# **Original Article**

# Effect of degree of conversion on in vivo biocompatibility of flowable resin used for bioprotection of mini-implants

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### ABSTRACT

**Objective:** To test the hypothesis that there is no difference between the biocompatibility and degree of monomer conversion of flowable resins used as bioprotective materials of orthodontic mini-implants.

**Materials and Methods:** Forty-eight male Wistar rats were divided into four groups (n = 12). Group Control (polyethylene), Group Wave, Group Top Comfort, and Group Filtek. The animals were sacrificed after time intervals of 7, 15, and 30 days and tissues were analyzed under optical microscopy for inflammatory infiltrate, edema, necrosis, granulation tissue, multinucleated giant cells, and collagen formation. The degree of conversion was evaluated by the Fourier method. Biocompatibility and degree of conversion were evaluated by the Kruskal-Wallis and Dunn tests, and analysis of variance and the Tukey test, respectively (P < .05).

**Results:** An intense inflammatory infiltrate was observed on the seventh day, with Groups Top Comfort and Filtek differing statistically from Group Control (P = .016). Edema, necrosis, granulation tissue, and giant cells showed greater expressiveness at 7 days, without statistical difference between them (P > .05). For the presence of collagen fibers, Group Top Comfort was shown to differ statistically from Group Control (P = .037) at 15 days and from Groups Filtek and Control (P = .008) at 30 days. Monomer conversion ranged from 62.3% in Group Top Comfort at 7 days to 79.1% in Group Filtek at 30 days.

**Conclusions:** The hypothesis was rejected. The resin Top Comfort demonstrated lower tissue repair capacity with a lower number of collagen fibers compared with Filtek and Wave resins. The resin Top Comfort showed the lowest conversion values during the experiment. (*Angle Orthod.* 2016;86:157–163.)

KEY WORDS: Orthodontics; Biocompatibility; Mini-implants; Resin

# INTRODUCTION

Flowable resin composites have been indicated as bioprotective materials on orthodontic mini-implant heads<sup>1</sup> to prevent the implants from traumatizing soft tissues. However, the leachable components from the resins, such as bisphenol A-diglycidyl dimethacrylate (Bis-GMA), triethylene glycol dimethacrylate, and urethane dimethacrylate (UDMA) found in a wide range of resins, have been shown to have a definite cytotoxic effect.<sup>2,3</sup> Because of the proximity of mini-implants to the gingiva and other oral tissues, a similar effect could occur in these tissues.<sup>1,2</sup>

The behavior of resins is directly linked to their chemical formulation, and the release of 25% to 45% of the monomers, which are not converted into polymers after polymerization,<sup>4,5</sup> occurs when using the conventional irradiation method.<sup>5</sup> Therefore, residual monomers<sup>6</sup> may trigger discrete to moderate—or even

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| Groups      | Resins                 | Composition*   | Manufacturer                      | Lot     |  |
|-------------|------------------------|--|-----------------------------------|---------|--|
| Filtek      | Filtek Z350 XT<br>Flow | 35% by weight of BisGMA, TEGMA, ytterbium fluoride, dimetha-<br>crylate-functionalized polymer and 65% by weight of ceramic<br>inorganic particles and silane-treated silica and titanium dioxide                      | 3M/Espe, St Paul, Minn            | N376841 |  |
| Top Comfort | Top Comfort<br>Flow    | 60% by weight of methacrylate monomers (BisGMA, UDMA, and TEGMA), stabilizer, camphorquinone, coinitiator, pigments, and 40% by weight of boron-aluminum-silicate glass inorganic particles and nanoparticulate silica | FGM, Joinville, SC,<br>Brazil     | 040112  |  |
| Wave        | Wave Flow              | 35% by weight multifunctional methacrylate ester and 65% by weight of inorganic particles (strontium and silica fill)  | SDI, Bayswater, VIC,<br>Australia | 110401N |  |

 Table 1.
 Composition of the Tested Flowable Resins

\* Bis-GMA indicates bisphenol A diglycidylmethacrylate; UDMA, urethane dimethacrylate; TEGDMA, triethylene glycoldimethacrylate.

severe—inflammatory reactions,<sup>7</sup> in addition to having a direct influence on the physical, mechanical, and biologic properties of the material.<sup>8,9</sup>

Studies have been conducted with adhesives and resin materials,<sup>7,10</sup> but there is a lack of studies that directly relate the degree of monomer conversion, polymerization, and the in vivo inflammatory events of flowable resins. Thus, the authors' aim was to test the hypothesis that there is no difference between the biocompatibility and degree of monomer conversion of flowable resins used as bioprotective materials of orthodontic mini-implants at different time intervals.

# MATERIALS AND METHODS

# **Animal Model and Experimental Groups**

For this study, 48 adult male Wistar rats with a mean weight of 250 g were used. The animals were divided into four experimental groups (12 rats per group): Group Control (polyethylene), Group Filtek, Group Top Comfort, and Group Wave (Table 1). The animal experiment was approved by the Ethics Committee on Animal Research, UACB\UFCG\0102012.

The rats were anesthetized with an intraperitoneal injection of sodium thiopental (50 mg/kg) (Cristália, Campinas, SP, Brazil). Then trichotomy was performed in the dorsal region, using 4% chlorhexidine gluconate for antisepsis of the operative field.<sup>10</sup> On the midline, equidistant from the base of the tail to the head of the animal, two incisions approximately 8 mm long were made using an No.15 scalpel blade (Embramac, Itapira, SP, Brazil).

The subcutaneous tissue was laterally parted to create a tunnel in the lateral direction, forming two surgical recesses, each approximately 18 mm deep. Each rat was fitted with two tube implants (1.5 mm inner diameter  $\times$  5 mm long) made of polyethylene (nontoxic Scalp Vein 19G, Embramac, Itapira, Brazil), which were washed with deionized water and autoclaved at a temperature of 110°C for 20 minutes and then used as inoculation vehicles for the tested materials.

The resins were introduced into the openings at the extremities of the tubes, using a syringe (Centrix, Shelton, Conn) supported on a glass slide at one extremity and a small glass slide at the other to flatten the material. Afterward, they were light polymerized for 40 seconds, using a LED appliance (Radii/SDI, Baywater, Victoria, Australia) fixed on a rod to ensure that the distance between the specimens remained constant and using a light intensity of 1000 mw/cm<sup>2</sup>.

After the materials were implanted, the surgical recesses were sutured with a 4.0 suture needle with thread, then the animals were given a 0.2-mL intramuscular dose of veterinary pentabiotic (Wyeth Laboratory, Collegeville, Pa) and an injection of sodium dipyrone (0.3 mL/100 g; Novalgina, São Paulo, Brazil). All the procedures of this study were performed in accordance with the Canadian Council on Animal Care (1981). The animals were kept in individual cages under adequate conditions with appropriate rations and water ad libitum.

After time intervals of 7, 15, and 30 days, the animals were anesthetized to obtain excisional biopsies of the implant area, including sufficient normal surrounding tissue. Afterward, the rats were sacrificed by the cervical dislocation technique after having been sedated with sodium thiopental (50 mg/kg).

# **Biocompatibility**

Then samples were taken and fixed in 4% formaldehyde (Milony solution) for 24 hours, embedded in paraffin to obtain serial histologic cuts 6  $\mu$ m thick, and stained with hematoxylin and eosin. The inflammatory reaction induced by the composites was evaluated by a blind examiner using a light microscope (BX40/ Olympus, Hamburg, Germany) at 100, 200, and 400× magnifications. The examiner was calibrated before data analysis (kappa = 0.78). For each sample of the study, five representative sections of the tissue adjacent to the implanted materials were evaluated histologically.

In terms of of inflammatory infiltrate, edema, necrosis, granulation tissue, multinuclear giant cells,

and collagen fibers, points were awarded according to the following scores: 1, absent (absent from the tissue); 2, scarce (scarcely present or in very small groups); 3, moderate (densely present or in some groups); and 4, intense (found in the entire field or present in large numbers, configuring high severity).

#### **Degree of Conversion**

To prepare and standardize the test samples that measured 5 mm in diameter and 1.5 mm thick, stainless steel bipartite matrices were used. These were placed on a glass slide, and the resin was injected using a syringe (Centrix), flattened with a small glass slide, and then polymerized. A total of 45 test samples (n = 5 per group) were stored in artificial saliva at 37°C and in lightproof boxes to prevent additional exposure to light.<sup>11</sup>

After intervals of 7, 15, and 30 days from polymerization and storage, each specimen was ground to obtain the resin powder, which was subsequently mixed with potassium bromide in a ratio of 1/10 by weight. This powder was placed in a tablet maker under an approximate pressure of 8 tons. A spectrophotometer (Bomen-MB-102, Dawson, Yukon, Canada) was used to assay the infrared spectrum measurements using the Fourier transformation method to determine the degree of conversion (DC) of the monomer.

#### **Statistical Analysis**

The cellular components of inflammatory infiltrate, edema, necrosis, granulation tissue, giant cells, and collagen were submitted to the Kruskal-Wallis non-parametric test, followed by Dunn's test to determine the differences among the groups (P < .05), because they did not present normal distribution. The parametric data of the degree of material conversion was submitted to an analysis of variance followed by the Tukey test (P < .05).

#### RESULTS

#### **Biocompatibility**

The flowable resins evaluated presented an intense inflammatory infiltrate, observed after 7 days, with Groups Top Comfort and Filtek (Figure 1A and B) differing statistically from Group Control (P = .016). All the groups presented circulatory alterations (edema) and granulation tissue with greater expressiveness after 7 days, showing gradual reduction in the subsequent periods, without statistical difference among them (P > .05). The resins demonstrated the presence of tissue degeneration (necrosis) around or within the cavity, as a result of resin implantation, in a more expressive manner after 7 days. Multinucleated giant

cells were rarely found in response to the nonpersistence of severe inflammatory infiltrate, and there was good tolerance of the body to the materials (Table 2).

Regarding the presence of collagen fibers during the repair process, Group Top Comfort demonstrated slower repair than did Group Wave (Figure 1C), differing statistically from the control group (P = .037) after 15 days (Figure 1D), which persisted after 30 days, with Group Top Comfort (Figure 1E and F) differing statistically from Groups Filtek and Control (P = .008) (Figure 1G; Table 2).

#### Degree of Conversion

Monomer conversion of the resins increased progressively up to the 30th day, with lower conversion of 62.3% in Group Top Comfort at 7 days and higher conversion of 79.1% in Group Filtek at 30 days. There were no statistically significant differences between the resins at 7 and 15 days (P > .05). The flowable resin Top Comfort showed the lowest conversion values during the experiment, with a statistically significant difference from Filtek (P = .006) after 30 days (Table 3). When the time intervals for the same resin were compared, there was significant difference between the times evaluated (P < .01) for all groups.

#### DISCUSSION

Orthodontic mini-implants have been increasingly used in orthodontics; however, complications during orthodontic loading, such as aphthous ulceration, soft tissue covering the miniscrew head, soft tissue inflammation, and infection in the neighboring tissues, arising from the small head of the mini-implant may also occur.<sup>12</sup> To minimize these effects, flowable resin composites have been used as protective material on the head of these devices.<sup>1</sup>

On the other hand, the monomers released by resins are cytotoxic to fibroblasts,<sup>13</sup> which can induce local and systemic reactions in patients,<sup>14</sup> causing epithelial proliferation, lichenoid reactions,<sup>15</sup> hypersensitivity, and allergic reactions.<sup>15,16</sup> The main cause of the cytotoxic effects of resins is the release of unpolymerized residual monomers,<sup>2,7,11,17</sup> which may cause severe cellular damage<sup>3,10,17</sup> and favor bacterial growth around the resin.<sup>18</sup> Studies<sup>19,20</sup> have demonstrated that flowable resins are more cytotoxic than traditional resin composites.

In this study, the biocompatibility of flowable resins was evaluated by means of inflammatory phenomena.<sup>10,11</sup> From the experiments, one may observe an intense inflammatory infiltrate for the resins evaluated, after 7 days, with a statistical difference between the resins Top Comfort and Filtek compared with the



**Figure 1.** Photomicrograph of histological sample. (A) Seven days after implantation, Group Top Comfort (GTC): intense inflammatory infiltrate composed predominantly of lymphocytes and plasmocytes (II) (H&E,  $\times$ 200 magnification; scale: 50 µm). (B) Seven days after implantation, Group Filtek (GF): extensive area of inflammatory infiltrate predominantly composed of lymphocytes and plasma cells (II) with presence of congested vessels (CV) (H&E,  $\times$ 200 magnification; scale: 50 µm). (C) 15 days after implantation, Group Wave (GW): moderate inflammatory infiltrate predominantly mononuclear with CV and presence of multinucleated giant cells (GC) near the foreign body (FB) material (H&E,  $\times$ 400 magnification; scale: 25 µm). (D) 15 days after implantation, GTC: area of moderate inflammatory infiltrate predominantly mononuclear with congested vessels (CV) and presence of an intense reaction of multinucleated GC near the nondigestible FB material (H&E,  $\times$ 400 magnification; scale: 25 µm). (E) 30 days after implantation, GTC: cavity surrounded by proliferation of young fibroblasts (YF) and areas of collagen fiber deposition (CFD) (H&E,  $\times$ 200 magnification; scale: 50 µm). (F) 30 days after implantation, GTC: shrouded cavity showing YF and areas of CFD (H&E,  $\times$ 400 magnification; scale: 25 µm). (G) 30 days after implantation, Group Control: cavity surrounded by areas of CFD of parallel form and uniform to the fullest extent (H&E  $\times$ 200 magnification; scale: 50 µm). Area of polyethylene tube implant.

control group. This suggests a similar release of monomers between them, as observed in the degree of DC, but of a more cytotoxic nature when compared with the resin Wave in this initial period. In this line of research, these findings support the idea that resin components show cytotoxic effects that are dependent on time and their concentration.<sup>21</sup>

One can observe the low level of expressiveness for the occurence of edema, tissue necrosis, and multinucleated giant cells, which demonstrates a low

|                         |            |                    | Groups              |                     |                    |       |  |
|-------------------------|------------|--------------------|---------------------|---------------------|--------------------|-------|--|
| Condition               | Time, Days | Top Comfort        | Wave                | Filtek              | Control            | P*    |  |
| Inflammatory infiltrate | 7          | 18.75^             | 17.50 <sup>AB</sup> | 18.75 <sup>^</sup>  | 10.00 <sup>в</sup> | .016  |  |
| ·                       | 15         | 12.50              | 10.00               | 12.50               | 10.00              | .171  |  |
|                         | 30         | 7.50               | 5.00                | 5.00                | 5.00               | .237  |  |
| Edema                   | 7          | 10.00              | 10.00               | 10.00               | 6.25               | .108  |  |
|                         | 15         | 6.25               | 6.25                | 5.00                | 5.00               | .543  |  |
|                         | 30         | 5.00               | 5.00                | 5.00                | 5.00               | 1.000 |  |
| Necrosis                | 7          | 8.75               | 7.50                | 8.75                | 5.00               | .131  |  |
|                         | 15         | 6.25               | 6.25                | 5.00                | 5.00               | .543  |  |
|                         | 30         | 5.00               | 5.00                | 5.00                | 5.00               | 1.000 |  |
| Granulation tissue      | 7          | 18.75              | 18.75               | 18.75               | 16.25              | .391  |  |
|                         | 15         | 12.50              | 10.00               | 12.50               | 7.50               | .071  |  |
|                         | 30         | 6.25               | 5.00                | 6.25                | 6.25               | .764  |  |
| Giant cells             | 7          | 10.00              | 8.75                | 7.50                | 5.00               | .127  |  |
|                         | 15         | 8.75               | 7.50                | 6.25                | 5.00               | .089  |  |
|                         | 30         | 5.00               | 5.00                | 5.00                | 5.00               | 1.000 |  |
| Collagen                | 7          | 11.25              | 10.00               | 12.50               | 12.50              | .391  |  |
| -                       | 15         | 12.50 <sup>A</sup> | 15.00 <sup>AB</sup> | 16.25 <sup>AB</sup> | 18.75 <sup>₿</sup> | .037  |  |
|                         | 30         | 15.00 <sup>A</sup> | 18.75 <sup>AB</sup> | 20.00 <sup>B</sup>  | 20.00 <sup>B</sup> | .008  |  |

Table 2. Mean of Scores Attributed to Resins After Time Intervals of 7, 15, and 30 Days, for the Six Conditions Evaluated<sup>a</sup>

<sup>a</sup> For each sample of the study, five representative sections of the histological condition of the tissue were analyzed, when all five sections of the tissue showed the same histological condition. Scores: 1, absent (5.00); 2, scarce (10.00); 3, moderate (15.00); and 4, intense (20.00).

\* P indicates nonparametric Kruskal-Wallis test, followed by Dunn's multiple comparisons test.

<sup>A or B</sup> Means followed by the same single letter do not express statistically significant difference (P > .05).

<sup>AB</sup> Means followed by different letters express statistically significant difference (P < .05).

level of concomitant aggressiveness of these resins. There was expressive presence of granulation tissue after 7 and 15 days, but there was no statistical difference between the materials. This, associated with the reduced inflammatory reaction in subsequent time intervals, demonstrated that the body tolerates the resins well.

The gradual reduction in inflammatory events and the ascendance of the polymerization process at 30 days corroborate the findings of other studies<sup>10,11</sup> and relate to the pattern of monomer conversion into polymers and consequent release of residual monomers<sup>22</sup> in the first 4 weeks.<sup>10,23</sup> Regarding the tissue repair process (presence of collagen fibers), the resins Wave and Filtek presented no statistical difference from the control group in any of the time intervals

 Table 3.
 Mean Values and Standard Deviation (SDs) of the Degree of Conversion (%) of Flowable Resins\*

|                   | Groups   |  |  |              |  |  |  |
|-------------------|--|--|--|--------------|--|--|--|
| Time              | Top Comfort  | Wave   | Filtek   | Р            |  |  |  |
| 7 Days<br>15 Days | 62.3 (2.6) <sup>Aa</sup><br>67.1 (3.2) <sup>Ab</sup> | 64.0 (2.9) <sup>Aa</sup><br>70.1 (3.4) <sup>Ab</sup> | 65.2 (3.8) <sup>Aa</sup><br>72.0 (4.1) <sup>Ab</sup> | .187<br>.061 |  |  |  |
| 30 Days           | 74.7 (2.7) <sup>Ac</sup>                             | 78.0 (3.9) <sup>ABc</sup>                            | 79.1 (2.0) <sup>Bc</sup>                             | .024         |  |  |  |
| Ρ                 | .001   | .001   | .001   | -            |  |  |  |

\* Means followed by different letters express statistically significant differences (P < .05) according to the analysis of variance and Tukey's post-hoc test represented by <sup>a,b,c</sup> (in columns, comparison between times) and <sup>A,B</sup> (in rows, comparison between resins for each time).

evaluated. The resin Top Comfort demonstrated statistical difference from the control group (P = .037) after 15 days, persisting after 30 days, and differing statistically from the resin Filtek and the control group (P = .008). The tissue exposed to the resins of Groups Wave and Filtek showed good healing capacity, with this process being slower and showing a lower quantity of collagen fibers for the resin Top Comfort.

As for the DC, other authors have shown immediate conversion values ranging from 60% to 68%.<sup>24</sup> In the present study, the values ranged from 62.3% for immediate conversion to 79.1% for conversion in 30 days. The DC depends on the light source used. Other investigators<sup>25</sup> have suggested that in order to obtain a similar DC among tested resins, the values could be achieved in 10 to 15 seconds by fast halogen (850 mW/cm<sup>2</sup>) polymerization. In this study, a LED system with a light intensity of 1000 mW/cm<sup>2</sup> was used for 40 seconds. The higher DC observed with this light source might be attributed to the greater light energy used on the material. This is in agreement with the findings of authors<sup>26</sup> who demonstrated that when light energy was decreased, the DC diminished considerably, as shown with different light energy densities for the same light source.

The flowable resin Top Comfort showed the lowest conversion values during the experiment, with a statistically significant difference from Filtek Z350 (P = .006) after an interval of 30 days. Flowable resins contain

a large quantity of a diluent monomer, commonly TEGDMA, added to a more voluminous and structurally rigid monomer base, such as Bis-GMA or UDMA, to reduce the viscosity.<sup>27</sup> This increases the number of polymerized double bonds; thus, DC increases when there is an increase in TEGDMA—up to a certain point.<sup>24</sup> Although most flowable resins have a proportion of TEGDMA that does not normally reach this critical concentration, the high proportion of monomer may be a limiting factor in the DC.<sup>25</sup>

The flowable resin Top Comfort is composed of 60% BisGMA, UDMA, and TEGMA monomers. This quantity of monomers is significantly higher than the 35% found in the Filtek and Wave resins. Based on the histological findings, we affirmed that the higher level of unconverted monomers released added to the cytotoxicity of these, influencing the slower reparative capacity of the resin Top Comfort, as demonstrated by the lower number of collagen fibers after 15 and 30 days compared with the other flowable resins. The difference in DC of the resins tested may also be attributed to differences in composition: In addition to Bis-GMA and TEGDMA, the resins contain UDMA, which modifies the rheology of the material and may influence the DC of the resin.<sup>24</sup>

Analysis of the inflammatory and polymerization phenomena in order to characterize and classify the experimental groups and compare them with the control allows one to affirm that the order of the bestto-worst performance was Filtek, Wave, and Top Comfort resin according to the tissue repair capacity after 30 days.

#### CONCLUSION

- The hypothesis was rejected. The flowable resin Top Comfort demonstrated lower tissue repair capacity with a lower number of collagen fibers compared with the Filtek and Wave resins.
- The DC ranged from 62.3% for immediate conversion to 79.1% for conversion in 30 days. The resin Top Comfort showed the lowest conversion values during the experiment.

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