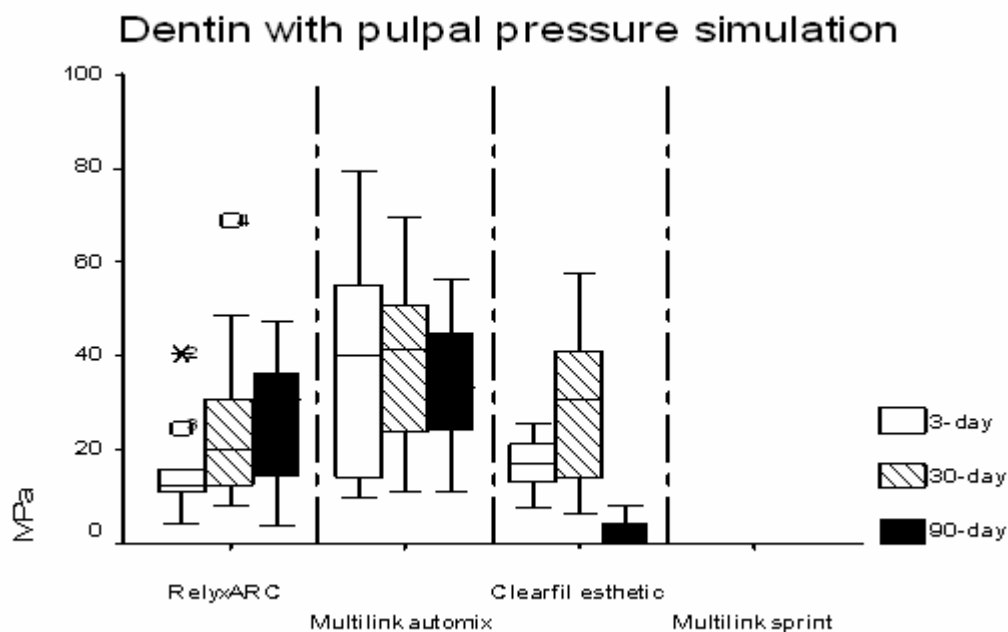


Fig.7 Box plots of μ TBS results of the dentin groups of four luting resins with simulated pulpal pressure for 3-days, 30-days with 5,000TC and 90-days with 15,000TC water storage. The box represents the spreading of the data between the first and third quartile (n=12 per group).

3.2 Micro-tensile bond strength (μ TBS) to human enamel after different storage conditions

Means and standard deviations of μ TBS of the enamel groups of the four luting resins are shown in Table 5. Statistical tests revealed that there were no significant differences in μ TBS of EMA and ECE after 90-d water storage with 15,000TC. But it was shown that for the ERA, the mean μ TBS was significantly higher than those of EMA, ECE, EMS and the mean μ TBS for EMS was significantly lower than those of EMA, ECE, ERA after 90-d water storage with 15,000TC ($p \leq 0.05$). Interestingly in EMA, ECE, EMS specimens TC mostly decreased all μ TBS values, whereas in the case of ERA TC increased the μ TBS significantly after 90-d water storage with 15,000 thermal cycles. Box plots of the μ TBS results of the enamel groups of four luting resins after 3-d, 30-d with 5,000TC and 90-d water storage with 15,000TC are shown in Fig. 8. The box represents the spreading of the data between the first and third quartile. The whiskers extend to the minimum and maximum value measured.

Table 5 Micro-tensile bond strength (μ TBS) of tested groups after different storage conditions. Means (standard deviations) are shown in MPa (n=8).

groups	Enamel (n=8)		
	3days	30days with 5,000TC	90 days with 15,000TC
RA	29(7) ^A _a	26(10) ^A _a	42(6) ^A _b
MA	21(4) ^B _a	24(9) ^A _a	26(5) ^B _a
CE	34(8) ^A _a	28(9) ^A _{a,b}	21(5) ^B _b
MS	17(7) ^B _a	19(6) ^A _b	16(6) ^C _a

Within the same vertical column, means with the same upper case superscript letter were not statistically different ($p > 0.05$) for each luting resin. Within the same horizontal row, means with the same lower case superscript letter were not statistically different ($p > 0.05$).). Kruskal-Wallis H multiple comparison tests followed

by Mann-Whitney-U tests for pairwise comparisons of groups at a confidence level of 95%.

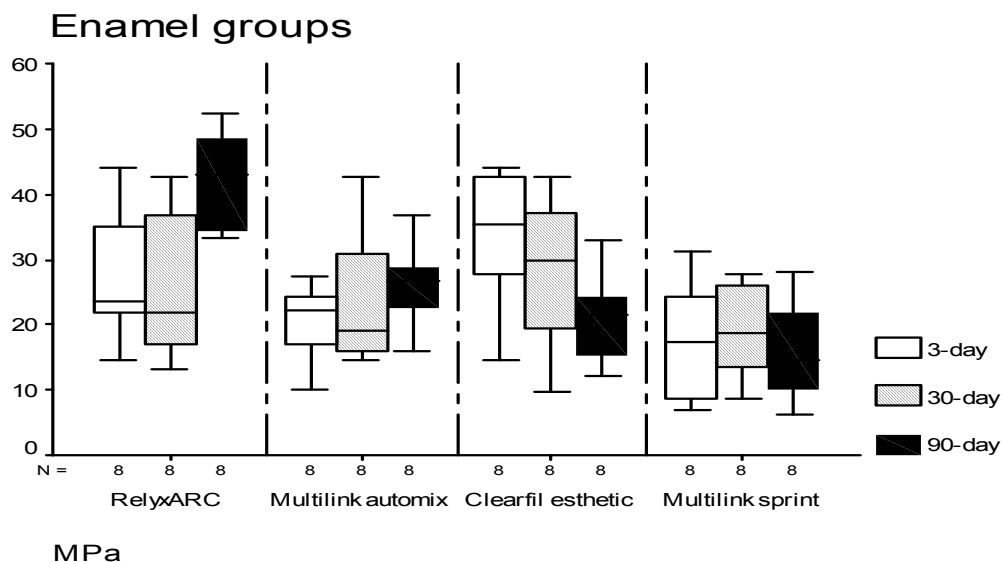


Fig.8 Box plots of μ TBS results of the enamel groups of the four luting resins after 3-days, 30-days with 5,000TC and 90-days water storage with 15,000TC. The box represents the spreading of the data between the first and third quartile (n=8 per group).

3.3 Fractographic analysis

Table 6 shows the distribution of failure modes for the dentin groups. Eight samples of each examined dentin group were then subjected into one of the following five failure mode groups:

A: Cohesive failure located in the dentin; B: Adhesive failure at the resin-dentin interface; C. Mixed adhesive and cohesive failure; D: Cohesive failure in the luting resin and E: Adhesive failure at the resin-composite interface. The portion of each failure mode on the debonded dentin surfaces was determined from the SEM micrographs with scale paper and expressed as a percentage of the total bonded surface area for each test group.

Five failure modes have been defined (see Fig. 9A). The percentage of each

fracture mode for all dentin specimens with pulpal pressure simulation are shown in Fig. 9B and without pulpal pressure simulation in Fig.9C. The numeral percentage of the fracture modes of the four luting resins are summarized in Table 6.

Figures 11-18 (for all SEM figures see appendices) present examples of the interface and fracture surfaces of dentin bonded to the composite blocks with the four luting resins under pulpal pressure and non-pulpal pressure simulated conditions using SEM. Figures 19 and 20 present examples of the interface and fracture surfaces of enamel bonded to the composite blocks with the four luting resins using SEM. Initially RA specimen failed solely at the resin-composite interface (Fig. 14a, b), but the failure modes diversified with lengthy storage. In contrast DRA-PP specimens (Fig. 17a, b, c) constantly failed adhesively either on the resin-dentin or the resin-composite interface.

In groups DMA-N (Fig. 12a, c, e) and DMA-PP (Fig. 16a, b, c) the main failure occurred at the resin-composite interface with a nearly constantly remaining percentage of the failure modes over storage time. Only in the groups DMA-N 30d (Fig. 12c, d) and DMA-N 90d (Fig. 12e, f) the percentage of a mixed adhesive and cohesive failure increased with longer storage time.

For debonding of specimens from the DCE-N (Fig. 11a, c, e) and DCE-PP group (Fig. 15a, b, c) adhesive failures were detected at the resin-dentin interface. In the DCE-N group the number of specimens debonding according to this mode increased with lengthy storage. For nearly all specimens of the DMS-N groups (Fig. 13a, c, e) the failure occurred to be at the resin-dentin interface. Most of the DMS-PP specimens (Fig. 18a, b, c) debonded prematurely prior to μ TBS testing.

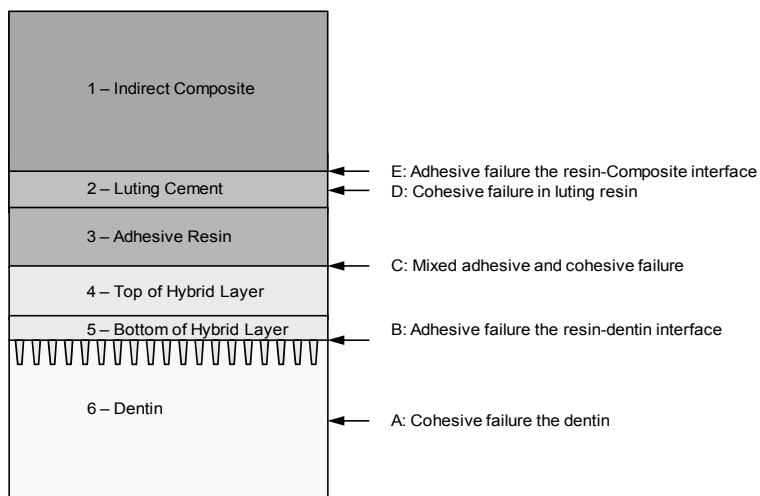


Fig. 9A Graphical presentation of the structure of the resin and dentin/dentin layer at the bonding interface of dentin groups.

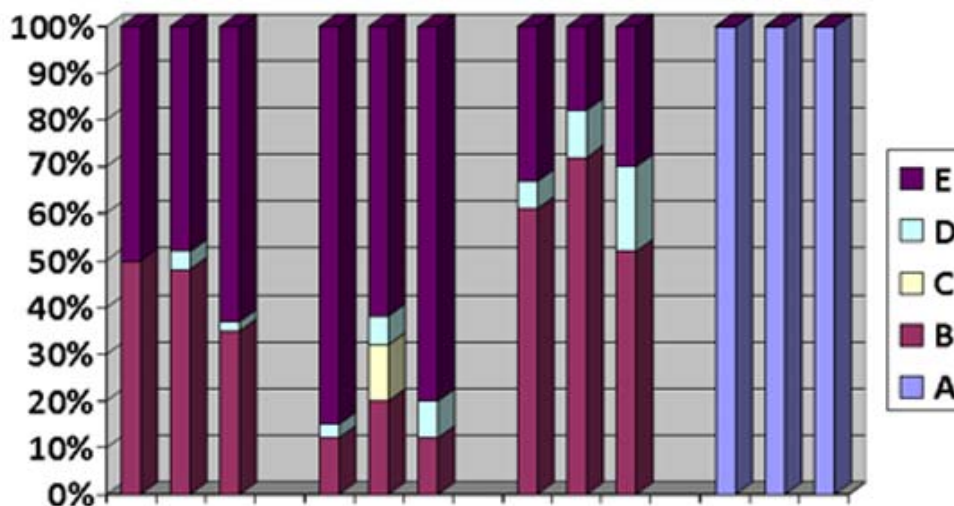


Fig. 9B Graphical presentation of proportional prevalence of fracture modes failure mode of debonded surfaces of dentin in dentin groups under pulpal pressure simulation. Definition of failure modes see Fig. 9 A.

Table 6. Distribution of the failure modes for all groups (%). Definition of failure modes see Fig. 9 A.

Failure mode of dentin groups after three storage times (n=12)		A	B	C	D	E
DRA- N	3 days	0	0	0	0	100
	30 days	0	10	10	10	70
	90 days	10	22	0	14	54
DRA-PP	3 days	0	50	0	0	50
	30 days	0	48	0	4	48
	90 days	0	36	0	4	60
DMA - N	3 days	0	7	0	5	88
	30 days	0	14	0	30	56
	90 days	0	12	0	27	63
DMA -PP	3 days	0	17	0	4	79
	30 days	0	21	14	6	56
	90 days	0	14	0	8	78
DCE - N	3 days	0	62	0	1	37
	30 days	0	29	0	17	54
	90 days	0	39	13	19	19
DCE -PP	3 days	0	65	0	5	30
	30 days	0	76	0	11	13
	90 days	0	55	0	19	26
DMS- N	3 days	0	0	83	17	0
	30 days	0	0	95	5	0
	90 days	0	0	93	7	0
DMS-PP	3 days	0	0	0	100	0
	30 days	0	0	0	100	0
	90 days	0	0	0	100	0

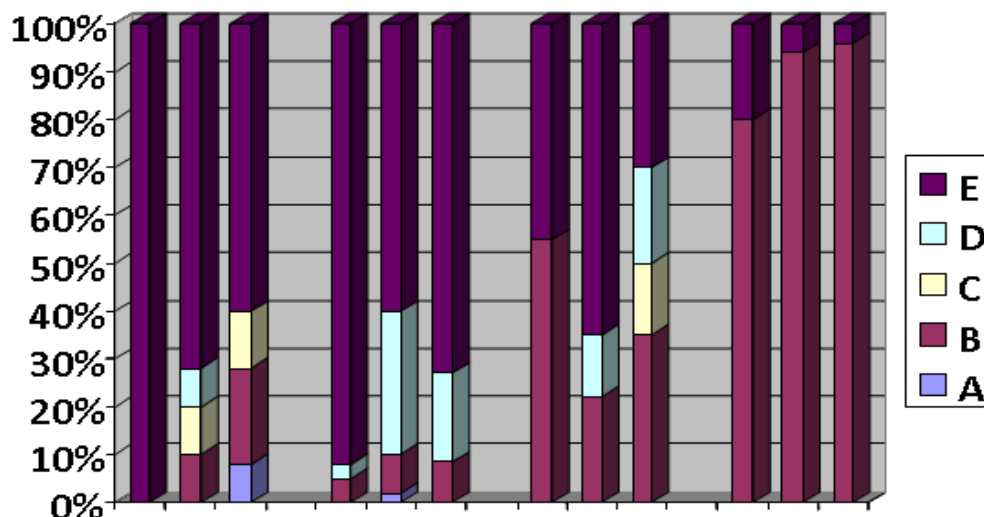


Fig. 9C Graphical presentation of proportional prevalence of fracture modes of debonded surfaces of dentin in the groups without simulated pulpal pressure. Definition of failure modes see Fig. 9 A.

Moreover, five failure modes have been defined for describing the debonding of the luting resin and enamel/enamel layer at the bonding interface of enamel groups (see Fig.10A). The percentage of fracture modes of enamel specimens of the four luting resins during μ TBS testing after 3-d, 30-d with 5,000TC and 90-d water storage with 15,000TC are shown in Fig.10B. For the ERA 26% (3-d) of failures were adhesive failures at the resin-composite interface and 60% for 30-d and 55% for 90-d. For EMA the percentages of adhesive failures at the resin-composite interface were for 3-d 15%, for 30-d 46% and for 90-d 29%. The percentages of adhesive failures for ECE were 30% (3-d), 20% (30-d) and 26% (90-d). For EMS the percentages for adhesive failures at the resin-composite interface were 0 % (3-d), 3% (30-d) and 26% (90-d). Eight specimens of each enamel group were categorized into one of the following five failure modes: A: Cohesive failure in the enamel. B: Adhesive failure at the resin-enamel interface. C: Mixed adhesive failure and cohesive failure in the enamel. D: Cohesive failure in the luting resin. E: Adhesive failure at resin-composite interface (See Fig.10A).

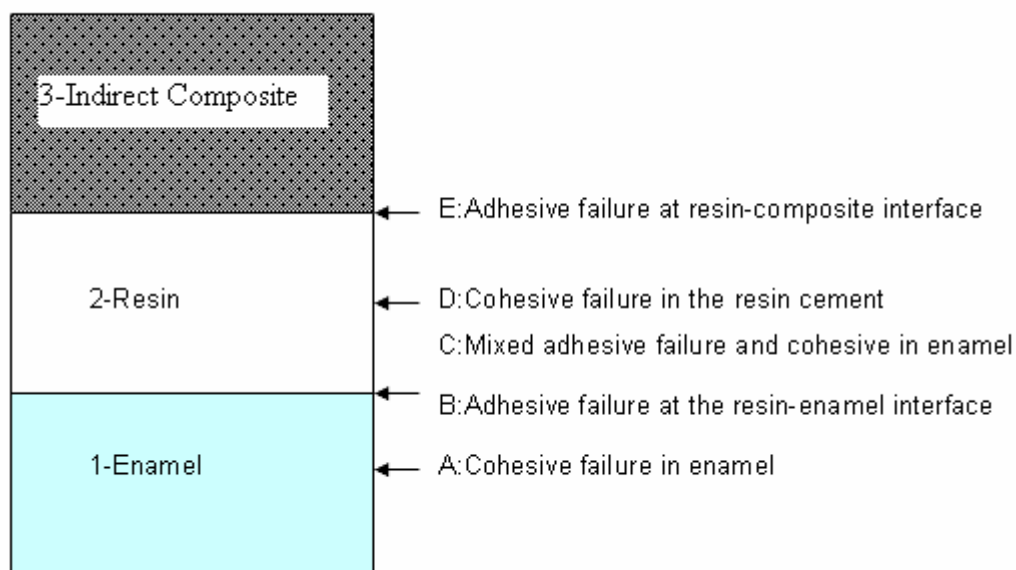


Fig. 10A Definition of the failure modes for the enamel groups

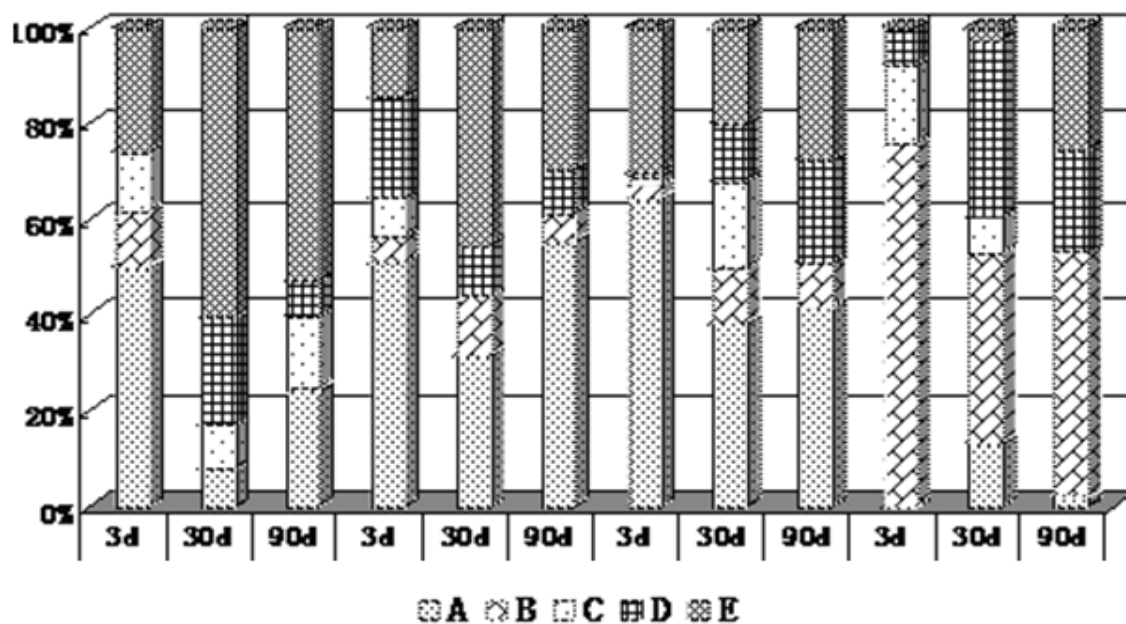


Fig. 10B Graphical presentation of the proportional prevalence of fracture modes in the enamel groups. Definition of failure modes see Fig. 10 A.

Table 8. Percentage of failures modes for the enamel groups (%). Definition of failure modes see Fig. 10 A.

Failure mode of enamel groups after three storage times (n=8)		A	B	C	D	E
ERA	3 days	50	12	12	0	26
	30 days	8	0	10	22	60
	90 days	25	1	15	8	55
EMA	3 days	50	6	9	20	15
	30 days	32	13	0	11	44
	90 days	54	6	0	10	30
ECE	3 days	64	3	2	1	30
	30 days	38	12	18	12	20
	90 days	39	9	0	20	26
EMS	3 days	0	76	16	8	0
	30 days	13	40	7	37	3
	90 days	2	50	0	22	26

4. Discussion

Simulating pulpal pressure represents a relatively accurate possibility to provide the researcher with in vitro conditions similar to the clinical environment when bonding luting resin to dentin. Moisture contamination is a clinically realistic concern, especially when luting resins are used on deep dentin proximal to the pulp horns. The ability of self-etching luting resin bonding to dentin in the presence of simulated pulpal pressure can provide valuable preclinical data for the clinical application.

In this study dentin surfaces were polished with 600 SiC grits to generate a smear layer simulating clinical conditions. The existence of a smear layer and smear plugs in dentinal tubules might limit excessive transudation at the dentin surface (30, 31).

The non-trimming μ TBS method used in this study can be used for the μ TBS of materials that produce low bond strength (23). Results demonstrated that the bonding ability of luting resins decreased when continuous fluid pulpal pressure was present during resin setting. The statistical analysis of the μ -TBS results using the Kruskal-Wallis H test followed by Mann-Whitney-U tests for multiple pair-wise comparisons of groups indicated that the effect of simulated pulpal pressure was different for each luting resin type.

The μ TBS values of dentin groups with PP significantly decreased during the whole setting procedure for RA, CE, MS ($p < 0.05$) with the only exception of MA (Table 3). Therefore, the first null hypothesis was rejected. A previous study indicated that pulpal pressure could have been responsible for lower μ TBS values (32). Moreover, hydrophilic resin monomers in adhesives attract water molecules and account for outward water movement (33). It can be speculated when water is exsuding from etched dentin into the adhesive, water could dilute the concentration of monomers and interfere with the conversion of monomers to polymers (34).

The application of three-step luting resin systems requires the smear layer to be dissolved and removed using an acidic conditioner and water rinsing. In the present study, the μ TBS of DRA-N was found to be the highest value, probably as the result of the primer, which is light-irradiated, and contains generated free radicals

increasing the surface energy and wetting ability. However, the μ TBS of DRA-PP decreased from 45.7 MPa to 26.6 MPa after 90-d water storage with 15,000 TC. As control group, the treatment with primer Adper Scotchbond and 0 cm H₂O pulpal pressure was chosen. Water movement could have been also induced by the primer. Thus the original accommodation and polymerization of the RA luting resin with simulated pulpal pressure could have been compromised. In theory to achieve optimal dentinal sealing resin monomers would flow into tubule orifices, diffuse into the interfibrillar collagen spaces and adequately polymerize forming hybridized resin tags (31).

Acid-etching of dentin is very likely to increase the permeability due to the removal of the smear layer and smear plugs from dentinal tubules. This potential influence was manifested by the SEM micrographs (Figs. 17e, f) that revealed adhesive failures between the adhesive and the dentin and in some areas with water globules inside the hybrid layer. These globules may have been the result of the emulsion of the luting resin's hydrophobic components once coming in contact with water (2, 35). This finding is supported by the fact that the bonding between the adhesive layer and the luting resin was weak when pulpal pressure was applied.

In the current study, pulpal pressure could also have played an important role in reducing the adhesion of bonding systems that require smear-layer removal. Due to continuous water uptake through the permeable adhesive layer, extended to an unsteady porous formation of the luting resin layer it can be assumed, that this accelerated the degradation along the interface between the adhesive and luting resin (8).

A further study revealed that fluid flow with self-etching adhesive systems was lower than that of total-etch adhesives (36). In the present work the reduction of μ TBS in the DMA-PP group with thermal cycling, showed slight but no significant difference ($p>0.05$). The interface of fractured beams in DMA-PP (Figs. 16a, d) was located between the luting resin and dentin, mostly more on the base than at the top of the hybrid layer. An intact structure was kept in conditioned collagen fibrils. The dentin near the dentinal tubules orifices was incompletely demineralized compare

with the better demineralization of intertubular dentin. It was assumed that dentinal tubules were obstructed with smear plugs and impeded or at least hindered the pulpal liquid flowing. Dentin specimens of the DMA-PP group were obviously not sensitive to a simulated pulpal pressure during slow bonding procedures. In a recently published article, it is stated that some of the latest bonding systems were less sensitive to the wetness of dentin (16). It is possible that the resin had a protective function around well-infiltrated collagen fibrils (7).

The reduction in μ TBS in CE specimens cured under simulated pulpal pressure was greater than reductions in the MA and RA after the 90-day storage with 15,000TC. Mixed failures of DCE-N occurred with the fractured surfaces located on the base of the hybrid layer, as shown in Figs. 11d, e, f. During the bonding procedure, the water transferring from dentin tubules made it difficult for the luting resin with a relatively high viscosity to penetrate the dentin. The mild demineralization effect of ED primer 2.0 was manifested by the SEM observations of the debonded dentin surfaces (Figs. 15e, d). The high concentrations of hydrophilic and ionic resin monomers in ED primer resulted in the formation of a highly permeable layer after polymerization (37). The components of ED primer contain water and solvents of ED primer are hydrophilic. Therefore, water penetration could have been responsible for the inferior bond strength, as e.g. compared to primers containing acetone. The application of a hydrophilic primer solution should infiltrate the exposed collagen network forming the hybrid layer (4).

The current results showed that the μ TBS of DCE-PP were decreased significantly in comparison with DCE-N after 90-days of water storage with 15,000TC. The advantage of water-based primers is that they are less technique-sensitive with respect to the wetness of the acid-etched dentin (38). However, excessive transudation of fluids from dentinal tubules and substrate moisture may exceed its water-chasing ability: with acetone evaporation exceeding that of water, the accumulation of the aqueous fraction accumulated in the adhesive film prior to polymerization tends to impair bonding. Additionally the increased permeability of the ED primer was found to be due to a fairly extensive nanoleakage at the bonded

interfaces (35). Self-etching primers are acidic by nature due to their increased concentrations of acidic hydrophilic resin monomers (39). Moreover, the entrapment of water or incompletely removed solvents within the adhesive resin may result in the subsequent hydrolytic degradation of hybrid layers and resins via the cleavage of ester bonds (40).

It was also found that much of the HL had disappeared over 1-3 years in function (41). The protective function of the adhesive resin may be affected by water resorption and hydrolytic degradation of the hydrophilic components in self-etching bonding systems. Water diffusion from dentinal tubules into the bonding interface may hasten hydrolytic degradation of resin components within the HL and adhesives, followed by hydrolysis of the naked collagen fibrils (9). It was speculated that the increased permeability of the primed dentin surface probably allowed water to diffuse from dentin across the hybrid layer, leaving exposed collagen fibrils without resin infiltration and form water droplets along the interface of dentin-resin resulting in lower bond strength.

Scanning electron microscopy of DMS-PP group specimens revealed that fracture modes were mainly at the resin/dentin interface. The poor demineralization effect of Multilink Sprint was shown by only a few exposed dentinal orifices (Figs. 18e, f). During one-step self-etching luting resin being conducted, water can diffuse from the hydrated dentin structure across the polymerized hydrophilic adhesive layer through an osmotic penetrating gradient (2). The few tubule orifices detected on the dentinal surface bonded with Multilink Sprint might be due to the slight diffusion of acidic monomers from the high viscosity luting resin penetrating through the smear layer (Figs. 13e, f).

For DMS-PP specimens, the μ TBS decreased to 0 MPa (Figs. 18a, d) confirming by not finding a hybrid layer even after just three days of water storage. The one-step adhesive system can behave as a semi-permeable membrane, permitting diffusion of water even after polymerization and can be ineffective in reducing dentin permeability when bonding under pulpal pressure (2). Fluid transudation through the adhesive would result in an emulsified polymerization of the luting resin, which can lead to

the forming of resin globs under the influence of water (37). It can be speculated that the dentinal fluid generated by the presence of pulpal pressure might have transudated from these slightly exposed orifices and that water droplets adversely diluted the concentration of the acid monomers.

All-in-one adhesive systems bonded to dentin showed lower bonding strength more due to a simulated pulpal pressure than due to TC (9). A TEM work demonstrated that water can pass from dentin around resin tags, to form water-filled channels that impede to form the hybrid layer (42). In contrast, a decrease of μ TBS for DRA-PP group specimens after 90-day water storage was not observed. This may reflect a certain affinity of this simplified luting resin with multi-step resin-based luting systems. Each class of an adhesive system has a different distribution of micropermeability. The higher the micropermeability, the higher the risk at the resin-dentin interface, which may represent the pathway for hydrolytic and enzymatic degradation of resin-dentin bonds over time (43).

The simulated pulpal pressure and thermal cycling is capable of diminishing the bond strength values of self-etch and etch-and-rinse adhesives. Thermocycling is a commonly used thermal fatigue loading method in bond strength studies. Although it cannot simulate chemical attacks from water into the interface, it is still able to imitate the effect of in vivo thermal stresses and prolong the water exposure on the bonding interface. Conflicting results have been often reported, since the effect of thermal cycling on dentin bonding strength is strongly related to the bonding systems, surface preparation, smear layer and storage time. Studies of the sensitivity of bonding systems to pulpal pressure could be regarded as the screening method for all future materials (44).

From the scanning electron microscopy photos, different modes of demineralization could be differentiated for the four bonding systems. However, deeper interaction with dentin does not always imply a superior bonding potential, as some monomers might preserve their etching ability affecting the polymerizing reaction and jeopardizing adhesion if not properly neutralized (30). This phenomenon was more evident on perfused dentin where the resin exerted a superior etching

potential, most likely due to acidic monomers dilution (10).

The results of this study require the partial rejection of the null hypotheses. Micro tensile bond strength was affected by the presence of simulated pulpal pressure (except Multilink Automix, where μ TBS was very low) and the bonding system, whereas the null hypothesis that thermal cycling does not influence the μ TBS could be partially accepted (except Multilink Sprint and Clearfil esthetic). Simplified systems of one-step may increase the possibility for hydrolytic and enzymatic degradation of bonding at the resin-dentin interface over time. Dentin and enamel groups showed different μ TBS results.

With regards to the bonding effectiveness to enamel, the lowest bond strength value was measured for the group MS. MS showed mainly adhesive failures 3-d (76%), 30-d (49%), 90-d (50%) at the enamel/resin interface. The highest bond strengths to enamel were obtained with RA and CE, where the highest μ TBS to enamel 90-d was produced by RA. Enamel specimens can crack in the interface of enamel-dentin during debonding, therefore the bond strength values could have been effected. Acid treatment modifies the enamel surface construction and produces a constant and regular distribution of removed enamel prisms and/or prism peripheries, resulting in characteristic enamel etching pattern. It is proposed that resin bond strengths to enamel may be related to the degree of etching of the enamel surface.

It has been reported that mild self-etch adhesives only shallowly demineralize enamel, resulting in a very thin micro-retentive pattern without formation of distinct macro- and micro-resin tags (45). The relatively low μ TBS values of specimens bonded to enamel can may be partially be explained brittleness and low elasticity of enamel rather than to a low bond strength of the luting resins to enamel. The sustainability of the enamel μ TBS during the TC can be explained by the more micro-retentive and dry enamel surface obtained when enamel etched with phosphoric acid as compared to which etched by the self-etch adhesive (46).

5. Summary

Successful attempts of bonding to dentin without the appliance of pulpal pressure conditions or thermal cycling in vitro have been reported extensively. However, the performance of adhesive luting systems when used for bonding to dentin under pulpal pressure in combination with thermal cycling is still an open question.

The current study aimed to investigate the influence of pulpal pressure (PP) and thermal cycling (TC) as a form of artificial aging on the bonding durability of one-step, two-step and a three-step adhesive luting systems. Dentinal surfaces were created by removing the occlusal enamel and a pulpal pressure was applied by removing the pulpal tissue and connecting the tooth with a device that created a pulpal pressure of 15 mm H₂O in the pulp cavity.

Independent of PP application, the two-step system Multilink Automix and the classic three-step system RelyX ARC showed significantly higher μ TBS than the two-step system Clearfil Esthetic and the one-step system Multilink Sprint ($P \leq 0.05$). A significant decrease in μ TBS was found for RelyX ARC and Multilink Sprint when subjected to PP ($P \leq 0.05$), whereas Clearfil Esthetic and Multilink Automix showed no significant difference ($P > 0.05$). TC had no significant influence on the μ TBS in RelyX ARC, Multilink Automix and Clearfil Esthetic without pulpal pressure application ($P > 0.05$), whereas Clearfil esthetic with pulpal pressure and Multilink Sprint showed a significant decrease in μ TBS ($P \leq 0.05$) when subjected to TC.

When bonding to enamel RelyX ARC showed the highest μ TBS values followed by Clearfil esthetic and Multilink Automix. The lowest μ TBS values were presented by Multilink Sprint.

With regards to the failure mode observation the RelyX ARC specimens failed adhesively solely at the resin-composite interface after 3 days storage and no thermal cycling, but failure modes diversified with 90 days storage and thermal cycling. In contrast RelyX ARC specimens bonded with the application of pulpal pressure constantly failed adhesively either on the resin-dentine or the resin-composite interface. In groups Multilink Automix and Multilink Automix with applied pulpal pressure the main failure occurred adhesively at the resin-cement interface

with a nearly constantly remaining percentage of the failure modes over storage time. For debonding of specimens from the Clearfil esthetic without and Clearfil esthetic with the application of pulpal pressure adhesive failures were detected at the resin-dentine interface. For nearly all specimens of the Multilink Sprint groups the failure occurred at the resin-dentine interface. Most of the Multilink Sprint specimens with pulpal pressure debonded prematurely prior to μ TBS testing. The specimens from the enamel groups exhibited mainly mixed failure mode with the exception of Multilink Sprint which specimens failed mostly cohesively in the cement.

Based on these results, there were significant differences between materials. Pulpal pressure and artificial ageing also seem to have an effect on the in-vitro bonding durability. If considered relevant to the materials' service performance then these conditions should be applied in the materials' testing.

5.1. Zusammenfassung

In der Literatur sind viele erfolgreiche in vitro Versuche beschrieben ohne Pulpendruck und bei fehlender Temperaturwechselbelastung erfolgreich zu Dentin zu kleben. Die Bewährung von adhäsiven Zementen, die unter appliziertem Pulpendruck zu Dentin geklebt wurden in Kombination mit nachfolgender Temperaturwechselbelastung ist bisher unerforscht.

Das Ziel der vorliegenden Studie war es den Einfluss von Pulpendruck und Temperaturwechselbelastung (als artifizielle Alterung) auf die Haltbarkeit des Klebeverbundes von einem ein-Schritt-System, zwei zwei-Schritt-Systemen und einem Drei-Schritt-System zu evaluieren. Dentinoberflächen wurden geschaffen, indem der okkluale Schmelz entfernt wurde. Der Pulpendruck wurde appliziert, indem das Pulpengewebe entfernt und in das leere Pulpenkavum ein Stahlrohr eingelegt wurde, was mit einer Apparatur verbunden wurde, die einen Pulpendruck von 15 mm H₂O aufbaute.

Unabhängig davon, ob Pulpendruck verwendet wurde oder nicht, zeigte das zwei-Schritt-System Multilink Automix and das klassische drei-Schritt-System RelyX ARC signifikant höhere μ TBS Werte zu Dentin als das zwei-Schritt-System Clearfil Esthetic and das ein-Schritt-System Multilink Sprint ($P \leq 0.05$). Einen signifikanten Abfall in μ TBS konnte für RelyX ARC und Multilink Sprint festgestellt werden, wenn sie Pulpendruck ausgesetzt wurden ($P \leq 0.05$), wohingegen Clearfil Esthetic and Multilink Automix keine signifikanten Unterschiede zeigten ($P > 0.05$). Temperaturwechselbelastung hatte keinen signifikanten Einfluss auf die μ TBS in RelyX ARC, Multilink Automix und Clearfil Esthetic ohne Applikation von Pulpendruck ($P > 0.05$). Clearfil Esthetic unter Pulpendruck und Multilink Sprint wiesen einen signifikanten Abfall in der μ TBS ($P \leq 0.05$) auf, wenn Sie Temperaturwechselbelastungen ausgesetzt waren. Wenn zu Schmelz geklebt wurde, zeigte RelyX ARC die höchsten μ TBS Werte gefolgt von Clearfil esthetic und Multilink Automix. Die niedrigsten μ TBS Werte wurden bei Multilink Sprint gemessen.

Bei der anschliessenden Fehleranalyse zeigte die meisten RelyX ARC Probekörper nach drei Tagen Lagerung und ohne Temperaturwechselbelastung ein

Versagen an der Zement-Komposit Kontaktfläche, aber die Art der Versagens diversifizierte sich mit zunehmender Lagerungsdauer. Dagegen versagten die RelyX ARC Probekörper, die mit Pulpendruck zementiert wurden, entweder an der Zement-Dentin oder Zement-Komposit Kontaktfläche. Bei den Multilink Automix Probekörpern mit und ohne appliziertem Pulpendruck, wurde meistens ein Versagen an der Zement-Komposit Kontaktfläche festgestellt, wobei der Prozentsatz der Versagensmodi bei veränderter Lagerungsdauer nahezu gleichblieb. Bei den Clearfil Esthetic Probekörpern mit und ohne appliziertem Pulpendruck konnte ein adhäsives Versagen an der Zement-Dentin Kontaktfläche identifiziert werden. Für nahezu alle Multilink Sprint Probekörper kam es zu einem Versagen an der Zement-Dentin Kontaktfläche. Die meisten der Multilink Sprint Probekörper, die unter applizierten Pulpendruck zementiert wurden, versagten vor dem eigentlichem Testen. Die Probekörper aus den Schmelzgruppen wiesen größtenteils einen gemischten Versagensmodus auf, mit der Ausnahme der Probekörper von Multilink Sprint, bei denen die Probekörper meistens kohäsiv versagten.

Basierend auf den Ergebnissen konnten signifikante Unterschiede zwischen den Materialien festgestellt werden. Pulpendruck und artifizielles Altern scheinen einen Effekt auf den Klebeverbund in-vitro zu haben. Wenn es als relevant für die Materialtestung eingeschätzt wird, sollten Pulpendruck und artifizielles Altern in der Materialstestung eingesetzt werden.

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7. Appendices

Appendix 1 SEM micrographs:

Figures 11-18 present examples of the interfaces and fractured surfaces of dentin bonded with the four luting resins under pulpal pressure and without pulpal pressure simulated conditions using SEM. Figures 19 and 20 present examples of the interfaces and fractured surfaces of enamel bonded with the four luting resins using SEM.

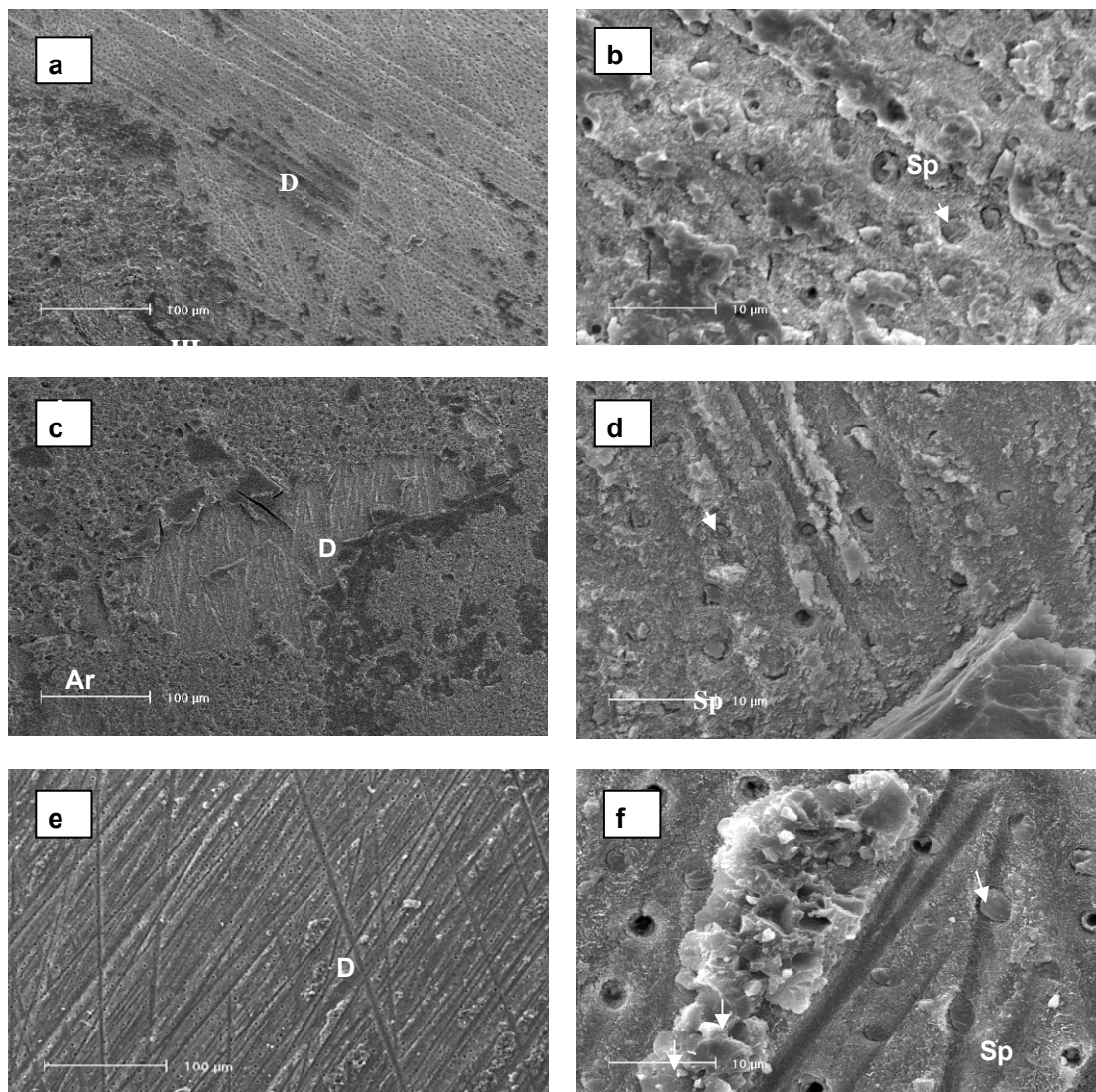


Fig. 11 SEM micrograph of the debonded dentin side of DCE-N group showing representative fractured beams after 3-days storage (Figs. 11a, b), after 30-days storage with 5,000 TC (Figs. 11c, d) and after 90-days with 15,000 TC (Figs. 11e, f). Low magnification view of 550x (Figs. 11a, c, e) revealed diagonal scratch lines coming from the smear-layer preparation confirming that the interface failed adhesively involving dentin and resin on the debonded surface of the specimen. Adhesive resin prevailed in some areas (Figs. 11a, c). Bar =100 µm. A high

magnification view of 5,000x of Figs. 11 b, d, f indicated that the hybrid layer were thin (Figs. 11a, c, e). Fracture occurred on the base of hybrid layer in the dentin-adhesive interface. Note the widened tubule orifices (Figs. 11f) and collagen around the tubule orifices (Figs. 11b, d, f). Smear plugs were observed in dentinal tubules (Figs. 11b, d). Ar: Adhesive resin. HL: hybrid layer. Dt: dentinal tubule. Sp: smear plug. Bar =10µm

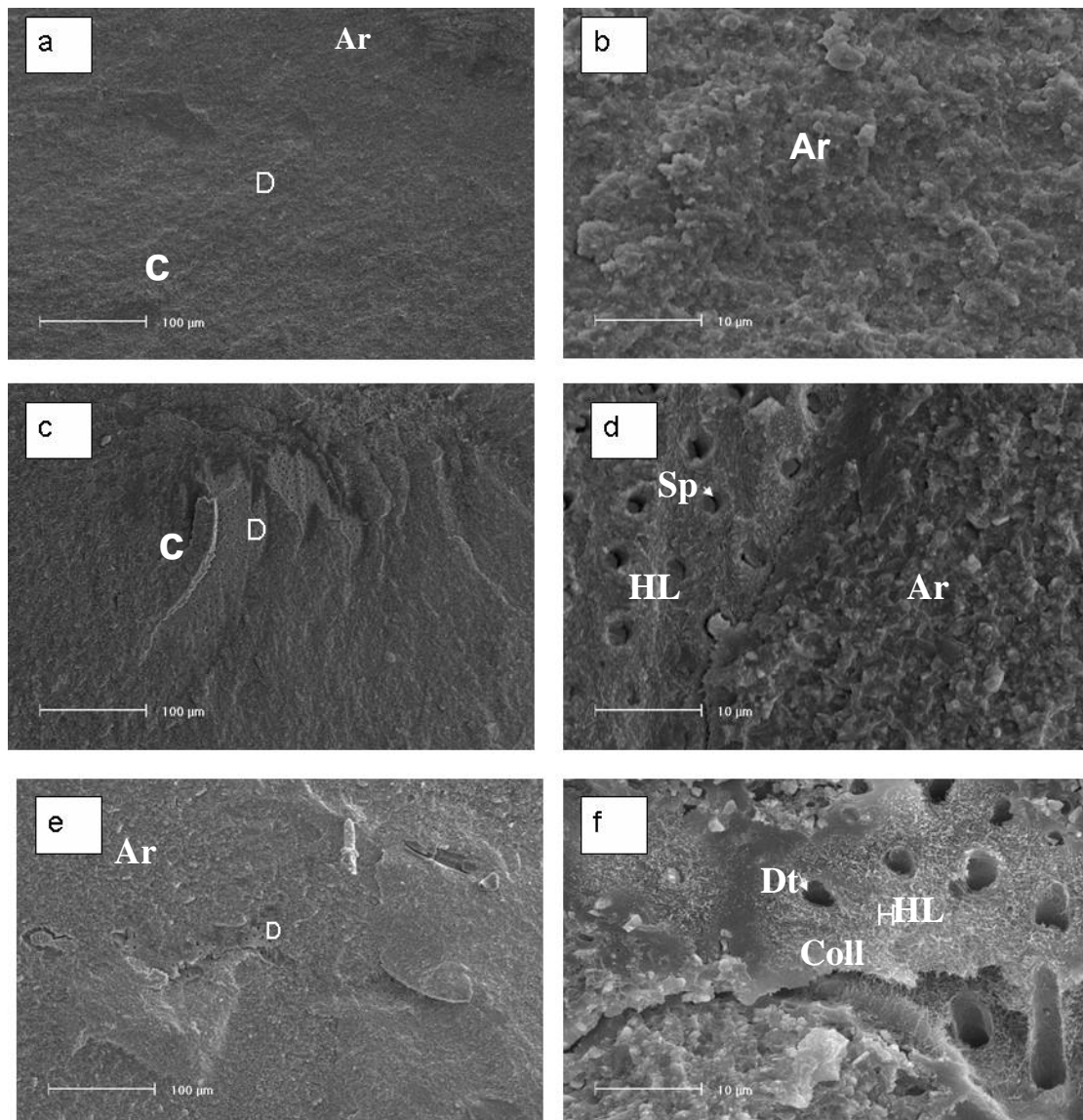


Fig. 12 SEM micrographs of the debonded dentinal side of DMA-N specimens. Representative fractured beams after 3-days storage (Figs. 12a, b), after 30-days storage with 5,000 TC (Figs. 12c, d) and after 90-days with 15,000 TC (Figs. 12e, f). Low magnification SEM micrograph 550x (Figs. 12a, c, e) showed the distribution of composite and the adhesive resin on the dentinal surface. A small area revealed traces of a hybrid layer. Bar =100µm. At a high magnification 5,000x (of Figs. 12b, d, f) failures were observed mostly

at the adhesive resin on the top of hybrid layer and small areas failed at the bottom of the hybrid layer and smear plugs blocked the tubules. The strong ability of the self-etching primer to demineralize dentin can be seen. Ar: Adhesive resin. HL: hybrid layer. Dt: dentinal tubule.

Sp: smear plug. Bar =10µm

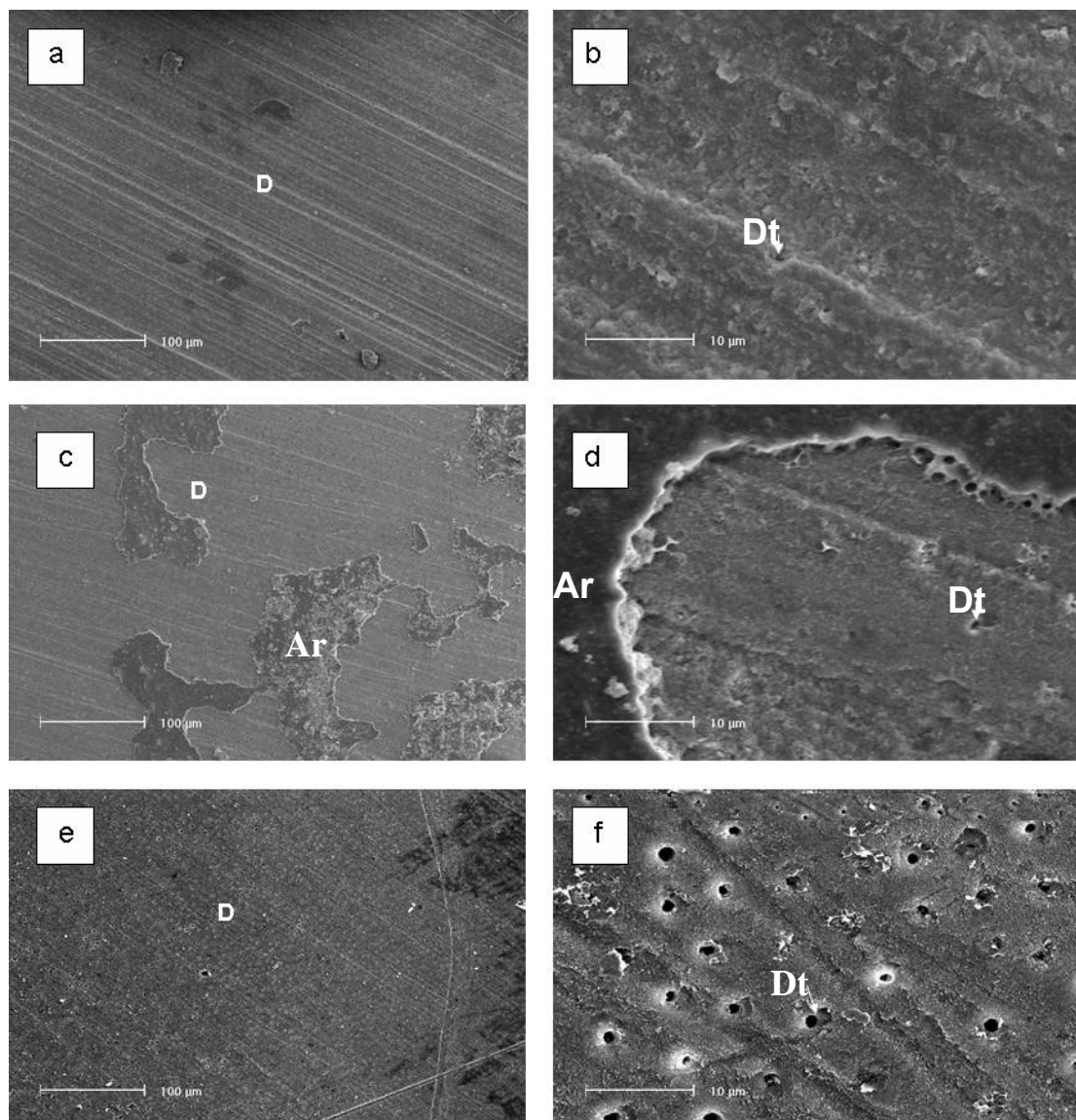


Fig.13 SEM micrographs of debonded dentinal sides of DMS-N specimens. Representative fractured beams after 3-days storage (Figs. 13a, b), after 30-days storage with 5,000 TC (Figs. 13c, d) and after 90-days storage with 15,000 TC (Figs. 13e, f). A low magnification view (Figs. 13a, c, e) showed the diagonal scratch lines on the surface of the specimen. Note some adhesive resin remaining on the dentinal surface after 30-days storage (Fig. 13c). Bar =100µm. High magnification view (Fig. 13f) indicated no obvious hybrid layer. The failure occurred on the top of hybrid layer and the luting resin layer (Fig. 13b, d). Higher

percentages of cohesive failures occurred within the luting resin. Scratches remaining from smear-layer preparation confirmed that the interface failed adhesively. Note that some tubules orifices are open after 90-days storage with 15,000 TC (Fig. 13f) and few obvious tubules orifices after 3-days and 30-days storage with 5,000 TC (Figs. 13b, d). MS shows a low demineralizing ability and infiltration of the dentinal tubules. Ar: Adhesive resin. Dt: dentinal tubule. Sp: smear plug. Bar =10µm

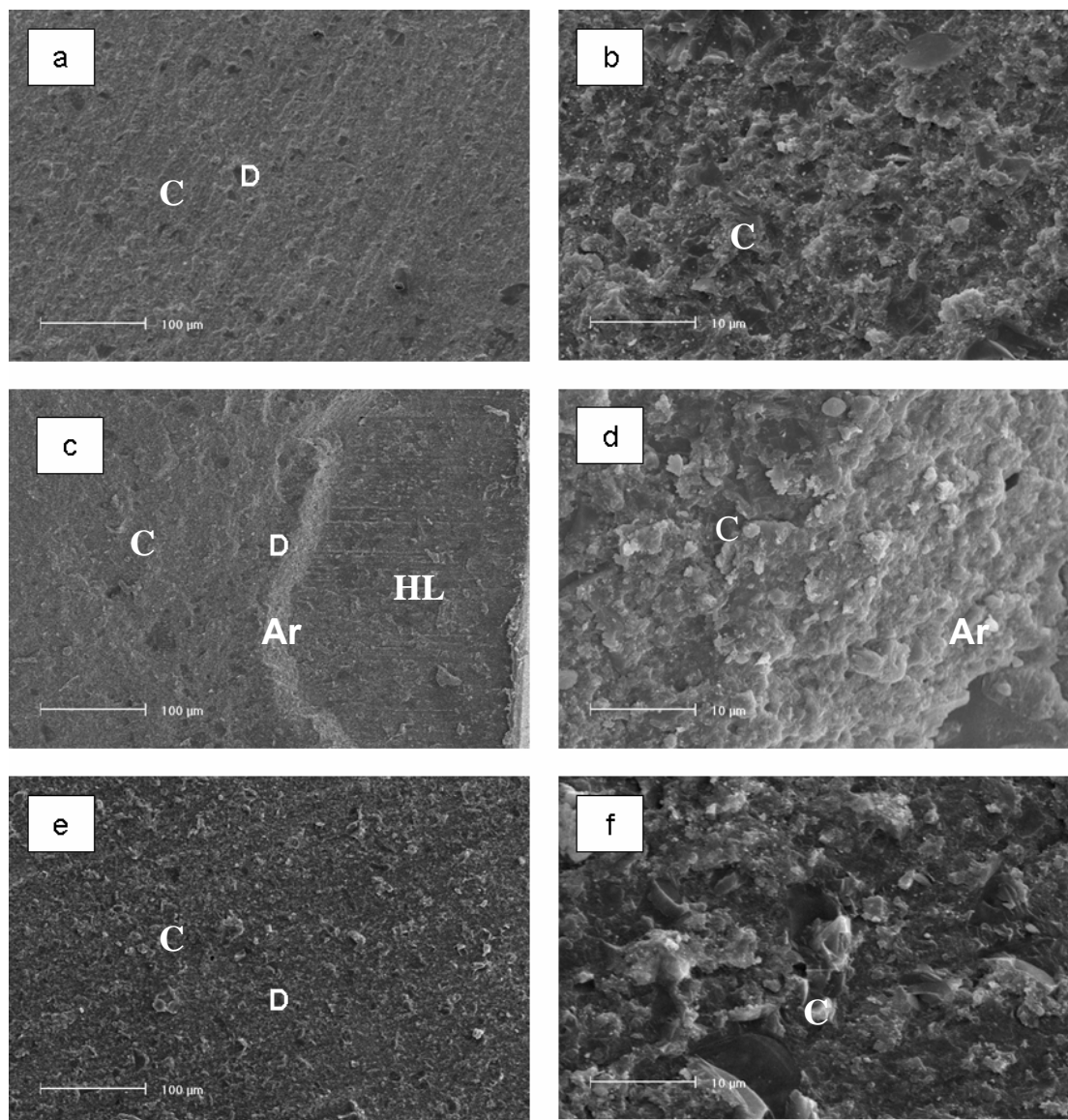


Fig. 14 SEM micrographs of debonded dentinal sides of a DRA-N specimens. Representative fractured beams after 3-days storage (Figs. 14a, b), after 30-days storage with 5,000 TC (Figs. 14c, d) and after 90-days storage with 15,000 TC (Figs. 14e, f). Low magnification view (Figs. 14a, c, e) observing the fractured area on the composite-resin interfaces. Fractures occurred on the top of hybrid layer (Fig. 14c). Bar =100µm. A high magnification view (Fig. 14b, d, f)

observing the region labeled “D” in Figs. 14 a, c, e revealed adhesive failures along the composite-resin interfaces. Etching and rinsing with the three-step luting resin system indicated strong demineralization on the dentinal surface. C: composite. Ar: Adhesive resin. HL: hybrid layer. Bar =10µm.

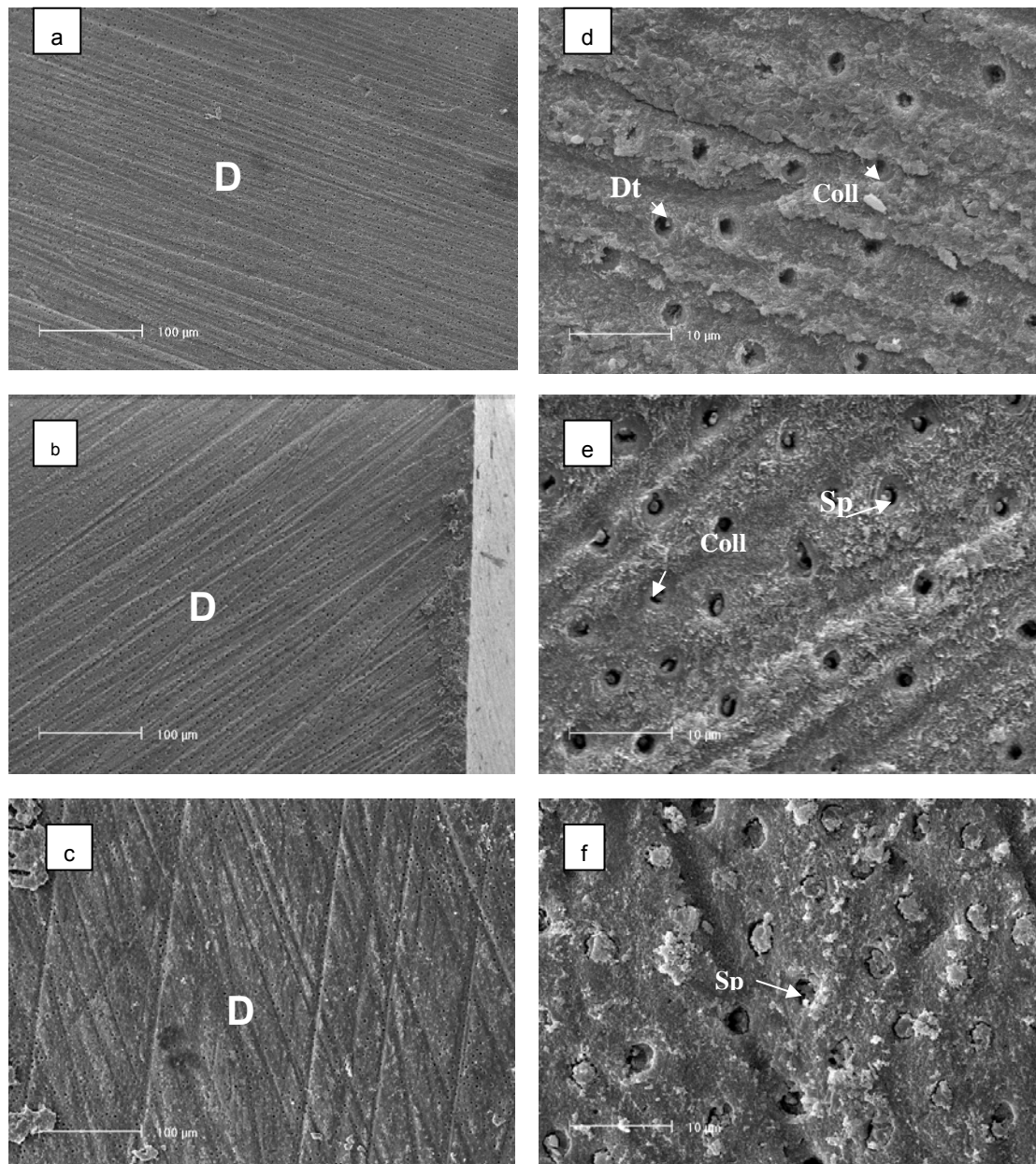


Fig. 15 SEM micrograph of debonded dentin sides of DCE-PP group. Representative fractured beams after 3-days storage (a, d) after 30-days storage with 5,000 TC (b, e) and in 90-days with 15,000 TC (c, f). A low magnification view (Figs. 15a, c, e) shows the diagonal scratch lines on the surface of specimen. Bar =100µm. A high magnification view for Figs. 15b, d, f indicated by the region labeled “D” in Fig. 15a, b, c dentinal tubules were obliterated with smear plugs. In Figs. 15d, e and f the dentin near the open dentinal tubules was

demineralized by the self-etching ED primer. The ED primer seemed to have dissolved the smear layer. Note the collagen of the dentinal surface in the 3-days group and in the 30-days group (Figs. 15d, e) and the absence of collagen, which was substituted by irregular debris in the 90-days group (Fig. 15f). Ar: Adhesive resin. Dt: dentinal tubule. Sp: smear plug Bar =10 μ m

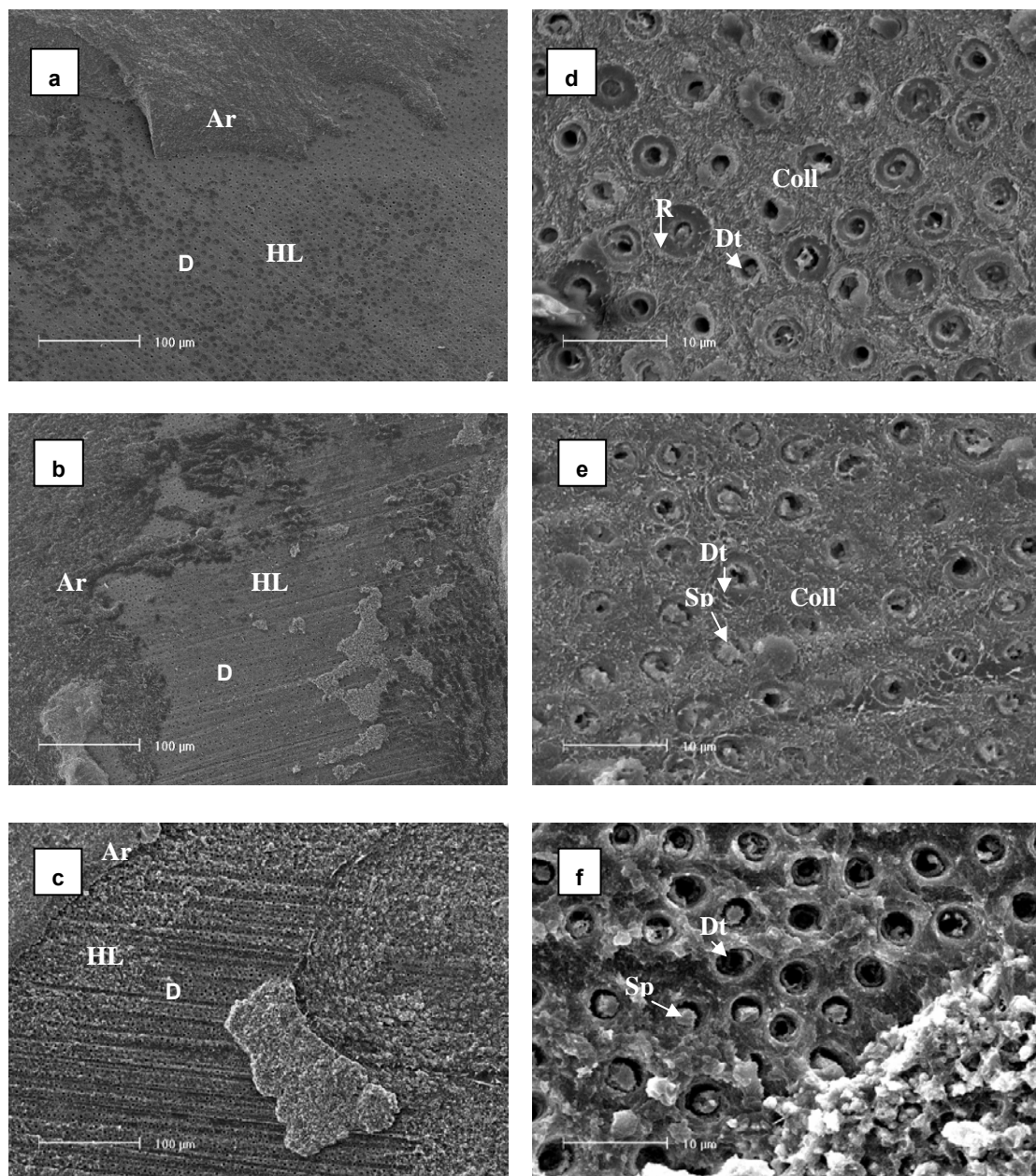


Fig. 16 SEM micrographs of the debonded dentinal side of DMA-PP group specimens. Representative fractured beams after 3-days storage (a, d), after 30-days storage with 5,000 TC (Figs. 16b, e) and after 90-days storage with 15,000 TC (Figs. 16c, f). Low magnification view (Figs. 16a, b, c) showed adhesive resin remnants on the dentinal surface. Bar =100 μ m. A high magnification view was taken from the region labeled “D” of Figs. 16a, b, c. A

presence of collagen fibrils free of resin in Figs. 16b, d indicated that the interface fractured more on the base than the top of hybrid layer. Dentinal tubules were obstructed with smear plugs (Sp), the dentin near the dentinal tubules (R, point) was demineralized more by the self-etching primer than the dentin of the periphery (Figs. 16d, e). Ar: Adhesive resin. HL hybrid layer. Dt: dentinal tubule. Sp: smear plug. Bar =10µm

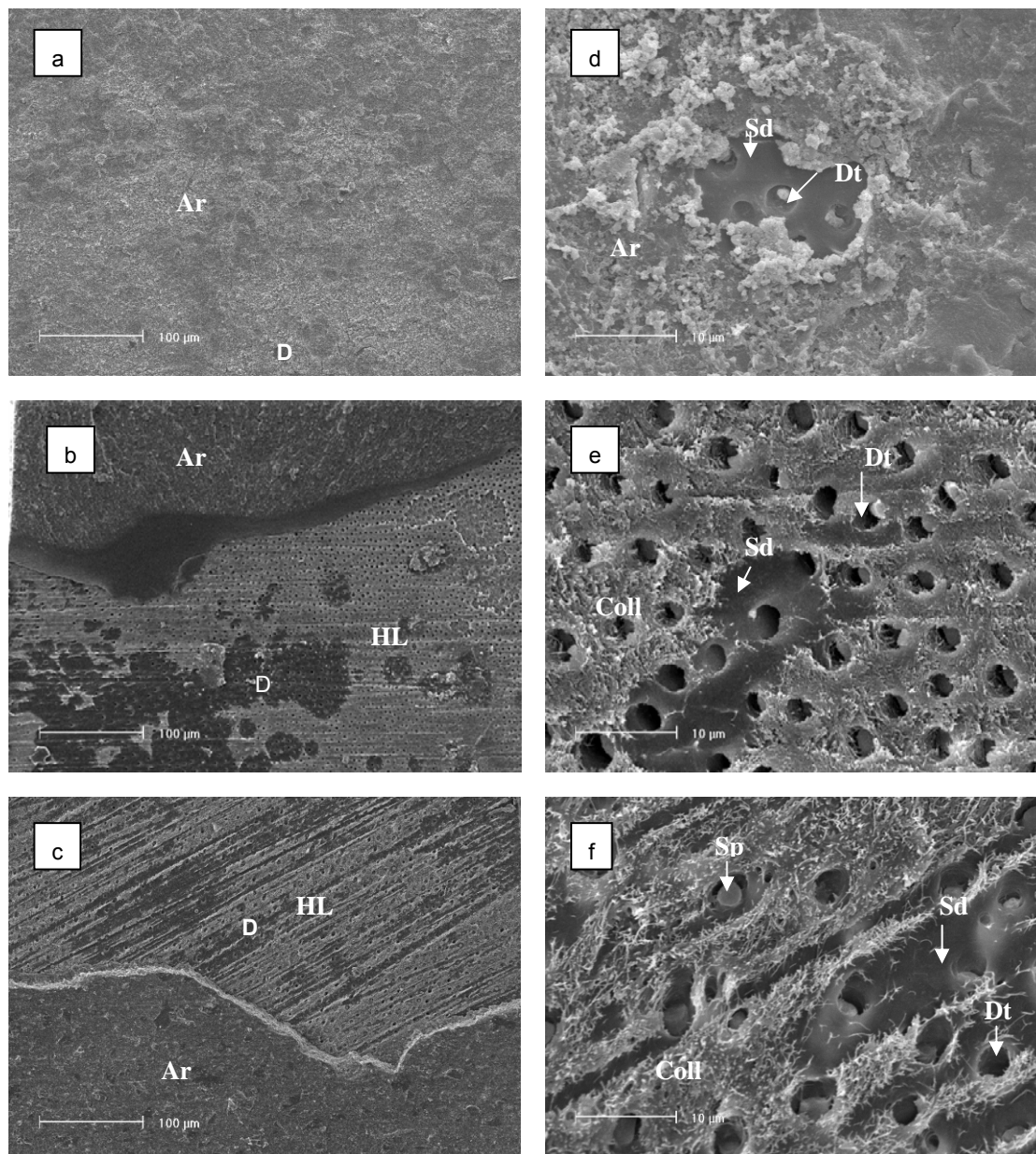


Fig.17 SEM micrographs of debonded dentinal sides of DRA-PP group specimens Representative fractured beams after 3-days storage (Figs. 17a, b), after 30-days storage with 5,000TC (Figs. 17c, d) and after 90-days storage with 15,000TC (Figs. 17 e, f). Low magnification view (Figs. 17a, b, c) revealed adhesive failures between the adhesive and the

luting resin and irregular small areas of empty voids of smooth dentin on the dentin surface. Bar =100µm. A high magnification view observed the region labeled “D” in (Fig. 17a, b, c). Some smear plugs appeared to be removed and the. Smooth dentin surfaces appeared to be connected with the open tubule orifices without collagen fibrils. The polymerization of the luting resins seems to have been interfered by water fluid on the dentin surface. Noted the widened dentinal tubular orifices (Fig. 17d, e, f). Ar: adhesive resin. HL: hybrid layer. Dt: dentinal tubule. Sp: smear plug, Sd: smooth dentin. Bar =10µm.

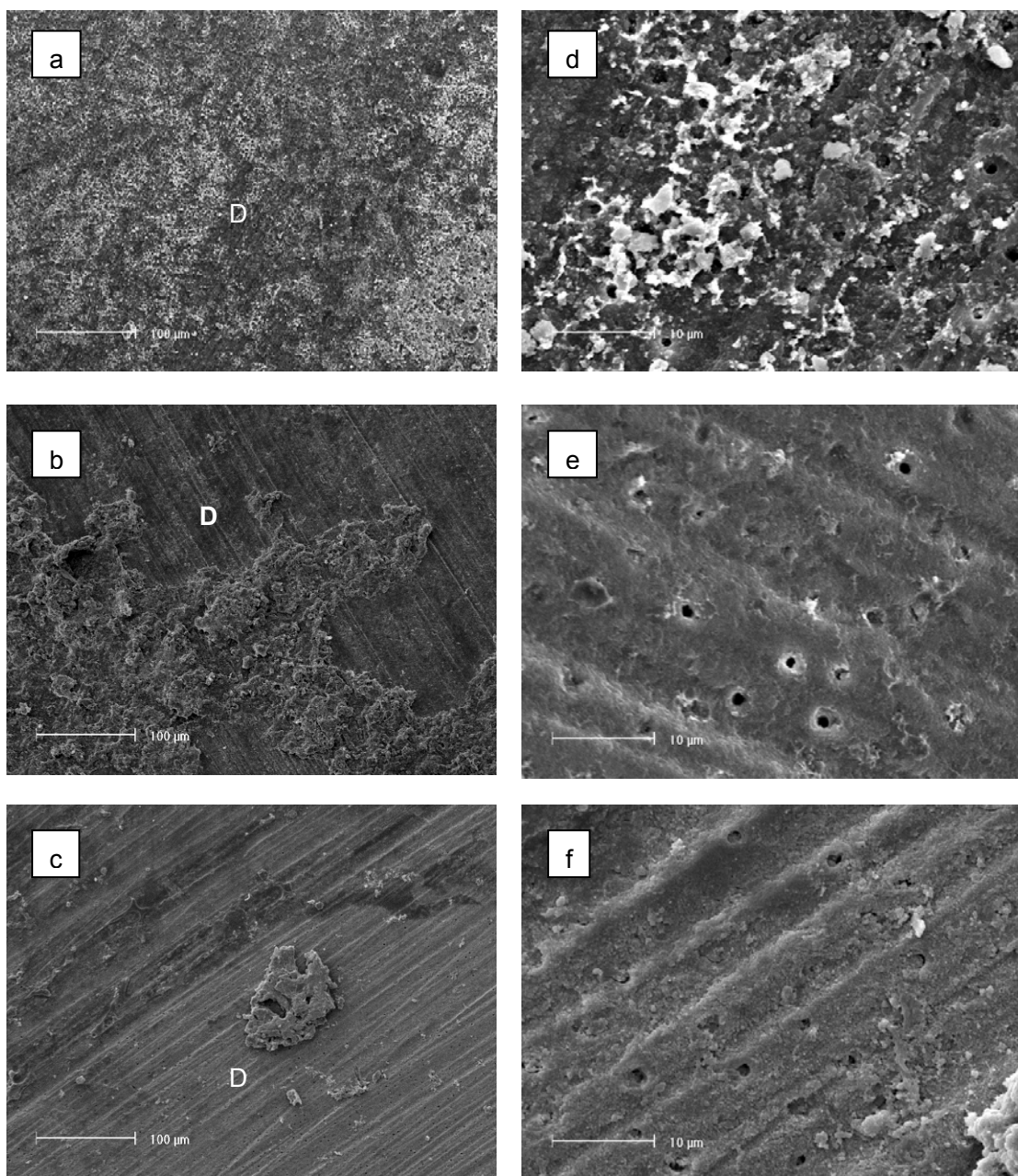


Fig.18 SEM micrographs of debonded dentinal sides of DMS-PP group specimens. Beams after 3-days storage (Figs. 18a, b), after 30-days storage with 5,000 TC (Figs. 18c, d) and after 90-days with 15,000 TC (Figs. 18e, f). Low magnification view (Figs 18a, b, c) showed after 30 days storage with 5,000TC (b) and 90-days storage with 15,000TC (c), MS resin was emulsionized and the diagonal scratch lines were obvious on the surface. Bar =100 μ m. High magnification views (Figs. 18d, e, f) were taken from the region labeled “D” of (a, b, c). The dentin was demineralized less with small opening of the dentinal tubules (Figs. 13d, e, f). Dentin debris indicated the incomplete dentin-resin bonding in the interface. Higher percentages of cohesive failure occurred within the luting resin. Scratches remaining from smear-layer preparation confirmed that a faintish degree of demineralization of MS on the dentinal surface. Dt: dentinal tubule. Rd: resin debris. Bar =10 μ m

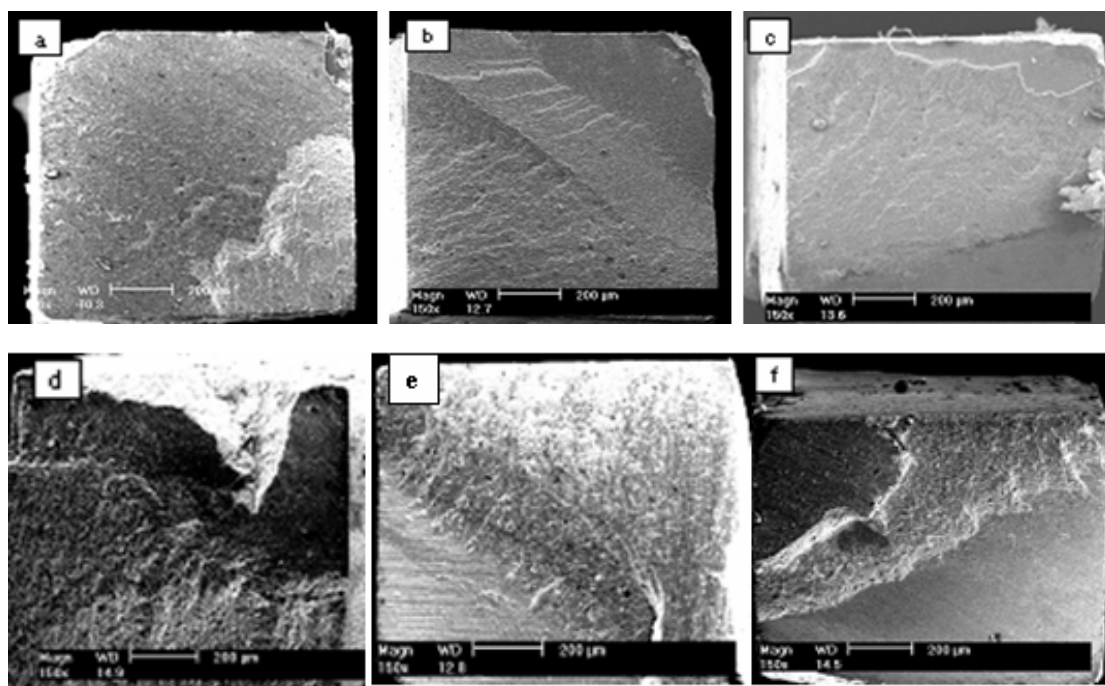


Fig.19 SEM micrograph of debonded enamel sides of EMA group specimens. A representative fractured beam after 3-days storage (Fig. 19a), after 30-days storage with 5,000 TC (Fig. 19b) and after 90-days storage with 15,000 TC (Fig. 19c). Low magnification view (Fig. 19a, b, c) showed the fracture failure mostly occurred on the mixed composite-resin interfaces and the luting resin layer. Bar =100 μ m

SEM micrograph of a debonded enamel side of group CE showing representative fractured

beams after 3-days water storage (Fig. 19d), after 30-days (Fig. 19e) and after 90-days (Fig. 19f). Low magnification view (Figs. 19a, b, c) showed, that approximately half of the failures were located at the luting resin and the resin-enamel interface. Bar =200 μ m.

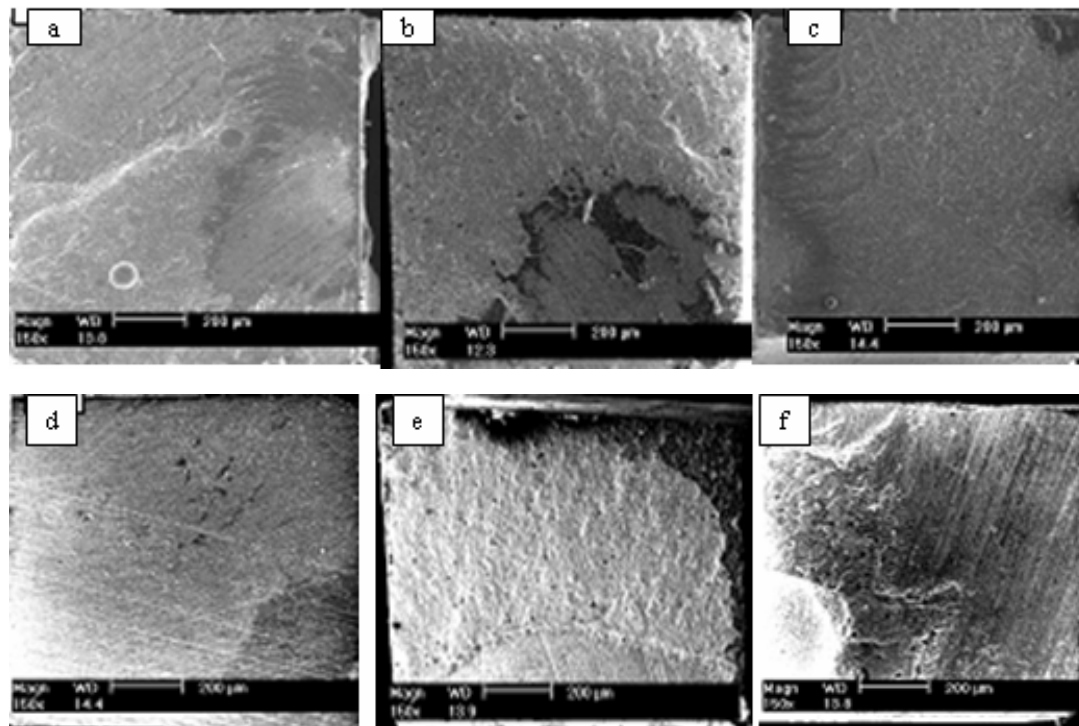


Fig. 20 SEM micrographs of debonded enamel sides of RA group specimens. Representative fractured beams in the 3-days group (Fig. 20a), in the 30-days group (Fig. 20b) and in the 90-days group (Fig. 20c). Low magnification view (Figs. 20a, b, c) showed the fracture failure mostly occurred on the composite-resin interfaces. The cohesive failure area increased in the 90-days group. Bar =200 μ m. SEM micrograph of the debonded enamel side of group MS showing a representative fractured beam in the 3-days group (Fig. 20d), in the 30-days group (Fig. 20e) and in the 90-days group (Fig. 20f). A low magnification view (Figs. 20d, f) showed that the fracture failures mostly occurred on the dentin-resin interfaces. Bar =200 μ m

Appendix 2 Bonding tests of Multilink Automix (Ivoclar Vivadent) dentin groups

Sample	Group: 001 MA +AB 3-days-dentin-Series				Sample	Group: 002 MA+AB 30-days dentin Series				Sample	Group: 003 MA+AB 90-days dentin Series			
	(22)	MPa	% - Failure mode			[N]	MPa	% - Failure mode			(22)	MPa	% - Failure mode	
			Adhesive*	Cohesive**				Adhesive*	Cohesive**				Adhesive*	Cohesive**
1	31.8	31.8	6.	93.9	1	62.8	62.88	0	100	1	28.9	28.9	4	96
2	41.5	41.5	5.	94.8	2	28.6	28.6	10.4	89.6	2	41.0	41.0	2.8	97.2
3	50.8	50.8	0	100	3	67.1	67.1	0	100	3	36.8	36.8	0	100
4	62.0	62.0	0	100	4	41.3	41.3	5	95	4	11.3	11.3	0	100
5	17.5	17.5	0	100	5	29.2	29.2	1	99	5	12.5	12.5	21	79
6	46.8	46.8	0.6	99.4	6	54.3	54.3	10.1	89.9	6	22.0	22.0	4	96
7	63.5	63.5	34.4	65.6	7	14.4	14.4	63.0	37	7	58.4	58.4	54.7	45.3
8	43.8	43.8	0	100	8	53.4	53.4	0	10	8	41.0	41.0	0	100
9	36.7	36.7			9	56.9	56.9			9	60.8	60.8		
10	58.0	58.0			10	21.6	21.6			10	30.5	30.5		
11	28.8	28.8			11	27.5	27.5			11	35.3	35.3		
12	43.8	43.8			12	48.2	48.2			12	41.6	41.6		
MA without PP		Mean value in MPa			SD		Med							
3d-dentin-Series		43.7			10.4		43.8							
30d-dentin-Series		42.1			15.0		44.8							
90d-dentin-Series		34.4			11.6		36.1							

Appendix 3 Bonding tests of Multilink Sprint (Ivoclar Vivadent) dentin groups

Sample	Group: 004 MS 3-days-dentin-Series				Sample	Group: 005 MS 30-days dentin Series				Sample	Group: 006 MS 90-days dentin Series			
	% - Failure mode					% - Failure mode					% - Failure mode			
	(22)	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	Cohesive**
1	18.5	18.5	100	00	1	5.1	5.1	100	0	1	1.7	1.7	100	00
2	15.8	15.8	100	00	2	20.9	20.9	85	15	2	11.3	11.3	100	00
3	44.4	44.4	94.1	4.9	3	3.7	3.7	100	0	3	1.4	1.4	100	00
4	25.9	25.9	100	90	4	12.3	12.3	94	6	4	26.2	26.2	40	60
5	39.2	39.2	100	0	5	9.1	9.1	100	0	5	1.6	1.6	100	0
6	14.8	14.8	100	0	6	24.1	24.1	87	13	6	0	0	100	0
7	31.7	31.7	100	0	7	4.9	4.9	100	0	7	0	0	100	0
8	5.0	5.0	100	0	8	9.6	9.6	100	0	8	0	0	100	0
9	10.5	10.5			9	2.9	2.9			9	0	0		
10	10.4	10.4			10	10.0	10.0			10	0	0		
11	12.4	12.4			11	9.3	9.3			11	0	0		
12	34.94	34.94			12	19.8	19.8			12	0	0		
MS without pp			Mean value	SD	Med									
3d-dentin-Series			21.9	11.0	17.1									
30d-dentin-Series			10.9	5.5	9.4									
90d-dentin-Series			3.5	5.1	0									

Appendix 4 Bonding tests of RelyX ARC(3M Espe) dentin groups

Sample	Group: 007 RA+ASB 3-days-dentin-Series				Sample	Group: 008 RA+ASB 30-days dentin Series				Sample	Group: 009 RA+ASB 90-days dentin Series				
			% - Failure mode					% - Failure mode					% - Failure mode		
	[N]	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	Cohesive**	
1	42.2	42.2	0	100	1	54.1	54.1	3	97	1	18.0	18.0	66.4	33.6	
2	55.6	55.6	0	100	2	65.4	65.4	11	89	2	60.8	60.8		100	
3	58.4	58.4	0	100	3	11.5	11.5	44.7	55.3	3	84.6	84.6		100	
4	24.4	24.4	0	100	4	47.1	47.10	1	99	4	53.8	53.8	25	75	
5	50.0	50.1	0	100	5	24.5	24.5	5	95	5	68.8	68.8		100	
6	50.5	50.6	0	100	6	53.6	53.6	3	97	6	54.0	54.0	22	78	
7	35.7	35.8	0	100	7	19.9	19.9	13	87	7	59.1	59.1		100	
8	64.6	64.7	0	100	8	67.6	67.6	5	95	8	15.0	15.0	58	35	
9	31.3	31.3			9	31.4	31.4			9	36.2	36.2			
10	53.9	53.9			10	63.0	63.0			10	16.6	16.6			
11	28.7	28.7			11	45.4	45.4			11	48.7	48.7			
12	43.0	43.0			12	30.0	30.0			12	32.6	32.6			
RA without pp				Mean value[N/mm ²]				SD				Med			
3d-dentine-Series				44.9				10.6				46.6			
30d-dentine-Series				42.8				16.1				46.3			
90d-dentine-Series				45.7				18.3				51.3			

Appendix 5 Bonding tests of Clearfil Esthetic cement (Kuraray) dentin

Sample	Group: 007 CE+ED 3-days-dentin-Series				Sample	Group: 008 CE+ED 30-days dentin Series				Sample	Group: 009 CE+ED 90-days dentin Series			
	[N]	MPa	% Failure mode			[N]	MPa	% Failure mode			[N]	MPa	% Failure mode	
			Adhesive*	Cohesive**				Adhesive*	Cohesive**				Adhesive*	Cohesive**
1	10.8	10.8	100		1	11.0	11.0	100	0	1	60.5	60.5	46	54
2	27.5	27.5	0	100	2	25.7	25.7	24	76	2	18.2	18.2	77	23
3	40.8	40.8	0	100	3	36.9	36.9	35	65	3	23.0	23.0	50	50
4	27.8	27.8	0	100	4	24.0	24.0	66	34	4	11.6	11.6	50	50
5	38.1	38.1	0	100	5	60.0	60.0	4	96	5	17.4	17.4	5	95
6	13.9	13.9	0	100	6	45.3	45.3	5	95	6	38.2	38.2	50	50
7	52.4	52.4	0	100	7	31.7	31.7	4	96	7	19.7	19.7	100	0
8	16.6	16.6	0	100	8	30.5	30.5	5	95	8	21.5	21.5	50	50
9	11.0	11.0			9	18.2	18.2			9	23.6	23.6		
10	10.7	10.7			10	12.0	12.0			10	1.9	1.9		
11	34.7	34.7			11	13.1	13.1			11	26.4	26.4		
12	47.8	47.8			12	14.1	14.1			12	34.6	1.9		
CLE without pp		Mean value in MPa			SD		Med							
3d-dentin-Series		27.7			12.7		27.6							
30d-dentin-Series		26.9			11.6		24.8							
90d-dentin-Series		24.8			10.1		22.3							

Appendix 6 Bonding tests of RelyX ARC (3M Espe) enamel groups

Sample	Group: 013 RAC+ASB 3-days-enamel-Series				Sample	Group: 014 RAC+ASB 30-days enamel Series				Sample	Group: 015 RAC+ASB 90-days enamel Series			
	% - Failure mode					% - Failure mode					% - Failure mode			
	[N]	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	Cohesive**
1	22.9	22.9	71.6	29.4	1	42.7	42.7	26	74	1	43.5	43.5	0	enamel
2	44.2	44.2	51.3	48.7	2	15.0	15.0	40.2	59.8	2	34.3	34.3	0	enamel
3	24.3	24.3	55.5	44.5	3	23.4	23.4	0	enamel	3	46	46	28.5	71.5
4	21.9	21.9	0	enamel	4	20.6	20.6	0	enamel	4	52.3	52.3	4.4	95.6
5	22.0	22.0	18	82	5	39.1	39.1	18	82	5	33.4	33.4	41.3	68.7
6	36.7	36.7	0	enamel	6	34.5	34.5	27.7	72.3	6	34.1	34.1	22.5	77.5
7	33.5	33.5	0	enamel	7	13.2	13.2	72.5	27.5	7	42.7	42.7	6.3	93.7
8	29.3	29.3	0	enamel	8	19.1	19.1	83	17	8	51.0	51.0	27.7	92.3
RA enamel group		Mean value		SD	Med									
3d- enamel -Series		29.3		6.5	26.8									
30d-enamel -Series		25.5		9.6	22									
90d-enamel -Series		42.1		6.1	43.1									

Appendix 7 Bonding test of Multilink Sprint (Ivoclar Vivadent)

Sample	Group: 016 MS 3-days-enamel-Series				Sample	Group: 017 MS 30- days enamel Series				Sample	Group: 018 MS 90-days enamel Series			
	% - Failure mode					% - Failure mode					% - Failure mode			
	[N]	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	Cohesive**
1	26.2	26.2	100	0	1	18.9	18.9	0	100	1	23.2	23.2	35.9	64.1
2	22.3	22.3	100	0	2	18.9	18.9	89.5	10.5	2	15.1	15.1	57.2	42.8
3	16.5	16.5	100	0	3	8.6	8.6	94.1	5.9	3	8.6	8.6	70	30
4	31.2	31.2	56.7	43.3	4	24.9	24.9	29.3	70.7	4	28.0	28.0	29.2	70.8
5	7.1	7.1	100	0	5	26.8	26.8	6.8	93.2	5	20.3	20.3	40.6	59.4
6	7.4	7.4	100	0	6	18.1	18.1	38.1	61.9	6	11.5	11.5	40.6	49.4
7	9.8	9.8	100	0	7	9.1	9.1	100	0	7	14.3	14.3	44.8	56.2
8	18.1	18.1	77.1	22.9	8	27.7	27.7	10.9	89.1	8	6.4	6.4	100	0
MS enamel group		Mean value in MPa		SD	Med									
3d-enamel-Series		17.3		7.1	17.3									
30d-enamel-Series		19.1		5.5	18.9									
90d-enamel-Series		15.9		5.9	14.7									

Appendix 8 Bonding tests of Clearfil Esthetic cement (Kuraray) enamel groups

Sample	Group: 019 CE+ED 3-days-enamel-Series				Sample	Group: 020 CE+ED 30-days enamel Series				Sample	Group: 021 CE+ED 90-days enamel Series				
	% - Failure mode					% - Failure mode					% - Failure mode				
	[N]	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	Cohesive**	
1	44.1	44.1	0	100	1	21.5	21.4	34.9	65.1	1	21.0	21.0	10	90	
2	37.7	37.7	33.8	67.2	2	17.4	17.4	0	enamel	2	25.0	25.0	17.2	82.8	
3	27.6	27.6	20.6	79.4	3	33.2	33.1	0	enamel	3	33.0	33.0	13.5	86.5	
4	14.5	14.5	0	enamel	4	9.7	9.7	100	0		4	12.3	12.3	67.8	32.2
5	32.8	32.8	0	enamel	5	26.6	26.6	70	30	5	13.6	13.6	81.9	18.9	
6	41.0	41.0	0	enamel	6	37.6	37.6	16.9	76.7	6	23.5	23.5	0	enamel	
7	27.0	27.0	0	enamel	7	42.8	42.8	22.6	77.4	7	16.7	16.7	0	enamel	
8	44.1	44.1	0	enamel	8	36.8	36.5	17.4	82.6	8	22.3	22.3	0	enamel	
CLE		Mean value MPa		SD	Med										
3d- enamel - Series		33.6		8.1	35.3										
30d-enamel- Series		28.2		9.4	29.9										
90d-enamel- Series		21.0		5.1	21.7										

Appendix 9 Bonding tests of Multilink Automix (Vivadent Ivoclar) enamel groups

Sample	Group: 022 MA+AB 3-days-enamel-Series				Sample	Group: 023 MA+AB 30-days enamel Series				Sample	Group: 024 MA+AB 90-days enamel Series			
	% Failure mode					% Failure mode					% Failure mode			
	[N]	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	Cohesive**
1	10.2	10.2	32.8	77.2	1	33.8	33.8	0	enamel	1	25.4	25.4	28.2	71.8
2	27.4	27.4	63.3	36.7	2	42.7	42.7	23.3	76.7	2	28.3	28.3	22.1	77.9
3	17.4	17.4	0	enamel	3	16.8	16.8	35.7	64.3	3	29.1	29.1	14.6	85.4
4	24.4	24.4	14.1	85.9	4	28.1	28.1	7.4	93.6	4	20.1	20.1	33.7	66.3
5	16.6	16.6	47.6	52.4	5	14.4	14.4	24.4	75.6	5	24.9	24.9	0	enamel
6	23.8	23.8	0	enamel	6	16.8	16.8	41.1	58.9	6	28.5	28.5	0	enamel
7	20.6	20.6	0	enamel	7	21.3	21.3	38.7	61.3	7	16.1	16.1	0	enamel
8	24.0	24.0	0	enamel	8	14.9	14.9	57.9	42.1	8	36.7	36.7	0	enamel
MA enamel group				Mean value in MPa	SD		Med							
3d- enamel -Series				20.5	4.3		22.2							
30d- enamel -Series				23.6	8.4		19.0							
90d- enamel -Series				26.1	4.5		26.8							

Appendix 10 Bonding tests of Multilink Automix (Ivoclar Vivadent) with pulpal pressure dentin group

Sample	Group: 025 MA+AB (With Pulpal pressure) 3-days-dentin-Series				Sample	Group: 026 MA+AB (With Pulpal pressure) 30-days dentin Series				Sample	Group: 027 MA+AB (With Pulpal pressure) 90-days dentin Series			
			% - Failure mode					% - Failure mode					% - Failure mode	
	[N]	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	Cohesive**
1	9.9	9.9	55	45	1	46.5	46.5	0	100	1	34.7	34.7	0	100
2	38.9	38.9	0	100	2	12.1	12.1	50	50	2	49.4	49.4	0	100
3	33.1	33.1	2	98	3	36.6	36.6	15	85	3	56.4	56.4	0	100
4	79.1	79.1	0	100	4	11.1	11.1	70	30	4	23.5	23.5	2	98
5	16.2	16.2	65	35	5	36.0	36.0	50	50	5	11.1	11.1	3	97
6	60.2	60.2	0	100	6	16.5	16.5	0	100	6	19.1	19.1	30	70
7	40.9	40.9	0	100	7	31.5	31.5	10	90	7	24.1	24.1	20	80
8	51.5	51.5	0	100	8	69.6	69.6	0	100	8	35.7	35.7	4	96
9	12.3	12.3			9	51.0	51.0			9	50.4	50.4		
10	59.21	59.21			10	51.0	51.0			10	24.1	24.1		
11	12.4	12.4			11	53.0	53.0			11	31.7	31.7		
12	42.64	42.64			12	49.5	49.5			12	40	40		
MA with pp				Mean value in MPa				SD		Med				
3d-dentin-Series				38.0				17.7		47.0				
30d-dentin-Series				41.1				13.3		46.5				
90d-dentin-Series				33.40				11.07		33.29				

Appendix 11 Bonding test of Clearfil Esthetic cement (Kuraray) with pulpal pressure dentin group

Sample	Group: 028 CLE+ED (With Pulpal pressure) 3-days-dentin-Series				Sample	Group: 029 CLE+ED (With Pulpal pressure) 30-days dentin- Series				Sample	Group: 029 CLE+ED (With Pulpal pressure) 90-days dentin- Series			
	% - Failure mode					% - Failure mode					% - Failure mode			
	[N]	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	Cohesive**		[N] ²	MPa	Adhesive*	Cohesive**
1	16.8	16.8	90	10	1	12.7	12.7	100	0	1	5.0	5.0	40	60
2	24.9	24.9	100	0	2	8.9	8.9	100	0	2	5.4	5.4	50	50
3	9.1	9.1	100	0	3	15.5	15.5	100	0	3	1.5	1.5	100	0
4	12.9	12.9	4	96	4	6.7	6.7	80	20	4	3.7	3.7	40	60
5	60.4	60.4	0	100	5	26.2	26.2	80	20	5	0.7	0.7	100	0
6	18.6	18.6	50	50	6	41.9	41.9	80	20	6	1.1	1.1	100	0
7	13.9	13.9	100	0	7	40.0	40.0	90	10	7	0	0	0	0
8	29.0	29.0	70	30	8	57.5	57.5	100	0	8	8.4	8.4	0	100
9	18.6	18.6			9	40.6	40.6			9	0	0	0	0
10	31.1	31.1			10	34.7	34.7			10	0	0	0	0
11	12.5	12.5			11	21.8	21.8			11	0	0	0	0
12	7.6	7.6			12	49.5	49.5			12	0	0	0	0

CLE with pp	Mean value MPa	SD	Med
3d-dentin-Series	21.3	10.0	17.3
30d-dentin-Series	29.6	14.3	30.4
90d-dentin-Series	2.1	2.3	0.9

Appendix 12 Bonding test of Multilink Sprint (Vivadent Ivoclar) with pulpal pressure dentin groups

Sample	Group: 031 MS (With Pulpal pressure) 3-days dentin- Series				Sample	Group: 032 MS (With Pulpal pressure) 30-days dentin- Series				Sample	Group: 033 MS (With Pulpal pressure) 90-days dentin- Series			
	% - Failure mode					% - Failure mode					% - Failure mode			
	[N]	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	cohesive**		[N]	MPa	Adhesive*	cohesive**
1	0	0	0	0	1	0	0	0	0	1	0	0	0	0
2	0	0	0	0	2	0	0	0	0	2	0	0	0	0
3	0	0	0	0	3	0	0	0	0	3	0	0	0	0
4	0	0	0	0	4	0	0	0	0	4	0	0	0	0
5	0	0	0	0	5	0	0	0	0	5	0	0	0	0
6	0	0	0	0	6	0	0	0	0	6	0	0	0	0
7	0	0	0	0	7	0	0	0	0	7	0	0	0	0
8	0	0	0	0	8	0	0	0	0	8	0	0	0	0
9	0	0	0	0	9	0	0	0	0	9	0	0	0	0
10	0	0	0	0	10	0	0	0	0	10	0	0	0	0
11	0	0	0	0	11	0	0	0	0	11	0	0	0	0
12	0	0	0	0	12	0	0	0	0	12	0	0	0	0
MS dentin group with pp				Mean value in MPa		SD		Med						
3d-dentin-Series				0		0		0						
30d-dentin-Series				0		0		0						
90d-dentin-Series				0		0		0						

Appendix 13 Bonding test of RelyX ARC (3M Espe) with pulpal pressure dentin groups

Sample	Group: 034 RAC+ASB (With Pulpal pressure) 3-days-dentin-Series				Sample	Group: 035 RAC+ASB (With Pulpal pressure) 30-days dentin Series				Sample	Group: 036 RAC+ASB (With Pulpal pressure) 90-days dentin Series			
	% - Failure mode					% - Failure mode					% - Failure mode			
	[N]	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	Cohesive**		[N]	MPa	Adhesive*	Cohesive**
1	11.7	11.7	72.0	28	1	10.5	10.5	70	30	1	47.8	47.8	0	100
2	40.3	40.3	5.6	94.6	2	9.1	9.1	100	0	2	36.4	36.4	0	100
3	24.7	24.7	30.2	69.8	3	32.5	32.5	0	100	3	26.7	26.7	15	70
4	11.4	11.4	8.6	91.4	4	68.8	68.8	30	70	4	3.9	3.9	73	27
5	15.8	15.8	100	0	5	29.2	29.2	60	40	5	17.7	17.7	10	90
6	4.2	4.2	100	0	6	23.9	23.9	40	60	6	39.1	39.1	15	85
7	9.6	9.6	100	0	7	48.5	48.5	50	50	7	12.2	12.2	37	63
8	10.9	10.9	10	90	8	15.6	15.6	4	96	8	11.0	11.0	100	0
9	15.8	15.8			9	8.4	8.4			9	35.0	35.0		
10	14.1	14.1			10	28.1	28.1			10	36.7	36.7		
11	13.7	13.7			11	16.1	16.1			11	36.2	36.2		
12	10.8	10.8			12	14.3	14.3			12	16.7	16.7		
RA dentin group withpp		Mean value MPa		SD		Med								
3d-dentin-Series		15.2		5.9		12.7								
30d-dentin-Series		26.2		13.7		23.9								
90d-dentin-Series		26.6		11.9		30.8								

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