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Class I Gap-formation in Highly-viscous
Glass-ionomer Restorations: Delayed vs
Immediate Polishing

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Class I gap-formation with highly-viscous glass-ionomer restorations minimized by delayed polishing.

RESEARCH MANUSCRIPTS

Short title: Delayed polishing technique on Class I restorations

Clinical relevance: Delaying polishing for one day resulted in improved gap-formation for Class I restoration of highly-viscous conventional and conventional glass-ionomer cements.

Abstract

This *in vitro* study evaluated the effects of delayed *versus* immediate polishing to permit maturation on interfacial gap-formation around highly-viscous conventional glass-ionomer cement (HV-GIC) in Class I restorations together with determination of associated mechanical properties. Cavity preparations were made in occlusal surfaces of premolar teeth. Three HV-GICs were studied (*Fuji IX GP*, *GlasIonomer FX-II* and *Ketac Molar*) and one conventional glass-ionomer cement (C-GIC, *Fuji II*, as a control), with specimen sub-groups (n = 10) for each property measured. After polishing, either: (i) immediately (6 min) after setting or (ii) after 24 h storage, the restored teeth were sectioned in a mesio-distal direction through the center of the model Class I restorations. The presence or absence of interfacial-gaps was measured at x 1000 magnification at 14 points (each 0.5-mm apart) along the cavity restoration interface; (n=10; total points measured per group =140). Marginal gaps were similarly measured in Teflon molds as swelling data, together with shear-bond-strengths to

22 enamel and dentin, and flexural strengths and moduli. For three HV-GICs and one C-GIC,
23 significant differences ($p < 0.05$) in gap-incidence were observed between polishing (i)
24 immediately and (ii) after one-day storage. In the former case, 80-100 gaps were found. In the
25 latter case, only 9-21 gaps were observed. For all materials, their shear-bond-strengths,
26 flexural strength and moduli increased significantly after 24 h storage.

27

28 **Introduction**

29 As a restorative material, conventional glass-ionomer cements (C-GIC) have certain desirable
30 properties. C-GIC include chemical bonding to enamel and dentin substrates, release of
31 anticariogenic fluoride into adjacent hard tissues and a low coefficient of thermal expansion
32 similar to that of dentine.^{1,2} However, C-GICs are susceptible to fracture and exhibit low wear
33 resistance. Therefore, these deficiencies have limited their use to areas subject to low
34 masticatory stresses.² Because of the low fracture toughness, mechanical strength and
35 brittleness of C-GICs, efforts were made to improve their mechanical properties by the
36 addition of powder.³⁻⁵ Highly-viscous C-GICs (HV-GICs) were developed to overcome
37 early moisture sensitivity and low mechanical properties associated with conventional
38 materials. And then they were designed as an alternative to amalgam for posterior preventive
39 restoration.^{1,2} Highly-viscous or high powder-liquid ratio C-GICs, such as Fuji IX GP, Ketac
40 Molar and GlasIonomer FX-II, provide a “condensable” feel and are particularly used for the
41 atraumatic restorative treatment (ART) technique introduced by the World Health
42 Organization (WHO) for use in developing countries.^{6, 7} Indications for these cements in

43 general practice are to small Class I cavities, deciduous teeth and long-term temporaries.^{2, 8-12}

44 The polishing period is another factor that may influence the seal-ability around a cervical or
45 a cavity restoration. Polishing after storage in water for one day resulted in improved gap
46 formation for cervical restorations or dentin cavities of a resin-modified glass-ionomer cement
47 (RM-GIC) or a C-CGI.¹³⁻¹⁷ Due to the structure of RM-GIC or C-GIC and their hydrophilic
48 nature, water sorption and subsequent swelling may lead to partial compensation of the
49 shrinkage. The preservation of sealing around a restoration would benefit most if water
50 sorption and setting shrinkage could proceed simultaneously. However, water sorption occurs
51 only at a later stage compared with setting shrinkage.¹⁸ Currently, no information is available
52 regarding the interfacial-gap formation behavior around Class I restoration of a C-GIC.

53 In the oral environment, C-GICs must also withstand masticatory and parafunctional
54 stresses. And these stresses vary markedly in different clinical situations. Thus, thresholds in
55 mechanical properties needed for success may vary considerably from case to case, with
56 stronger C-GICs being required where greater stresses are anticipated. Flexural test are
57 appropriate to assess the mechanical properties of restorative and luting cements.^{9, 13, 16} In
58 previous studies, C-GICs were proposed to improve their marginal seal by enhancement of
59 their flexural strength and bonding ability after 24 h water-storage.¹³ Appropriate elastic
60 moduli and proportional limit values are also desirable.¹⁶

61 The principal aims of the present study, therefore, were: 1) to evaluate both gap-formation
62 integrity around but-joints in model restorations, analogous to Class I restoration of HV-GICs
63 and 2) determination of the early development of their flexural and adhesive properties,

64 compared with those of a C-GIC. An important clinical variable was to be assessed in this
65 connection: namely, the effect on these properties of an immediate *versus* a 24 h -delayed
66 finishing procedure. Hence, a major hypothesis to be tested was that premature finishing
67 would significantly reduce interfacial integrity, relative to delayed finishing. Several
68 additional properties, including shear-bond-strengths, were also to be measured, to further
69 elucidate the effects of water-uptake over 24 h upon intrinsic and interfacial material behavior,
70 and to discriminate between the different material types.

71

72 **Materials and Methods**

73 The basic properties of three HV-GICs and one C-GIC, as a control, are summarized in Tables
74 1. Human premolars, extracted for orthodontic reasons, were used for the experiment. After
75 extraction, each tooth was immediately stored in distilled water at 4°C for one to two months
76 before use.

77 Four C-GICs were investigated (Table 1), which were placed according to the
78 manufacturers' instructions. Dentin Conditioner was applied for 20 s, and rinsed with water.
79 Ketac Conditioner was applied for 10 s, and rinsed with water. The cavity was filled with
80 mixed GIC using a syringe tip (Centrix C-R Syringe System, Centrix, Connecticut, USA) and
81 covered with a plastic strip and was stored in an incubator at 37 °C and 100% relative
82 humidity for 4 min after mixing as setting procedure. The restored teeth were then coated with
83 a varnish (Fuji Varnish, GC, Tokyo, Japan).

84

85 **Gap-Formation around Class I restoration:**

86 **1. Preparing and Polishing Procedures:**

87 A Class I cavity was prepared in the human premolar surface, having a length of 3.5 mm, a
88 width of approximately 2 mm with a depth of 1.5 mm, using a tungsten carbide bur
89 (200,000-rpm) and a fissure bur (8,000-rpm) under wet conditions (Figure 1). Cavosurface
90 walls were finished to a butt joint. This design differed from a Class I clinical cavity in that
91 cavity corners were geometric-box angles to prepare a constant-volume model. One cavity
92 was prepared in each of 80 teeth; (4 materials x 2 polishing or inspecting times x 10 repeats =
93 80). The surfaces of designated restorations were polished immediately after setting, with
94 abrasive points (Silicone Mide, Shofu, Kyoto, Japan) while rinsing with distilled water in an
95 effort to avoid desiccation and breakdown. The other designated specimens were stored after
96 setting in distilled water at 37°C for 24 h. Then the surfaces of the restorations were polished,
97 as described above.

98 **2: Inspection Procedures**

99 Each tooth was sectioned in a buccolingual direction through the center of the restoration with
100 a low-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL). The presence or absence of
101 marginal gaps was measured at 14 points (each 0.5-mm apart) along the cavity restoration
102 interface (n=10; total points measured=140) using a traveling microscope (X1,000,
103 Measurescope, MM-11, Nikon, Tokyo, Japan) (Figure 1). The number of gaps in each position
104 was totaled and expressed as a sum for each sample.^{14, 15, 17}

105

106 **Marginal Gap in Teflon Cavity**

107 Since Teflon does not react with GICs, it was used as a mold to measure the degree of setting
108 shrinkage (immediately after setting) and any hygroscopic expansion (after 1-day storage in
109 water) of the GICs. Each prepared Teflon mold (n = 10), with a depth of 1.5 mm and a
110 diameter of 3.5 mm, was placed on a silicone oil-coated glass plate, and filled with GIC using
111 a syringe tip, then covered with a plastic strip until set. The sum of the maximum gap-width
112 and the opposing gap width (if any) was expressed as the marginal gap in the Teflon cavity.

113

114 **Shear Bond Strengths to Enamel and to Dentin**

115 Wet grinding of buccal surfaces was performed with up to 1000 grit silicon carbide abrasive
116 paper until a flat enamel or superficial dentin area of at least 4 mm in diameter was exposed.
117 The surface was pretreated as described above. A split Teflon mold with a cylindrical hole
118 (diameter, 3.6 mm; height, 2 mm) was clamped to the prepared enamel or dentin surface. The
119 Teflon mold was filled with various restorative materials using a Centrix syringe tip (Centrix
120 C-R Syringe System, Centrix, Connecticut, USA). It was covered with a plastic strip and the
121 material was hardened by light irradiation, as described above. For each material, 10
122 specimens were prepared. Prepared specimens were secured in a mounting jig. At a time of
123 either 6 minutes from start of mixing procedure, or after 24 h water-storage, the shear force
124 was transmitted by a flat (blunt) 1 mm broad shearing edge making a 90° angle to the
125 direction of the load (or the back of the load plate). The shear force was applied (Autograph
126 DCS-2000, Shimadzu, Kyoto, Japan) at a cross-head speed of 0.5 mm/min. The stress at

127 failure was calculated and recorded as the shear-bond strength. The failed specimens were
128 examined under a light microscope (x 4; SMZ-10, Nikon, Tokyo, Japan) to determine the total
129 number of adhesive failure surfaces.¹⁶

130

131 **Flexural strength and flexural modulus of elasticity.**

132 Teflon molds (25×2×2 mm) were used to prepare flexural specimens (n=10 /group). Each GIC
133 was setting as described above. Flexural properties were measured, both immediately after
134 setting and after 24 h storage, using the three-point bending method with a 20 mm-span and a
135 load speed of 0.5 mm/min (5565, Instron, Canton, MA, USA) outlined in ISO 9917-2 (1996)
136 and the flexural modulus was calculated (Software Series IX, Instron, Canton, MA, USA).

137

138 All procedures, except for cavity preparation, were performed in a thermo-hygrostatic room
139 kept at 23±0.5 °C and 50±2 % relative humidity. Ten specimens were made for each material,
140 storage period and property investigated. The results were analyzed statistically using the
141 Mann-Whitney U test, Tukey Test (non-parametric),^{14 - 17, 19} Tukey Test, *t*-Test, or Fisher Exact
142 Test (Sigmastat 3.1, Systat software, Inc., Point Richmond, CA).

143

144 **RESULTS**

145 Table 2 summarizes the interfacial gap formation observed in the Class I with three HV-GICs
146 and a normal C-GIC (as a control), when the specimen was polished immediately after
147 light-activation and after delayed polishing. For all materials, the sums of gaps were

148 significantly fewer with delayed polishing, compared to immediate polishing.

149 Table 3 summarizes the marginal gap width between the four GICs and Teflon molds under
150 two conditions. The two columns represent the linear (diametral) setting shrinkage-strain
151 immediately after setting and after one-day storage. The data for polishing after one-day
152 storage was significantly better compared with that for polishing immediately. With
153 immediate and after one-day stages, the values of Fuji IX GP and GlasIonomer FX-II were
154 significantly less than for Fuji II.

155 Tables 4 summarize the shear bond strength to the enamel surface and the mode of fracture,
156 respectively. Immediately after setting, the value of shear bond strength of Ketac-Molar was
157 significantly less than that of the normal C-GIC (Fuji II). However there was no difference
158 between the four GICs after one-day storage. The data for polishing after one-day storage
159 were significantly better compared with that for polishing immediately. For all groups, no
160 significant differences in fracture mode were observed between immediate and 24 h.

161 Tables 5 summarize the shear bond strength to the dentin surface and the mode of fracture,
162 respectively. Immediately after setting, the value of shear bond strength of Fuji IX GP and
163 Ketac-Molar were significantly less than that of Fuji II. It was not significantly different
164 between the three C-GICs, except GlasIonomer FX-II, after one-day storage. The data for
165 polishing after one-day storage were significantly better compared with that for polishing
166 immediately. For all groups, no significant differences in fracture mode were observed
167 between immediate and 24 h.

168 All materials showed significantly higher flexural strengths after 1 day than immediately

169 after setting (Table 6).

170 Table 7 summarizes the flexural modulus under two conditions. The tendency of results
171 was similar to the flexural strength, increasing after storage. All materials showed
172 significantly higher value than when the specimens were measured immediately after setting.

173

174 **DISCUSSION**

175 This study used a model cavity for the geometry of typical Class I cavities. This only
176 approximates the Class I morphology and is not the typical morphology for a C-GIC, but has
177 the advantage of a constant volume, reproducible geometry that is beneficial for an in vitro
178 scientific study.

179 This study demonstrated that polishing of four GICs should not be performed immediately
180 after the filling and setting procedure but should be delayed to a later time to prevent
181 interfacial gap-formation between the material and the Class I cavity. In contrast to the
182 marginal gap of approximately 80-100 μ m when the specimen was polished immediately
183 after setting, the gap was near zero when the specimen was polished after storage in water for
184 1 day. The GICs shrink during the setting reaction, so interfacial gaps form as the adhesion
185 between tooth-cavity and glass-ionomers does not resist the shrinkage-stress.^{13, 20, 21} However,
186 after 1-day water storage the shrinkage-stresses of the materials are effectively compensated
187 for or even converted into expansion-stress due to water uptake and swelling.^{1, 2, 4, 18} Water
188 absorption of C-GICs reportedly affects cavity adaptation and reduces microleakage.^{3, 13}
189 Although the hygroscopic expansion may not be enough to compensate for the setting

190 shrinkage, it plays an important role in reducing the shrinkage caused by the cement setting
191 reaction and thus improves the interfacial gap-formation in the restoration.^{14, 15}

192 The marginal gap measured using the Teflon mold showed a similar pattern, with respect to
193 the polishing condition, to that obtained using the Class I restoration, as mentioned above. The
194 marginal gap observed even after the specimen was stored in water for 1 day indicated that the
195 hygroscopic expansion did not fully compensate for the setting-shrinkage.

196 The bond-strength, flexural-strength and flexural-modulus of 1-day storage were
197 significantly higher than those measured immediately for the all C-GICs, and
198 inter-relationships have been reported previously.^{3, 13, 22} As expected, cements show higher
199 bond and mechanical strengths when fully set than during the setting reaction. Also the pH, an
200 index of the extent of the hardening reaction for GICs, gradually increases for 24 hours.^{1, 2, 23}
201 Therefore it can be presumed that completing of the setting reaction of a GIC requires at least
202 24 hours.

203 After 1-day storage a HV-GIC (Fuji IX GP) performed significantly better than its
204 corresponding conventional C-GIC (Fuji II). Increasing powder-liquid ratio is the main reason
205 for improving these results, as the two C-GICs are otherwise very similar. This improvement
206 is achieved by a reduction in the glass particle-size. However GlasIonomer FX-II and Ketac
207 Molar Aplicap did not clearly show this pattern. This may be explained by differences in
208 density, distribution or content of the powder, and the polyacrylic or maleic acid concentration
209 or molecular weight of polyacrylic or maleic acid of the liquid. A number of variations led to a
210 HV-GIC with improved physical properties.²⁴

211 Investigating interfacial gap-formation after 24 h storage, for C-GICs, had value, as was
212 also found in studying various types of restorative filling materials.¹³⁻¹⁷ The greater interfacial
213 integrity of GICs resulted from harmony between: good bond-strength, low setting shrinkage
214 or possibly some hygroscopic expansion. With HV-GICs it is thus generally advisable to
215 adjust of occlusion immediately after initial setting and perform a final contouring and
216 finishing by delayed polishing procedure. And, it is thought that a HV-GIC is the most useful
217 and significant restorative material for some pediatric or geriatric patients.

218 A more extensive approach to the evaluation of sealing efficacy with C-GICs would require
219 longer-term durability testing or load-cycling.

220

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289 **Caption to Figure**

290 Figure Class I restoration and each measurement location for gap-formation.

291 E: Enamel substrate, D: Dentin substrate, G: Glass ionomer restorative material

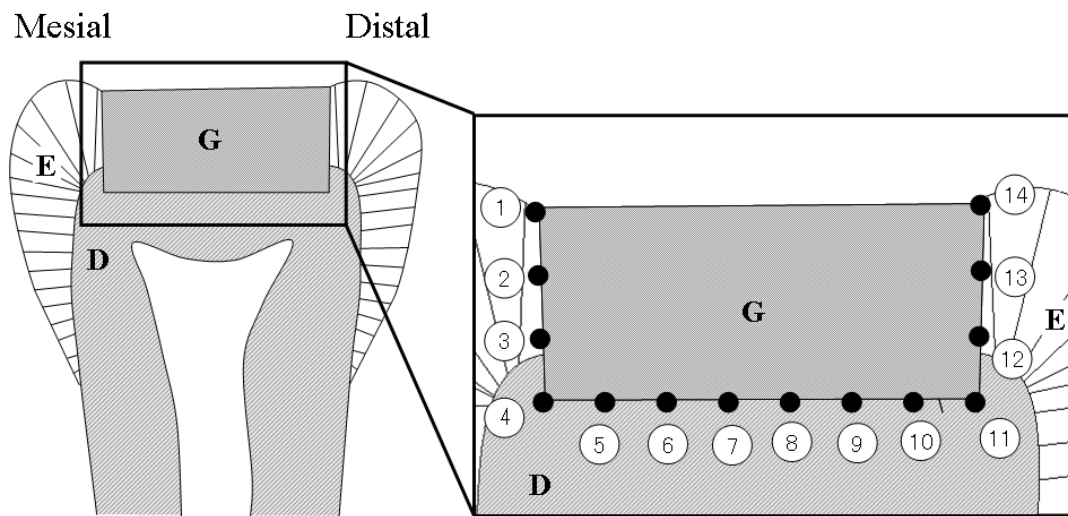


Table 1 Restorative Materials and conditioner agents investigated

| Material | Manufacturer | Batch No. | Powder/Liquid, Components, Surface treatment |
|---------------------------|--------------------------------|--------------------------|---|
| Restorative materials | | | |
| Fuji IX GP | GC Corp. Tokyo, Japan | P: 0404301 L: 0404301 | 3.6 P: fluoroaluminosilicate glass L: copolymer of acrylic and maleic acids, polybasic carboxylic acid, water |
| GlasIonomer FX-II | Shofu Corp. | P: 120304 L: 050302 | 2.6 P: fluoroaluminosilicate glass L: copolymer of acrylic acid and tri-carboxylic acid, water |
| Ketac Molar Aplicap | 3M ESPE AG Seefeld, Germany | 169574 | Precapsulated P: fluoroaluminosilicate glass L: polycarbonic acid, tartaric acid, oligomers, water |
| Fuji II (as a control) | GC Corp. Tokyo, Japan | P: 0309091 L: 0309121 | 2.7 P: fluoroaluminosilicate glass L: copolymer of acrylic and maleic acids, polybasic carboxylic acid, water |
| Conditioner agents | | | |
| Dentin Conditioner | GC Corp. Tokyo, Japan | 151021 | Polyacrylic acid, water. Apply with brush 20 seconds - rinse - gently dry 5 seconds |
| Ketac Conditioner | 3M ESPE AG Seefeld, Germany | 00026 | Polyacrylic acid, water Apply with brush 10 seconds - rinse - gently dry 5 seconds |

Table 2 Effect of polishing time on gap formation around Class I restoration

| Restoration | Polishing time | Number of specimens showing gaps | | | | | | | | | | | | | | Sum |
|----------------------------|-----------------------|----------------------------------|---|---|---|--------|---|----|----|---|--------|----|----|----|----|---------------------------------|
| | | Medial | | | | Bottom | | | | | Distal | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | |
| Fuji IX GP | | | | | | | | | | | | | | | | |
| | Immediate | 10 | 5 | 2 | 4 | 2 | 1 | 7 | 7 | 7 | 3 | 7 | 6 | 9 | 10 | 80 (NS) * a [#] |
| | After one-day storage | 2 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 9 (S) b |
| GlasIonomer FX-II | | | | | | | | | | | | | | | | |
| | Immediate | 9 | 5 | 4 | 4 | 2 | 5 | 6 | 5 | 7 | 7 | 7 | 3 | 6 | 10 | 80 (NS) a |
| | After one-day storage | 5 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 5 | 12 (NS) b |
| Ketac Molar Aplicap | | | | | | | | | | | | | | | | |
| | Immediate | 10 | 6 | 6 | 5 | 6 | 5 | 7 | 9 | 5 | 7 | 8 | 9 | 6 | 10 | 99 (NS) c |
| | After one-day storage | 4 | 0 | 0 | 2 | 0 | 1 | 0 | 2 | 2 | 1 | 4 | 2 | 0 | 3 | 21 (NS) d |
| Fuji II | | | | | | | | | | | | | | | | |
| (as a control) | Immediate | 10 | 5 | 4 | 4 | 5 | 7 | 10 | 10 | 6 | 3 | 7 | 6 | 7 | 10 | 94 |
| | After one-day storage | 7 | 0 | 0 | 2 | 0 | 2 | 1 | 1 | 0 | 2 | 1 | 0 | 1 | 4 | 21 |

N=10 (total measuring points, 1 - 14 = 140)

* : vs. Fuji II (Man-Whitney U-Test, S: Significant difference, NS: Not significant difference, alpha=0.05)

[#] : Means with the same letters were not significantly different by Tukey test. (p>0.05, non-parametric^{14-17, 19}).

** : Immediate vs. After 1-day storage (Man-Whitney U-Test, S: Significant difference, alpha=0.05)

Table 3 Effect of polishing time on marginal gap-width in the Teflon mold (micrometer).

| Restoration | Mean (S.D.) | | p value* |
|---------------------|-------------------------|------------------------|----------|
| | Immediately | After one-day storage | |
| Fuji IX GP | 14.3 (2.3) (S) # | 9.3 (2.2) (S) | <0.001 |
| GlasIonomer FX-II | 14.3 (3.5) (S) | 9.5 (1.7) (S) | <0.05 |
| Ketac Molar Aplicap | 17.0 (2.4) (S) | 11.8 (3.0) (NS) | <0.001 |
| Fuji II | 20.0 (3.3) | 12.9 (3.0) | <0.001 |

N=10, Diameter in Teflon mold: 3.5 mm. *: *t*-test.

: vs. Fuji II (*t*-test, S: Significant difference, NS: Not significant difference, $p>0.05$)

Table 4 Shear bond strength to enamel surface (MPa) immediately after setting and one-day storage.

| Restoration | Mean (S.D.) | | p value* |
|------------------------|---------------------------------------|------------------------------------|--------------|
| | Immediately | After one-day storage | p value# |
| Fuji IX GP | 2.50 (0.64) a b 0 / 2 / 8** | 8.29 (1.87) c 0 / 3 / 7 | <0.001 NS |
| GlasIonomer FX-II | 2.78 (0.58) a 0 / 0 / 10 | 8.41 (1.52) c 0 / 0 / 10 | <0.001 NS |
| Ketac Molar Aplicap | 1.83 (0.53) b 0 / 8 / 2 | 5.99 (2.90) c 0 / 7 / 3 | <0.001 NS |
| Fuji II (as a control) | 2.93 (0.90) a 0 / 2 / 8 | 6.44 (1.97) c 0 / 3 / 7 | <0.001 NS |

*: *t*-test. #: Fisher Exact test. NS: Not significantly different (p>0.05)

** : Number with each fracture mode, adhesive failure at the bonding site / mixed failure / cohesive failure, N=10

Means with the same letters were not significantly different by Tukey test. (p>0.05).

Table 5 Shear bond strength to dentin surface (MPa) immediately after setting and after one-day storage.

| Restoration | Mean (S.D.) | | p value* |
|------------------------|--------------------------------------|--------------------------------------|--------------|
| | Immediately | After one-day storage | p value# |
| Fuji IX GP | 1.38 (0.55) a 0 / 0 / 10** | 8.80 (1.12) c 0 / 0 / 10 | <0.001 NS |
| GlasIonomer FX-II | 2.12 (0.45) b 0 / 0 / 10 | 5.45 (1.03) d 0 / 0 / 10 | <0.001 NS |
| Ketac Molar Aplicap | 1.42 (0.59) a 0 / 0 / 10 | 7.17 (1.99) c d 0 / 0 / 10 | <0.001 NS |
| Fuji II (as a control) | 2.20 (0.67) b 0 / 0 / 10 | 8.59 (2.00) c 0 / 0 / 10 | <0.001 NS |

*: *t*-test. #: Fisher Exact test. NS: Not significantly different (p>0.05)

** : Number with each fracture mode, adhesive failure at the bonding site / mixed failure / cohesive failure, N=10

Means with the same letters were not significantly different by Tukey test. (p>0.05).

Table 6 Flexural strength (MPa) immediately after setting and after one-day storage.

| Restoration | Mean (S.D.) | | p value * |
|------------------------|----------------------|-----------------------|-----------|
| | Immediately | After one-day storage | |
| Fuji IX GP | 1.83 (0.79) a | 29.18 (5.39) b | <0.001 |
| GlasIonomer FX-II | 1.70 (0.53) a | 17.29 (1.87) c | <0.001 |
| Ketac Molar Aplicap | 1.89 (0.88) a | 19.33 (5.38) c | <0.001 |
| Fuji II (as a control) | 2.00 (1.59) a | 15.33 (2.07) c | <0.001 |

*: *t*-test, N=10

Means with the same letters were not significantly different by Tukey test. ($p>0.05$).

Table 7 Flexural modulus (GPa) immediately after setting and after one-day storage.

| Restoration | Mean (S.D.) | | p value * |
|------------------------|----------------------|-----------------------|-----------|
| | Immediately | After one-day storage | |
| Fuji IX GP | 1.30 (0.34) a | 14.54 (1.97) b | <0.001 |
| GlasIonomer FX-II | 1.82 (0.43) a | 12.63 (1.92) b | <0.001 |
| Ketac Molar Aplicap | 1.98 (0.95) a | 14.43 (4.34) b | <0.001 |
| Fuji II (as a control) | 1.57 (1.01) a | 12.63 (4.10) b | <0.001 |

*: *t*-test, N=10

Means with the same letters were not significantly different by Tukey test. ($p>0.05$).