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In-vitro Study Analysing the Impact of Gluma Desensitizer on Long-term Shear Bond Strength to Dentin

INAUGURAL-DISSERTATION
zur Erlangung der Doktorwürde der Zahnmedizin
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1. Abstract

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2. Introduction

A series of studies have reported that the use of adhesive cementation techniques employing resin cements enhances clinical performance based on the increased resistence for fracture in glass-ceramic [1-8]. Resin cements improve retension and exhibit reduced dissolution in the oral environment in comparison to other traditional cements such as glass ionomer, zinc phosphate, and polycarboxylate cements [9,10]. Furthermore, they lead to less micro-leakage and have excellent asthetic shade-matching potential [10-14].

Conventional resin cements require a pretreatment of the dentin. Hence, there is a tendency in the dental field to move to self-adhesive resin cements. This is, because they are more simple and efficient to handle, thus saving the dentist's time [15]. Self-adhesive resin cements do not require a separate conditioning of the dentin, since their adhesive mechanism is based on the partial retention of the smear layer. The applied procedures are intended to provide sufficient acidity to penetrate the dentin through the smear layer and allow infiltration of the monomers inside the demineralised collagen network [16]. Due to this effect, priming and bonding can be eliminated. The initial shear bond strength on dentin of self-adhesive resin cements is comparable with conventional resin cements [17].

Depending on the size of the tooth, and the amount of tooth reduction, millions of dental tubules are exposed, after the enamel has been removed to allow room for indirect reconstructions [18]. Through the exposed dentin, and because of the open dentin tubules, the potential risk of pulpal injuries is increased [19]. This is due to the applied grinding pressure, the heat caused by the grinding process or the chemicals used. One method to reduce the sensitivity of a prepared tooth is by pretreatment with desensitizing solution. It has also been hypothesized that the sealing of dentin decreases the sensitivity of a prepared tooth resulting in less post-operative pain [20-23].

One of the most broadly studied desensitizer, Gluma Desensitizer, is a glutaraldehyde-based substance. The glutaraldehyde is used as a fixative [24]. It reduces dentin permeability and therby the dentin sensitivity. Moreover, glutaraldehyde-containing solutions disinfect dentin in vitro [25,26]. When Glutaraldehyde was combined with HEMA it showed a high shear bond strength [27]. The diffusion of monomers into dentin is likely to be accelerated by HEMA despite the precipitations [28]. As soon as the dentin tubules are closed, the hydrodynamic of dentin liquidity is reduced and the sensitivity decreases. The dentin adhesives build a hybrid layer and seal the dentin surface in one application. The obliteration of dentin is relevant for desensitization and Panavia21 with the dentin pre-treatment (ED Primer) is sealing the dentin surface and reduced the sensitivity. Both systems, Gluma Desensitizer and ED Primer (Panavia21), contain HEMA, which is characterized by a high wettability and good infiltration to the dentin tubules that produces a resin reinforced layer of dentin which, in turn, is assumed to be responsible for the improvements in shear bond strength as previously noted [29,30].

A study reported the effect of using higher shear bond strength self-adhesive resin cements when combined with Gluma Desensitizer than conventional resin cements combined with Gluma Desensitizer [31]. The conventional resin cement Panavia21 shows excellent shear bond strength to dentin [32,33]. However, several in vitro studies [11,31,34] have concluded that Panavia21 / ED Primer, combined with Gluma Desensitizer, causes a coagulation of the dentin fluid protein and plugs the tubule. Thus, a significant reduction of the bond strength values occurred. It is assumed that

ED Primer reacts directly with dentin, but when applied with soluble desensitizer, this reaction with dentin is blocked [34].

The aim of the present study was to investigate the initial and long-term effect of self-adhesive resin cements combined with and without Gluma Desensitizer on the shear bond strength. The primary aim was to test the hypothesis that the shear bond strength of self-adhesive resin cements combined with Gluma Desensitizer has higher bond strength and better long-term stability compared to conventional resin cement. The secondary aim was to test the hypothesis that the shear bond strength after thermocycling is lower than after water storage.

3. <u>Material and methods</u>

Two dual-cured self-adhesive resin cements (Rely X Unicem and G-CEM), and as a control one conventional resin cement including a pretreatment of the dentin (Panavia 21), were investigated in this study. Gluma Desensitizer was used for desensitizing of dentin according to the manufacturer's instructions (Table 1). Both self-adhesive cements were tested combined with and without Gluma Desensitizer pretreatment. Initial shear bond strength and subsequently long-term shear bond strength with different aging times in water storage and thermocycling (1d, 4d, 9d, 16d, 25d) were measured (Fig. 1).

3.1 Specimens preparation

For this study 550 extracted caries-free molars were collected. The teeth were cleaned from pariodontal tissue residues by using a scaler and kept in 0,5% Chloramin T at room temperature for a maximum of 7 days. They were then stored in distilled water for a maximum of six months at 5°C [35].

In order to get a flat surface for embedding, the teeth were levelled out parallel to the tooth axis using a polishing machine with P400 silicon carbide polishing paper (SCAN DIA, Hagen, Germany). The teeth were embedded in an acrylic resin (ScandiQuick, ScanDia, Hagen, Germany) and polished with P400 carbide polishing paper until a sufficient bond area of 4x4 mm dentin was exposed. The specimens were then randomly divided into 5 main groups of 110 each, according to the bonding treatment (PAN, RXU, RXU-G, GCM, GCM-G). Of each group, 10 specimens were tested after the initial bonding, and the remaining 100 specimens were subject to aging. The aging was carried out either in a constant temperature water bath (37°C), or by thermocycling between two baths. 5 aging periods were tested (1d, 4d, 9d, 16d, 25d). Thus, for each bonding treatment, and each aging exposure, there were 10 samples (N=550, n=10 per group).

3.2 Bonding procedure

Prior to the application of the cements, the dentin test area was grounded with P500 silicon carbide polishing paper. The specimen was inserted into a special holding device designed to keep the bonding surface horizontal. Dentin pretreatment with Gluma Desensitizer was applied according to the manufacturer's instruction prior to the use of the self-adhesive resin cements (Table 2). An acrylic cylinder with an inner diameter of 2.9 mm (D+R Tec, Birmensdorf, Switzerland) was pressed onto the dentin surface by a holding device, thus defining the bond area for the resin cement (Fig. 2A). The cements were activated and put in the acrylic cylinder (Fig. 2B) as recommended by the manufacturers (Table 2). In order to achieve as homogeneous as possible dispersion of the cement, a steel screw with an inner hexagon and an outer diameter of 2.8 mm was inserted parallel to the axis of the acrylic cylinder and at its center (Fig. 2C). The steel screw was put parallel into the acrylic cylinder with a weight of 100 g. The excess cement was completely removed (Fig. 2D). A cement thickness of 0.5 mm was attained in all specimens. The specimens were luted (Fig. 2E) according to the manufacturer's instructions (Table 2). After bonding, the specimens were removed from the device and prepared for aging (Fig. 2F). In addition, the initial shear bond strength (Fig. 2G and Fig. 2H) of all five main groups was tested.

3.3 Aging

The water storage aging was carried out in distilled water at 37° C in an incubator (UMS, Memmert, Schwabach, Germany).

The specimens were stored for 24 hours in distilled water at 37° C in the incubator (UMS, Germany) before the aging in the thermocycling machine. The two water baths of the thermocycling had a temperature of 5° C and 55° C with a dwell time of 20 s in each bath.

All specimens were aged either in constant temperature water or thermocycled between the two baths. The aging periods were either 1 day (24 hours), 4 days, 9 days, 16 days and 25 days.

3.4 Shear bond strength measurement

The shear bond strength was measured using a universal testing machine (Zwick/Roell Z010, Ulm, Germany) with a crosshead speed of 1 mm/min. For this purpose, the specimens were positioned in the sample's holder with the tooth surface parallel to the loading piston and the chisel of the loading piston was adjusted (Fig. 2G). The load was applied on the outer diameter of the cylinder and the force recorded at which the cylinders debonded (Fig. 2H). The shear bond strength was calculed using the formula: force at failure / bond area. Units of N/mm^2 = MPa. Specimens which did not survive the aging process were recorded as having a bond strength of 0 MPa.

3.5 Fracture type

Three failure types were observed: i) adhesive (no cement remnants on the dentin surface), ii) mixed failure (cement remnant and dentin surface exposed), and iii) cohesive failure in the dentin. The failure types were observed by one operator under a 25x optical microscope (Wild M3B, Heerbrugg, Switzerland).

3.6 Statistical analysis

Descriptive statistics (mean and standard deviations) and 95% confidence intervals (95% CI) were computed. In order to detect the differences between the means of the shear bond strength, the one-way ANOVA together with Scheffé post-hoc test was applied. The difference in mean shear bond strength between water storage and thermocycling for every aging time and every cement with or without pretreatment was analysed by means of the two-sample Student's t-test. Mean shear bond strength differences between water storage and thermocycling together with the corresponding 95% CI were estimated and compared. Relative frequences of adhesive fracture types together with the 95 % CI were provided [36].

The data was entered into an Excel spreadsheet and analysed with SPSS Version 15. Results of the analysis with p-values smaller than 5% were considered to be statistically significant.

4. Results

4.1 Shear bond strength between the five cement groups at specific aging times

Table 3 presents the descriptive statistics (mean, SD, 95%CI) of the measured shear bond strength for each test group according to their applied aging type and period. The results are graphically summarized in Fig. 3 and Fig. 4.

Among the initial groups ($p<0.001$) significantly higher mean shear bond strength was observed from both self-adhesive resin cements combined with Gluma Desensitizer compared to self-adhesive resin cements without Gluma Desensitizer. GCM-G showed significantly higher results than the control group PAN. GCM revealed the lowest mean shear bond strength. The control group PAN being higher than those of the self-adhesive cements groups RXU and GCM.

Water storage (37°C)

(Table 3: statistically differences are denoted by different small letters a,b,c)

After one day aging with water storage ($p=0.001$), significantly highest shear bond strength was obtained from groups GCM-G and RXU than from control group PAN and GCM. After four days water storage ($p<0.001$), the highest mean shear bond strength was obtained from RXU-G and GCM-G. These two groups are significantly higher than all other groups, RXU lies in the middle since it has significantly higher shear bond strength than the self-adhesive resin cement GCM. The control group PAN showed lower shear bond strength than those of both self-adhesive resin cements combined with Gluma Desensitizer. After nine days of water aging ($p<0.001$), the highest mean shear bond strength was obtained from both self-adhesive resin cements combined with Gluma Desensitizer RXU-G and GCM-G. RXU-G showed significantly higher results than those of the control group PAN, and of the self-adhesive cements without desensitizer RXU and GCM. GCM revealed the lowest mean shear bond strength. At the next aging period (16 days, p<0.001), again the highest results were observed from both self-adhesive resin cements combined with Gluma Desensitizer being only different from those of control group PAN and GCM. RXU showed lower shear bond strength than RXU-G. After 25 days water storage (p<0.001), the highest shear bond strength was obtained from GCM-G and RXU-G being different from those of control group PAN. GCM showed lower shear bond strength than GCM-G (p = 0.001). No difference in mean shear bond strength between RXU and RXU-G could be found.

Thermocycling (5°C and 55°C)

(Table 3: statistically significant differences are denoted by different capital letters a,b,c)

After one day thermocycling aging (p<0.001), a significantly lower shear bond strength was obtained from group RXU than from those of RXU-G and GCM-G, but not different of the control group PAN and GCM. In the next aging level (4 days, p<0.001) the significantly highest shear bond strength was obtained from both self-adhesive resin cements combined with Gluma Desensitizer. After nine days of thermocycling (p<0.001) the lowest bond values were observed in the control group PAN, GCM and RXU, but RXU was not different from GCM-G. RXU-G provided the highest shear bond strength. Among the 16 and 25 days thermocycling (p<0.001), the significantly highest shear bond strength was observed in the self-adhesive resin cement groups combined with Gluma Desensitizer.

4.2 Long-term shear bond strength stability for every main cement group

Water storage (37°C)

(Table 3: statistically significant differences are denoted by different capital letters A,B,C)

Within the control group PAN (p=0.009), after 9 aging days a significantly higher shear bond strength was observed compared with 25 days of water aging. The self-adhesive resin cement group RXU ($p<0.001$) showed initially significantly lower values than after 1 day of aging and remained constant until 25 day water storage. In the group RXU-G ($p<0.001$), the lowest results were observed from initial and 1 day of aging. The shear bond strength increased with further aging. GCM ($p=0.011$) showed increased shear bond strength, which was significantly higher after 25 days of water storage than the initial shear bond strength. The group GCM-G ($p=0.025$) showed in all aging times stable shear bond strength.

Thermocycling (5°C and 55°C)

(Table 3: statistically significant differences are denoted by different capital letters A,B,C)

The control group PAN ($p=0.115$), RXU ($p=0.089$), GCM ($p=0.197$) and GCM-G ($p=0.083$) showed at all aging times stable shear bond strength with thermocycling. Among the RXU-G group ($p<0.001$), an increase of the shear bond values was observed. Initial shear bond strength showed significantly lower values than the groups with 4 and 9 days of thermocycling.

4.3 Effect of aging type

Table 4 provides p-values, mean difference, and 95% CI of the two-sample t-test between the two aging types including water storage and thermocycling.

Statistical differences were observed in four out of the analysed five cement groups. Thermocycling showed in group RXU after 1 day ($p = 0.003$) and 4 days of aging ($p = 0.023$) and in group PAN after 16 days ($p = 0.023$) lower values compared to the according days of water storage. In group RXU-G after 9 days of aging, the values with thermocycling showed significantly higher shear bond strength than the water aging ($p = 0.011$).

4.4 Fracture type

The frequency of the failure types with 95% CI for relative frequency of adhesive failure are shown in Table 5.

In all cases, most of the failures were adhesive rather than mixed.

Water storage (37°C)

With GCM and GCM-G, only adhesive failure types occurred at all aging times. The remaining groups showed scattered mixed failure.

Thermocycling (5°C and 55°C)

All failures occurring with thermocycling were adhesive apart from PAN initial showing 4 mixed failures and after 16 days of aging showing 1 mixed failure.

5. Discussion

5.1 Shear bond strength

The self-adhesive resin cement RelyX Unicem exhibited similar initial shear bond strength to a conventional resin cement with a dentin primer. In the literature it is documented that the self-adhesive, partly hydrophilic, resin cements do not require any preconditioning of enamel and/or dentin and still obtain bond strength values similar to conventinal resin cements [15,37-40]. In the present study, the self-adhesive resin cement G-Cem showed the lowest initial shear bond strength, but already after 24 h of aging it is similar to the conventional resin cement. This phenomena can be explained with the fact that G-Cem keeps polymerizing after luting achieving similar value range as the conventional resin cement after 1 day of aging. This suggests that the after-polymerization plays a key role with this self-adhesive resin cement. As both the self-adhesive resin cements combined with Gluma Desensitizer demonstrated a higher shear bond strength than the conventional resin cement, at all aging times, the primary hypothesis of higher shear bond strength and better stability is confirmed.

Numerous in vitro studies observed the impact of Gluma Desensitizer on the resin/dentin interface of conventional resin cements [31,34,41-44]. Three studies reported no impact of the desensitization of dentin on the bond strength with conventinal resin cements including dentin pretreatment [42-44]. Other studies determined a negative effect of the desensitizer on the bond strength of the conventional resin cement Panavia21 [31,34,41]. An additional study stated that the resin cement was not able to polymerize with the dentin desensitizer [41]. In this study the long-term bond strength of both self-adhesive resin cements tended to be positively influenced by the application of desensitizers. Another study hypothesized that the bond strength of self-adhesive resin cements and desensitizers, and between desensitizers and dentin, exceeded the bond strength of self-adhesive resin cement and dentin itself [31]. This might occur because the Gluma Desensitizer contains glutaraldehyde and HEMA, and thus has hydrophilic properties, which improve the bonding to hydrophilic dentin. Self-adhesive resin cements contain phosphate groups; RelyX Unicem - methacrylated phosphoric esters; G-CEM - 4-META, to improve the bonding to dentin. The above observations may be explained by a possible condensation reaction between HEMA and phosphate through the elimination of water [27]. Alternatively, a reaction between glutaraldehyde and phosphate may lead to a very strong and stable bonding of the Gluma Desensitizer and the self-adhesive resin cements.

Different results were found for the effect of water storage and thermocycling aging. Thermocycling affected only the group RelyX Unicem (1 day and 4 days of aging) and the control group Panavia21 (16 days of aging) leading to significantly lower shear bond strength than with water aging. Water storage significantly decreased shear bond strength of RelyX Unicem with pretreament with Gluma Desensitizer after 9 days compared to thermocycling. No differences of shear bond strength between the two aging types were found for the other groups. However, as the majority of these results were not significantly different between the two aging types, no final conclusions can be drawn. Therefore, the secondary null-hypothesis of current study was rejected. One other study presented significant influence of thermocycling after 1500 cycles (5°C/55°C, transfer time of 10 s, dwelling time of 20 s) by resin cements [17]. This study reported on shear bond strength results of two different centers. All factors such as curing mode, dentin quality, and thermocycling influences within all test series were operated in a similar manner as the two participating centers followed the same study protocol, but by different operators. Thermocycling was reported to deliver different results in a recent study.

5.2 Failure type

Sailer et al [31] found that the self-adhesive cement RelyX Unicem, after debonding without pretreatment of dentin and after desensitization with Gluma Desensitizer, showed only cohesive failures within the cement. The Panavia21 group showed both with and without Gluma Desensitizer mixed failures in 80% and 70% of the tested specimens, respectively. Another study observed over 80% (RelyX Unicem 80% and G-Cem 90%) adhesive failure between dentin and luting agent using self-adhesive cements without pretreatment [45]. In this study G-Cem tended to show higher µTBS values (16.9 ± 10.3 MPa) than RelyX Unicem (12.5 ± 2.4 MPa), but with a large standard deviation. In our study, the self-adhesive resin cements with and without pretreatment of Gluma Desensitizer showed only adhesive failures after thermocycling, as well as G-Cem without and with desensitizing fractured adhesive in water aged specimens. The remaining groups predominantely showed adhesive failures, whereas mixed failures were rare. Therefore, adhesive failures were more often observed with self-adhesive resin cements, even though they occurred at higher shear bond strength compared with conventional cements. This adhesion mechanism is based on the partial retention of the smear layer and, therefore, it can be assumed that the infiltration ability is reduced and a cohesive fracture is less likely.

5.3 Limitation of the test method

One possible reason for the observed variations of the bond strength values could be the quality of the human teeth. It has been demonstrated that the bond strength of resin cements is dependent on the micromorphology of the dentin that is used for the bond strength test [39,46]. Additionally, the bonding performance of resin cements is dependent on the quality of the hybridisation layer, which is established during the pretreatmet of dentin [47]. If this layer is porous, H_2O molecules may penetrate and allow hydrolysis to occur. Another limitation of this study was the use of extracted teeth, which probably caused some loss of dentin fluid protein and such an environment could have prevented Gluma Desensitizer from reaction with dentin fluid protein. Furthermore, it must be realized that apart from all efforts to standardize the test procedures, a possibility for error remains during the application of the bonding agents or the cements [48].

The shear bond test assesses the quality of adhesion. Retention form of the preparation, marginal integrity, and clinical micro-leakage are the key parameters used to judge the effectiveness of a resin cement system. Once a cementing system passes the in vitro testing, a clinical trial with a controlled standardized study design should evaluate the clinical long-term performance.

5.4 Clinical relevance

In addition to the densibilisation of the dentin, the pretreatment of the dentin surface with Gluma Desenitizer in combination with self-adhesive resin cements showed a positive long-term effect on shear bond strength.

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7. Tables and Figures

Table 1 Summary of the used resin cements and desensitizer.

Cements and all steps	Short name	Company	Lot-Nr.
Panavia 21 Clearfil Porcelain Bond Activator Clearfil SE Bond Primer	PAN	Kuraray Dental Co Ltd., Osaka, Japan	00406C UNI TC / 00647C CAT 00208B 00769A
RelyX Unicem	RXU	3M ESPE , Seefeld, Germany	352388
G-CEM Capsule	GCM	GC, Leuven, Belgium	803061
Gluma Desensitizer	G	Haereus Kulzer, Hanau, Germany	20088

Table 2 Composition and application steps of the bonding agents and cements.

Composition of the bonding agents and cements		
Bonding agent and cement	**composition**	**Application steps as recommended by the manufacturer**
Pretreatment of the dentin		
Panavia21, ED Primer A	MDP, HEMA, water, MASA, accelator, water	1. Mix one drop of ED Primer A with one drop of ED Primer B for 5 s
Panavia21, ED Primer B	MASA, Na-benzene sulfonate, accelator,water	2. Apply on dired dentin, leave 60 s and blow the remnants away leaving the surface shiny
Panavia 21, cement catalyst	Hydrophobic aromatic dimethacrylate, hydrophibic alipathic dimethacrylate, MDP, fillers, BPO	1. Dispence equal amounts of Panavia21 Catalyst and Universal pastes 2. Slowly turn the dispencer knob one complete turn to the right until it clicks
Panavia 21, cement base	Hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, hydrophilic dimethacrylate, fillers, DEPT, sodium aromatic sulfonate	3. Mix the paste for 20 – 30 s until a smooth, uniform paste results 4. Oxyguard II to all margins for 3 min remove by rinsing with water
RelyX Unicem Aplicap	Powder: glass fillers, silica, calciumhydroxide, self-cure initiators, pigments, lightcure initiators Liquid: methacrylated phosphoric esters, dimethacrylates, acetate, stabilizers, self-cure initiators	1. Insert capsule into Activator, press handle and hold for 2 – 4 s 2. Mix 10 s with RotoMix Capsule Mixing Unit 3. Insert capsule into applier
G-CEM Capsule	4-META, UDMA, alumino-silicate glass, pigments, dimethacrylates, water, phosphoric ester monomer, initiators, campherquinone	1. Shake the capsule and push the plunger until it flush with the body 2. Place the capsule into an Applier and click the lever once 3. Mix for 10 s 4. Insert capsule into Applier
Gluma Desensitizer	HEMA, glutaraldehyde, distilled water	1. Apply on dried dentin and leave for 30 – 60 s 2. Dry and spray with air

BPO = benzoylperoxid, HEMA = 2-hydroxyethyl-methacrylate, MASA = N-methacrylolyl-5-aminosalicylic acid, MDP = 10-methacrylate oxydecyl dihydrogen phosphate, 4-META = 4-Methacryloyloxyethyl-trimellitat-anhydrid, UDMA = urethane-dimethacrylate

Table 3 Mean, standard deviation (SD) and 95% CI of the mean shear bond strength and significant difference in the resin cement groups of each aging time range and each aging type and in a aging time range of each cement and each aging type.

	initial		1 day		4 days		9 days		16 days		25 days	
	MW (SD) MPa	(95% CI) MPa	MW (SD) MPa	(95% CI) MPa	MW (SD) MPa	(95% CI) MPa	MW (SD) MPa	(95% CI) MPa	MW (SD) MPa	(95% C) MPal	MW (SD) MPa	(95% CI) MPa
Water store (37°C)												
PAN	6.2 (1.6)^{AB,bc}	(5.0,7.3)	5.0 (1.6)^{AB,a}	(3.9,6.2)	5.7 (2.1)^{AB,ab}	(4.2,7.2)	6.6 (1.4)^{B,ab}	(5.6,7.5)	6.4 (1.8)^{AB,a}	(5.1,7.7)	3.9 (1.6)^{A,a}	(2.8,5.1)
RXU	4.5 (0.7)^{A,ab}	(3.9,5.1)	8.4 (2.1)^{B,b}	(6.9,9.9)	8.0 (1.6)^{B,bc}	(6.8,9.1)	8.4 (1.5)^{B,b}	(7.3,9.4)	8.8 (2.9)^{B,ab}	(6.7,10.9)	6.8 (3.1)^{AB,ab}	(4.5,9.0)
RXU-G	7.4 (1.4)^{A,cd}	(6.4,8.4)	7.6 (1.5)^{A,ab}	(6.5,8.6)	11.2 (1.9)^{AB,c}	(9.8,12.5)	11.7 (2.5)^{B,c}	(9.9,13.5)	13.1 (4.3)^{B,c}	(10.0,16.2)	10.2 (2.4)^{AB,bc}	(8.4,11.9)
GCM	2.6 (1.2)^{A,a}	(1.7,3.5)	4.7 (1.6)^{AB,a}	(3.5,5.9)	3.6 (1.9)^{AB,a}	(2.2,4.9)	4.2 (1.5)^{AB,a}	(3.2,5.3)	5.1 (2.9)^{AB,a}	(3.0,7.2)	6.1 (3.0)^{B,ab}	(3.9,8.3)
GCM-G	8.6 (2.3)^{A,d}	(6.8,10.2)	8.9 (3.9)^{A,b}	(6.1,11.7)	9.5 (4.5)^{A,c}	(6.3,12.8)	9.5 (3.2)^{A,bc}	(7.2,11.8)	12.7 (2.2)^{A,bc}	(11.0,14.3)	12.8 (4.6)^{A,c}	(9.5,16.1)
Thermocycling (5°C / 55°C)												
PAN	6.2 (1.6)^{A,bc}	(5.0,7.3)	5.8 (2.2)^{A,ab}	(4.2,7.4)	4.8 (2.3)^{A,a}	(3.1,6.4)	5.2 (2.7)^{A,a}	(3.2,7.1)	4.1 (2.2)^{A,a}	(2.5,5.7)	2.7 (3.6)^{A,a}	(0.1,5.3)
RXU	4.5 (0.7)^{A,a}	(3.9,5.1)	4.7 (2.6)^{A,a}	(2.7,6.6)	5.9 (2.1) ^{A,a}	(4.3,7.4)	7.7 (2.3)^{A,ab}	(6.0,9.4)	6.9 (3.3)^{A,ab}	(4.5,9.3)	5.8 (1.4)^{A,a}	(4.7,6.8)
RXU-G	7.4 (1.4)^{A,cd}	(6.4,8.4)	8.9 (3.8)^{AB,b}	(6.1,11.6)	11.6 (1.6)^{BC,b}	(10.5,12.8)	15.2 (3.0)^{C,c}	(13.1,17.3)	10.4 (2.9)^{AB,b}	(8.3,12.5)	10.6 (2.7)^{AB,bc}	(8.6,12.5)
GCM	2.6 (1.2)^{A,a}	(1.7,3.5)	5.5 (1.9)^{A,ab}	(4.1,6.9)	5.0 (2.0)^{A,a}	(3.5,6.4)	4.1 (3.3)^{A,a}	(1.7,6.5)	3.5 (2.5)^{A,a}	(1.7,5.3)	4.3 (2.7)^{A,a}	(2.3,6.2)
GCM-G	8.6 (2.3)^{A,d}	(6.8,10.2)	9.3 (3.3)^{A,b}	(6.9,11.7)	13.1 (3.3)^{A,b}	(10.7,15.4)	10.4 (4.0)^{A,b}	(7.5,13.3)	10.7 (3.0)^{A,b}	(8.6,12.9)	11.2 (2.5)^{A,b}	(9.3,13.0)
a,b,c…: within each aging time; A,B,C…: within each resin cement group												

Table 4 Results: p-value, mean difference (MD) and 95% CI of the two-sample t-test between the two aging modes water and thermocycling.

	1 day		4 day		9 day		16 day		25 day	
	p-value	MD (95% CI)	p-value	MD (95% CI)	p-value	MD (95% CI)	p-value	MD (95% CI)	p-value	MD (95% CI)
PAN	0.392	-0.8 (-2.5,1.1)	0.351	0.9 (-1.1,3.0)	0.160	1.4 (-0.6,3.5)	0.023	2.3 (0.3,4.2)	0.330	1.3 (-1.3,3.9)
RXU	0.003	3.7 (1.4,6.0)	0.023	2.1 (0.3,3.9)	0.468	0.6 (-1.1,2.5)	0.189	1.9 (-1.0,4.8)	0.368	1.0 (-1.2,3.3)
RXU-G	0.340	-1.3 (-3.9,1.5)	0.558	-0.5 (-2.1,1.2)	0.011	-3.5 (-6.0,-0.9)	0.124	2.7 (-0.7,6.1)	0.732	-0.4 (-2.8,2.0)
GCM	0.307	-0.8 (-2.4,0.8)	0.124	-1.4 (-3.2,0.4)	0.879	0.2 (-2.2,2.6)	0.202	1.6 (-0.9,4.1)	0.166	1.9 (-0.8,4.6)
GCM-G	0.809	-0.4 (-3.8,3.0)	0.061	-3.5 (-7.2,0.2)	0.574	-0.9 (-4.3,2.5)	0.119	1.9 (-0.5,4.4)	0.333	1.7 (-1.8,5.2)
Positive significant differences mean that H_2O is better than TC										
Negative significant differences mean that TC is better than H_2O										

Table 5 Frequency of failure types together with the corresponding 95% for relative frequency.

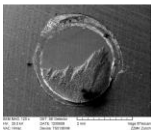
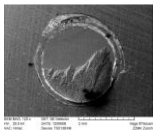
Water (37°C)				**Thermocycling (5°C / 55)**			
Aging time (days)	adhesive 	mix 	Relative frequency of adhesive failure (%) (95% CI)	Aging time (days)	adhesive 	mix 	Relative frequency of adhesive failure (%) (95% CI)
PAN							
0	6	4	60 (26.2;87.9)	0	6	4	60 (26.2;87.9)
1	10	0	100 (69.1;100)	1	10	0	100 (69.1;100)
4	9	1	90 (55.5;99.8)	4	10	0	100 (69.1;100)
9	9	1	90 (55.5;99.8)	9	10	0	100 (69.1;100)
16	7	3	70 (34.7;93.4)	16	9	1	90 (55.5;99.8)
25	10	0	100 (69.1;100)	25	10	0	100 (69.1;100)
RXU							
0	10	0	100 (69.1;100)	0	10	0	100 (69.1;100)
1	9	1	90 (55.5;99.8)	1	10	0	100 (69.1;100)
4	10	0	100 (69.1;100)	4	10	0	100 (69.1;100)
9	9	1	90 (55.5;99.8)	9	10	0	100 (69.1;100)
16	10	0	100 (69.1;100)	16	10	0	100 (69.1;100)
25	10	0	100 (69.1;100)	25	10	0	100 (69.1;100)
RXU-G							
0	8	2	80 (44.3;97.5)	0	10	0	100 (69.1;100)
1	10	0	100 (69.1;100)	1	10	0	100 (69.1;100)
4	9	1	90 (55.5;99.8)	4	10	0	100 (69.1;100)
9	9	1	90 (55.5;99.8)	9	10	0	100 (69.1;100)
16	8	2	80 (44.3;97.5)	16	10	0	100 (69.1;100)
25	8	2	80 (44.3;97.5)	25	10	0	100 (69.1;100)
GCM							
0	10	0	100 (69.1;100)	0	10	0	100 (69.1;100)
1	10	0	100 (69.1;100)	1	10	0	100 (69.1;100)
4	10	0	100 (69.1;100)	4	10	0	100 (69.1;100)
9	10	0	100 (69.1;100)	9	10	0	100 (69.1;100)
16	10	0	100 (69.1;100)	16	10	0	100 (69.1;100)
25	10	0	100 (69.1;100)	25	10	0	100 (69.1;100)
GCM-G							
0	10	0	100 (69.1;100)	0	10	0	100 (69.1;100)
1	10	0	100 (69.1;100)	1	10	0	100 (69.1;100)
4	10	0	100 (69.1;100)	4	10	0	100 (69.1;100)
9	10	0	100 (69.1;100)	9	10	0	100 (69.1;100)
16	10	0	100 (69.1;100)	16	10	0	100 (69.1;100)
25	10	0	100 (69.1;100)	25	10	0	100 (69.1;100)

Figure 1 Involved cements and their pretreatment.

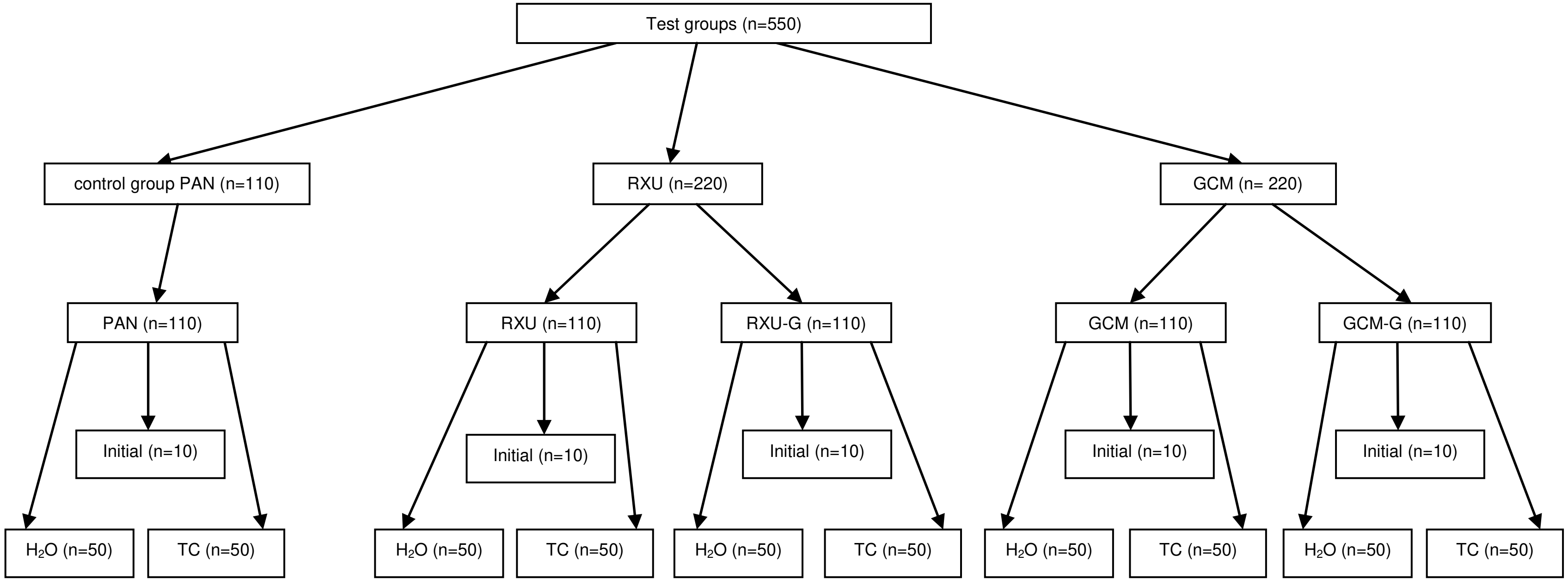


Figure 2 Production process of the specimens. A: Acrylic cylinder pressed on the the dentin surface B:cement put in the acrylic cylinder C: steel screw put into the acrylic cylinder D: removed of excess cement E: cement luted F: finishes speciment G: speciment in sample´s holder H: test design of shear bond strength

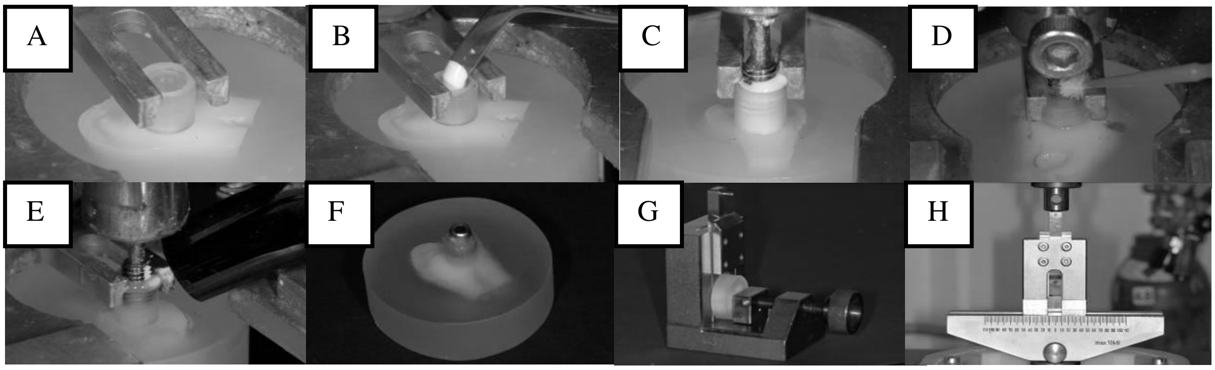


Figure 3 Diagram of shear bond strength after waterstorage (1, 4, 9, 16, 25 days).

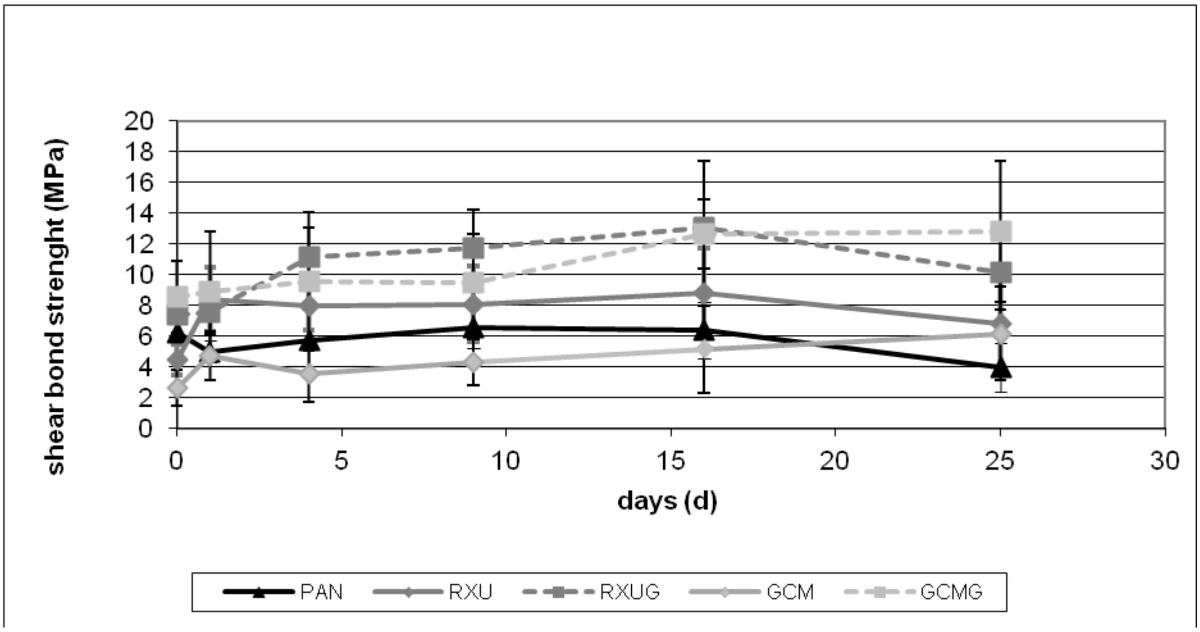


Figure 4 Diagram of shear bond strenght after thermocycling (1, 4, 9, 16, 25 days).