

## Marginal filling and adhesive resistance of bulk fill resin applying 18% edta gel compared with 37% phosphoric acid gel *in vitro* dental conditioning.

Sellado marginal y resistencia adhesiva de resina bulk fill aplicando gel de edta al 18% comparado con el gel de ácido fosfórico al 37% en el acondicionamiento dental *in vitro*.

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**Receipt:** 11/28/2018 **Revised:** 04/13/2019  
**Acceptance:** 05/01/2019 **Online:** 08/05/2019

**Abstract:** Objective. To evaluate the degree of marginal sealing and adhesive strength in Bulk-Fill resin by applying 18% EDTA gel compared to 37% phosphoric acid gel in dental conditioning. Materials and methods. The study was transversal, experimental comparative, and applied. The sample group consisted of 60 teeth with Class I cavities, which were later subjected to conditioning. This was carried out separately with phosphoric acid gel for 15 seconds, and 18% EDTA gel within two different time frames: 60 and 90 seconds. All samples were incubated at 37°C for a period of 24 hours and thermocycled between 5 to 55°C for 500 cycles. To assess microfiltration, they were immersed in methylene blue at 37°C for 4 hours, and were later sectioned and observed through a stereomicroscope. In order to perform the traction, the conventional testing machine was used at a speed of 0.75mm/min until the screw with the resin detached. Results: Samples conditioned with 37% phosphoric acid showed a greater degree 1 microfiltration and a mean adhesive resistance of 7.97 MPa. The same number of microfiltration grades 1 and 2 was found when using EDTA gel for 60 seconds, with a mean tensile strength of 8.8 MPa. On the other hand, applying EDTA gel for 90 seconds showed mostly grade 1 microfiltrations, and to a lesser extent, grade 0 and 2, with a mean resistance of 9.2 MPa. Conclusion: There are no statistically significant differences regarding adhesive strength between the two gels; however, better marginal filling was observed when samples were conditioned with 18% EDTA gel for 90 seconds.

**Keywords:** *Microstraining; dental bonding; acid etching, dental; edetic acid; dental materials.*

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**Resumen:** Objetivo. Evaluar el grado de sellado marginal y resistencia adhesiva en resina Bulk – Fill aplicando gel de EDTA al 18% en comparación al gel de ácido fosfórico al 37% en el acondicionamiento dental. Materiales y métodos. El tipo de estudio fue transversal, comparativo, de nivel aplicativo y diseño experimental. La muestra estuvo conformada por 60 dientes con cavidades clase I; se realizó el acondicionamiento con gel de ácido fosfórico, gel del EDTA al 18% en 60 segundos y 90 segundos. El total de la muestras fueron incubados a 37°C por 24 horas, se termocicló con 500 ciclos entre 5 a 55°C. Para evaluar microfiltración, fueron sumergidos en azul de metileno a 37°C por 4 horas, fueron seccionadas y observados al estereomicroscopio, para realizar la tracción se empleó la máquina de ensayos universales a una velocidad de 0.75mm/min hasta que se desprendiera el tornillo con la resina. Resultados. Las muestras acondicionadas con ácido fosfórico al 37% presenta mayor microfiltración grado 1 y el promedio de su resistencia adhesiva es de 7.97Mpa, la microfiltración obtenida con EDTA en 60 segundos presenta equidad

**Cite as:**

César Cayo, Llancari L, Mendoza R & Cervantes L. Marginal filling and adhesive resistance of bulk fill resin applying 18% edta gel compared with 37% phosphoric acid gel *in vitro* dental conditioning. J Oral Res 2019; 8(3):228-235. Doi:10.17126/joralres.2019.034

entre grado 1 y 2; promedio de resistencia a la tracción de 8.8 Mpa y el EDTA 90 segundos presentó mayor microfiltración grado 1 y el resto 0 y 2; el promedio de resistencia fue 9.2 Mpa. Conclusión. No existen diferencias estadísticamente significativas al comparar los acondicionantes para la prueba

de resistencia adhesiva, sin embargo se observó mejor sellado marginal cuando se acondicionó con gel de EDTA al 18 % por 90 segundos.

**Palabras Clave:** Microcribado; recubrimiento dental adhesivo; grabado ácido dental; ácido edético; materiales dentales.

## INTRODUCTION.

The bonding process of restorative materials on dental structures is complex and has always been an open question for researchers. Traditionally, marginal filling and adhesive resistance tests are assessed in studies related to this subject. In 2015, Pérez<sup>1</sup> analyzed the adhesion strength of surface dentin previously conditioned using self-etching techniques and 18% EDTA gel, concluding that the EDTA technique results in better adhesion strength than self-etching. Likewise, in 2013 Barzallo<sup>2</sup> studied the feasibility of replacing 37% phosphoric acid with 18% EDTA in dental conditioning, obtaining favorable results when 18% EDTA was applied for 90 seconds.

In 2015, Arce<sup>3</sup> assessed marginal filling in two different types of resins and adhesive techniques, obtaining favorable results for the Tetric N - Ceram Bulk Fill® resin with both adhesive techniques. In order to achieve this bonding, the modification of dental structures -both enamel and dentine- along with their respective characteristics, is necessary. Regarding enamel, the structural loss is approximately 10 microns in depth of the inorganic matrix, and its surface looks rough when observed under a scanning electron microscope, thus receptive to the adhesive system.<sup>4</sup> In dentin this process occurs in the intratubular and intertubular zones, dissolving between 3 and 7 microns of the inorganic component, which is composed of 50% minerals, 30% collagen and 20% water.<sup>5</sup> In this structure, the process is even more complex due to the high prevalence of type I collagen within the matrix.<sup>6</sup> The interaction of etching acid on dentin produces the exposure of collagen fibers, which must be ideally merged in their entirety by the adhesive system, which in turn, when polymerized, gives rise to a hybrid layer. If this is not accomplished, then the metalloproteinases (MMPs) found within the dentin matrix,<sup>7</sup> which preferentially degrade collagen, proteoglycans and fibronectin, are activated. When this process is complete, they become inactive.<sup>8</sup> Metalloproteinases (MMPs) are zinc-dependent enzymes

that are capable of activating growth factors, surface receptors and adhesion molecules. Twenty-five types of MMPs have been found, grouped into 5 classes according to their function and structure.<sup>9</sup> Notable ones include collagenases, gelatinases and stromelysins.<sup>8</sup>

When dentine is faced with acids produced by the metabolism of cariogenic bacteria<sup>10</sup> or the acidic attack of restorative materials such as etching gel, it produces the activation of the latent forms of MMPs 2 and 9, while MMPs 8, 2, 9, 3 and 20 intervene in the degradation of the hybrid layer.

The activation of said MMPs is due to the low pH involved in both of the processes mentioned that also result in the availability of calcium and zinc, thus resulting in the degradation of unprotected collagen.<sup>9,11</sup>

The proteolytic activity of MMPs is regulated by tissue inhibitors of metalloproteinases (TIMPs),<sup>12</sup> which can be specific and non-specific.<sup>11</sup> Theoretically, the presence of TIMPs stabilizes enzyme activity and the increase of growth factors, leading to the reorganization and repair of dentine in situations of favorable pH.<sup>8</sup> The application of artificial inhibitors of MMPs on the dentin surface after acid etching or their incorporation into the adhesive system promotes the integrity and stability of long term restoration.<sup>13</sup>

The most used non-specific inhibitors in restorative dentistry are chlorhexidine digluconate and EDTA.<sup>14</sup> Chlorhexidine in this form allows dissociation at physiological pH releasing cationic molecules thus having a bacteriostatic and bactericidal effect,<sup>11</sup> with the added inhibitory effect of MMPs at low concentrations and with a preference for MMPs 2, 8, and 9.<sup>15</sup> The application of chlorhexidine delays the degradation of the resin-dentine interface,<sup>14</sup> and can protect this bonding for up to 12 months.<sup>16</sup>

EDTA is an organic tetracarboxylic acid derived from ethane with the ability to chelate metal ions, with a preference for Ca, Mg, Mo, Fe, Cu and Zn<sup>17</sup> ions and a weak dentine demineralizer. This results in a milder alteration of dentine

proteins which, compared to phosphoric acid conditioning, allows collagen to retain more apatite crystals.<sup>18</sup> The focal point of its action on MMPs lies on enzymatic inhibition, as EDTA chelates the ion co-factors needed for the catalytic activity of enzymes.<sup>17</sup> As a hypothesis we proposed that the application of 18% EDTA gel for 90 seconds in dental conditioning improves the degree of marginal filling and adhesive strength of Bulk Fill resin compared to using 37% phosphoric acid gel.

Therefore, the objective of this study was to compare the degree of marginal filling and adhesive strength of Bulk-Fill resin with the application of 18% EDTA gel for 60 and 90 seconds, and the application of 37% phosphoric acid gel for 15 seconds. The optimal use of 18% EDTA gel is carried out in order to limit the activation of MMPs that degrade the hybrid layer and to thus extend the duration of dental restoration. The STROBE guidelines were used in the present study.

## MATERIALS AND METHODS.

A double-blind study was carried out at an applicable, experimental, prospective, cross-sectional and comparative level (one statistical examiner and another who performed stereomicroscope observations). The Kappa index was used by the examiner using the stereomicroscope (who had over 10 years of experience) for the inter-examiner and intra-examiner calibration tests, considering 0.74 and 0.81 as good and very good, respectively. For preservation, sample number and experimentation tests, ISO TS11405:2015 was applied. Sample collection began three months before performing the experiment by immersing specimens in normal saline solution and replacing the solution twice a week. Groups of ten teeth each were selected for each test, from a total of 60 upper and lower third molars of average length, free of carious lesions and without previous restorations.<sup>19</sup>

The surrounding soft tissues were removed from all teeth with a Woodpecker® scaler and a periodontal curette No. 13-14, and then, with a Kavo Extra torque 600® high-speed device and a medium-grain cylindrical burr, cusps and pits were removed in order to obtain a flat surface. Then, dental apices were filled with an I-Seal® photocurable ionomer. A G.V. Black class I cavity was made in all teeth with a medium-sized cylindrical milling burr and the Kavo Extra

torque 600 high-speed device, 3mm in diameter and 4mm deep, measured with a digital vernier caliper.<sup>19</sup>

Once the preparation of cavities was completed, the teeth were randomly divided into six groups of 10 teeth each. The first two groups were conditioned with 37% phosphoric acid (Total Etch - Ivoclar Vivadent®) for 15 seconds, the third and fourth groups were conditioned with 18% EDTA (Ultradent®) for 60 seconds, and the final two groups were conditioned with 18% EDTA for 90 seconds. All of the cavities were disinfected with 2% chlorhexidine for 30 seconds and the excess material was removed with sterile absorbent paper.

Two layers of the Tetric N - Bond adhesive (Ivoclar - Vivadent®) were applied for 10 seconds and the solvent was allowed to evaporate by applying a blast of air for 5 seconds. The adhesives were light-cured for 20 seconds with a Woodpecker LED lamp, and the emitted radiation was calibrated at each activation with the built-in radiometer.

The filling of teeth was as follows: for the adhesive resistance test, Tetric N-Ceram Bulk Fill resin (Ivoclar - Vivadent®) was inserted through a screw, to a depth of 4mm, and light-cured for 20 seconds; while, for the marginal filling test, Tetric N-Ceram Bulk Fill® resin was used in a traditional manner and restorations were polished with differently graded Soflex Discs, water and a Denflex® micro-engine. Once the specimens were filled, samples were incubated at 37°C for 24 hours, using the Memmert® INE700 stove and bi-distilled water to simulate oral conditions.

For thermocycling, teeth were placed in stainless steel containers lined with aluminum foil and subjected to 500 cycles of temperature for a period of 20 seconds: 5°C±2°C and 55°C±2°C, and at room temperature for 10 seconds. Two Boeco digital thermometers were utilized for temperature monitoring. For the marginal filling test, two layers of nail varnish were employed, leaving the 2mm margin around the restoration unvarnished.

Dental samples were immersed for 4 hours in 0.5% methylene blue at 37°C. Teeth were sectioned in half in a mesial - lingual direction with a 0.20mm thick diamond cutting disc and a Dremel 3000 marking machine (at the High Technology Laboratory Certificate S.A.C.). Sectioned teeth were observed using a Unico - 24 V stereomicroscope and, following ISO TS11405:2015

regulations, the criteria taken into account was: grade 0 (without microfiltration), grade 1 (microfiltration up to enamel), grade 2 (microfiltration up to dentin) and grade 3 (microfiltration up to pulp floor).

In order to evaluate adhesive resistance, samples were subjected to the tensile test, supported by an LG CMT - 5L® universal testing machine (at the High Technology Laboratory Certificate S.A.C.) used at a speed of 0.75mm/min. Previously, teeth selected for testing were placed in Vitalloy self-curing acrylic cubes in three different colors, up to the amelocemental junction. For data analysis, a database was prepared in Microsoft Excel and then imported into SPSS software version 23.0. In order to analyze results from the adhesive strength test, the Shapiro-Wilk normality test was carried out.

Regarding inferential analysis, the nonparametric Mann - Whitney U test was applied, with the objective of contrasting the marginal filling difference hypothesis between the groups under study. To compare adhesive

strength, a one-way ANOVA test for independent samples was used. All statistical tests considered a confidence level of 95%, accepting a 5% type I error ( $p < 0.05$ ).

## RESULTS.

Most samples that were conditioned for 15 seconds with 37% phosphoric acid presented microfiltration with dye penetration extending into the cavity's enamel wall (grade 1), while the remaining suffered microfiltration with dye penetration reaching into the cavity's dentin wall (grade 2). In terms of adhesive strength, a mean value of 7.97 MPa was obtained.

Regarding dental samples conditioned for 60 seconds with 18% EDTA gel, half showed microfiltration with dye penetration reaching into the cavity's enamel wall (grade 1), while the other half experienced microfiltration with dye penetration extending into the cavity's dentine wall (grade 2). For the tensile strength test, the mean value obtained was 8.8 MPa.

**Table 1.** Comparison of marginal *in vitro* filling in Bulk Fill resins conditioned with 37% phosphoric acid in 15 seconds of application and 18% EDTA gel in 60 seconds of application.

| Group               | Grade 0<br>( Without penetration ) |      | Grade 1<br>( Penetration in enamel ) |       | Grade 2<br>( Penetration in dentin ) |       | p-value* |
|---------------------|------------------------------------|------|--------------------------------------|-------|--------------------------------------|-------|----------|
|                     | Absolute frequency                 | %    | Absolute frequency                   | %     | Absolute frequency                   | %     |          |
| Phosphoric acid 37% |                                    |      |                                      |       |                                      |       |          |
| 15 seconds          | 0                                  | 0.0% | 7                                    | 70.0% | 3                                    | 30.0% | 1.000    |
| EDTA 18%            | 0                                  | 0.0% | 5                                    | 50.0% | 5                                    | 50.0% |          |
| 60 seconds          |                                    |      |                                      |       |                                      |       |          |

\*: Based on the non-parametric Mann Whitney U test.

**Table 2.** Comparison of marginal *in vitro* filling in Bulk Fill resins conditioned with 37% phosphoric acid in 15 seconds of application and 18% EDTA gel in 90 seconds of application.

| Group               | Grade 0<br>( Without penetration ) |       | Grade 1<br>( Penetration in enamel ) |       | Grade 2<br>( Penetration in dentin ) |       | p-value* |
|---------------------|------------------------------------|-------|--------------------------------------|-------|--------------------------------------|-------|----------|
|                     | Absolute frequency                 | %     | Absolute frequency                   | %     | Absolute frequency                   | %     |          |
| Phosphoric acid 37% |                                    |       |                                      |       |                                      |       |          |
| 15 seconds          | 0                                  | 0.0%  | 7                                    | 70.0% | 3                                    | 30.0% | 0.263    |
| EDTA 18%            | 3                                  | 30.0% | 6                                    | 60.0% | 1                                    | 10.0% |          |
| 90 seconds          |                                    |       |                                      |       |                                      |       |          |

\*: Based on the non-parametric Mann Whitney U test.

**Table 3.** Comparison of *in vitro* adhesive strength in Bulk Fill resin conditioned with 37% phosphoric acid gel in 15 seconds of application and 18% EDTA gel in 60 seconds of application.

| Group                             | Mean (MPa) | CI95%    |          | Median (MPa) | SD   | p-value* |
|-----------------------------------|------------|----------|----------|--------------|------|----------|
|                                   |            | LL (MPa) | UL (MPa) |              |      |          |
| Phosphoric acid 37%<br>15 seconds | 7.98       | 5.99     | 9.96     | 7.91         | 2.78 | 0.692    |
| EDTA 18%<br>60 seconds            | 8.80       | 7.38     | 10.22    | 8.26         | 1.98 |          |

\* : Based on the non-parametric Mann Whitney U test. **CI** : Confidence interval of the mean. **LL** : Lower limit of the CI. **UL** : Upper limit of the CI. **SD** : Standard Deviation.

**Table 4.** Comparison of *in vitro* adhesive strength in Bulk Fill resin conditioned with 37% phosphoric acid gel in 15 seconds of application and 18% EDTA gel in 90 seconds of application.

| Group                             | Mean (MPa) | CI95%    |          | Median (MPa) | SD   | p-value* |
|-----------------------------------|------------|----------|----------|--------------|------|----------|
|                                   |            | LL (MPa) | UL (MPa) |              |      |          |
| Phosphoric acid 37%<br>15 seconds | 7.98       | 5.99     | 9.96     | 7.91         | 2.78 | 0.451    |
| EDTA 18%<br>90 seconds            | 9.20       | 7.88     | 10.52    | 8.83         | 1.84 |          |

\* : Based on the non-parametric Mann Whitney U test. **CI** : Confidence interval of the mean. **LL** : Lower limit of the CI. **UL** : Upper limit of the CI. **SD** : Standard Deviation.

**Table 5.** Comparison of *in vitro* marginal filling in Bulk Fill resin conditioned with EDTA at 18% in 60 and 90 seconds of application.

| Group                    | Grade 0<br>( Without penetration ) |       | Grade 1<br>( Penetration in enamel ) |       | Grade 2<br>( Penetration in dentin ) |       | p-value* |
|--------------------------|------------------------------------|-------|--------------------------------------|-------|--------------------------------------|-------|----------|
|                          | Absolute                           | %     | Absolute                             | %     | Absolute                             | %     |          |
|                          | frequency                          |       | frequency                            |       | frequency                            |       |          |
| EDTA 18% -<br>60 seconds | 0                                  | 0.0%  | 5                                    | 50.0% | 5                                    | 50.0% | 0.038*   |
| EDTA 18%<br>90 seconds   | 3                                  | 30.0% | 6                                    | 60.0% | 1                                    | 10.0% |          |

\*: Based on the non-parametric Mann Whitney U test.

The majority of teeth treated for 90 seconds with 18% EDTA gel presented microfiltration with dye penetration extending into the cavity's enamel wall (grade 1). To a lesser extent, some samples suffered microfiltration reaching into the cavity's dentine wall (grade 2) and the absence of dye penetration (grade 0). Regarding tensile strength, the

recorded mean was 9.2 MPa, the highest result obtained between sample groups. Only for this group a grade 0 was observed. When comparing the marginal filling effect of applying 37% phosphoric acid for 15 seconds and 18% EDTA gel for 60 seconds, similar values were found for both groups (Table 1).



After analyzing the marginal filling effect of applying 37% phosphoric acid gel for 15 seconds and 18% EDTA gel for 90 seconds, microfiltration grade 1 and 2 was found in the first group, while the latter recorded microfiltration grades 0, 1, and 2. The differences between the 18% EDTA gel group and the 37% phosphoric acid group are not statistically significant (Table 2).

When comparing the adhesive strength effect of applying 37% phosphoric acid gel for 15 seconds with administering 18% EDTA gel for 60 seconds, the latter obtained slightly higher values, but these were not considered statistically significant (Table 3).

The adhesive strength effect of applying 37% phosphoric acid gel for 15 seconds was compared with administering 18% EDTA gel for 90 seconds, resulting in significantly higher values obtained for the latter (Table 4). Significantly better results were obtained when administering 18% EDTA gel for 90 seconds compared with 60 seconds (Table 5).

When comparing results regarding the adhesive strength effect of 18% EDTA gel in 60 and 90 seconds of application, a higher mean was obtained for the 90-second group, but no significant differences were found (Table 6).

## DISCUSSION.

Results indicate that there are no statistically significant differences related to adhesive resistance. However, samples treated for 90 seconds with 18% EDTA gel have higher adhesion strength (9.2 MPa) compared to 37% phosphoric acid gel (7.9MPa). Regarding marginal filling, statistically significant differences were only found when contrasting the two groups conditioned with 18% EDTA gel (60 versus 90 seconds), resulting in a better outcome when 18% EDTA gel conditioning was applied for 90 seconds.

In 2017, Mandava *et al.*,<sup>21</sup> assessed and compared adhesive strength by performing microtraction on three Bulk Fill compounds and on one conventional one, considering an experimental methodology similar to the one used in this study. They concluded that Bulk Fill resins present adequate dentin adhesion strength, results that agree with the data obtained in this study about samples conditioned with 37% phosphoric acid.

In 2017, Charamba *et al.*,<sup>22</sup> analyzed the bonding strength of two Bulk Fill resins (packable and flow) and one nanohybrid resin, concluding that composite Bulk Fill resins has higher binding resistance values (expressed in MPa). These results comply with the data found in the present study when acid etching was carried out, obtaining a mean value of 7.97 MPa.

In 2015, Pérez<sup>1</sup> analyzed adhesive strength applying 18% EDTA gel for 90 seconds and a self-etching technique, concluding that strength was greater in the EDTA group, in agreement with the present study. In 2014, Monsalves *et al.*,<sup>20</sup> evaluated marginal filling and adhesive resistance in deciduous and permanent teeth with a two-step system. The data obtained for permanent dentition in the adhesive resistance test (9.52 MPa) contradicts what was obtained in the present study (7.97 MPa).

Finally, in 2013 Barzallo<sup>2</sup> analyzed the adhesive strength of applying 18% EDTA as a substitute for phosphoric acid by carrying out the tensile test and considering various application times, a similar methodology to the one employed in this study. Results showed that applying 18% EDTA gel for 90 seconds was indeed an effective substitute for 37% phosphoric acid gel, differing with the present study as we found no statistically significant differences.

In 2016 Nascimento *et al.*,<sup>25</sup> evaluated microfiltration in Bulk Fill resin using acid etching and self-etching techniques, employing a scale ranging from 0 to 3, with none of the systems eliminating microfiltration. The present study utilized etching acid and 18% EDTA for periods of 60 and 90 seconds, obtaining results which differ slightly from the aforementioned research since statistically significant differences were found between the 60 and 90 seconds 18% EDTA groups.

In 2015, Arce<sup>3</sup> compared the degree of microfiltration between two different Bulk Fill resin brands and using two adhesive systems, concluding that there were no significant differences between the techniques employed regarding microfiltration. However, Tetric N-Ceram Bulk Fill® resin showed a lower percentage of microfiltration. The present study obtained similar results when the aforementioned resin was employed and conditioned with 37% phosphoric acid gel.

## CONCLUSION.

There were no statistically significant differences in adhesive strength between conditioners. The only statistically significant differences were found in the marginal filling test, between the 60 seconds and the 90 seconds 18% EDTA gel groups.

To conclude, 37% phosphoric acid gel and 18% EDTA gel, applied for 60 or 90 seconds, are good dental conditioners for use in the dental filling process, with 18% EDTA gel applied for 90 seconds being the best conditioner.

**Conflict of interests:** The authors declare no conflicts of interest.

**Ethics approval:** Approved by the Research Committee of the Faculty of Dentistry of the National University Federico Villarreal.

**Funding:** None.

**Authors' contributions:** Cayo C, conceived the research idea and designed the methodological part, supervised the experimental part and read the stereomicroscope, reviewed the draft and developed the final version. Llancari L, did the literature review search, executed the experimental part and wrote the draft. Mendoza R, supervised the experimental part and thermocycling. Cervantes L, reviewed the statistics and helped to prepare the final version of the manuscript.

**Acknowledgements:** To Robert Eusebio, who facilitated the use of his laboratory to perform the extraction and sectioning of dental pieces. To Segundo García for the facilities provided to perform the microfiltration test readings at Alas Peruanas University, Huacho Branch, Peru.

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