

## PAPER DETAILS

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# Evaluation of the Protective Effect of Silver Diamine Fluoride Against Secondary Caries Development in Composite Resin Restorations Comparatively with Sodium Fluoride and Titanium Tetrafluoride by Micro Computed Tomography

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Kompozit Resin Restorasyonlarda Gümüş Diamin Florürün Sekonder Çürük Gelişimine Karşı Koruyucu Etkisinin Sodyum Florür ve Titanyum Tetraflorür ile Karşılaştırmalı Olarak Mikro Bilgisayarlı Tomografi ile Değerlendirilmesi



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## ABSTRACT

**Objective:** The aim of this study is to evaluate the effectiveness of 38% silver diamine fluoride (SDF) in preventing secondary caries formation by comparing it with other fluoride-containing remineralization agents such as sodium fluoride and titanium tetrafluoride in vitro.

**Material and Methods:** Cavities of 4x2x2 mm were prepared on the mesial, distal, buccal and lingual surfaces of the extracted 12 intact human third molars. 38% silver diamine fluoride solution was applied to the first group, 2% NaF solution to the 2nd group, 2% TiF<sub>4</sub> solution to the 3rd group and deionized water to the 4th group. After the solutions were applied, the cavities were restored with composite resin. Then, all samples were aged by thermal cycle method and sterilized by autoclave. The volume of the lesions formed by keeping the samples in 5% sucrose solution containing *Streptococcus mutans* and *Lactobacillus acidophilus* for 28 days was evaluated by micro computed tomography method. Data analysis of the study was performed using SPSS 21.0 V (IBM, Chicago, USA) statistical package program. Group results were compared using one-way analysis of variance (ANOVA) test ( $p>0.05$ ).

**Results:** Although the secondary caries lesion volume was found to be lower in the silver diamine fluoride applied group compared to the other groups, there was no statistically significant difference between the groups.

**Conclusion:** These results show that the effectiveness of silver diamine fluoride to prevent secondary caries formation in composite resin restorations should be investigated with longer-term studies.

**Keywords:** Silver diamine fluoride; Secondary caries; Micro-computed tomography; Sodium fluoride; Titanium tetrafluoride

## ÖZ

**Amaç:** Bu çalışmanın amacı %38'lik gümüş diamin florürün (SDF) sekonder çürük oluşumunu önleme etkinliğinin sodyum florür ve titanyum tetraflorür gibi flor içeren diğer remineralizasyon ajanları ile in vitro ortamda karşılaştırılarak değerlendirilmesidir.

**Yöntem:** Çekilmiş 12 adet sağlam insan 3. molar dişlerinin mezial, distal, bukkal, lingual yüzeylerine 4x2x2 mm boyutlarında kaviteler hazırlandı. İlk gruba %38'lik gümüş diamin florür solüsyonu, 2. gruba %2'lik NaF solüsyonu, 3. gruba %2'lik TiF<sub>4</sub> solüsyonu ve 4. gruba deiyonize su uygulandı. Solüsyonlar uygulandıktan sonra kaviteler kompozit rezin ile restore edildi. Daha sonra bütün örnekler termal siklus yöntemi ile yaşlandırılıp otoklav ile sterilize edildi. Örneklerin 28 gün boyunca *Streptococcus mutans* ve *Lactobacillus acidophilus* içeren %5'lik şeker solüsyonunda bekletilmesiyle oluşturulan lezyonların hacmi mikro bilgisayarlı tomografi yöntemi ile değerlendirildi. Çalışmaya ait verilerin analizleri SPSS 21.0 V (IBM, Chicago, ABD) istatistik paket programı kullanılarak yapıldı. Tek yönlü varyans analizi (ANOVA) testi kullanılarak grup sonuçları karşılaştırıldı ( $P>0.05$ ).

**Bulgular:** Gümüş diamin florür uygulanan grupta sekonder çürük lezyon hacmi diğer gruplara göre daha az bulunsa da gruplar arasında istatistiksel olarak anlamlı fark yoktu.

**Sonuç:** Bu sonuçlar, gümüş diamin florürün kompozit rezin restorasyonlarda sekonder çürük oluşumunu önleyici etkinliğinin daha uzun süreli çalışmalarla araştırılması gerektiğini göstermektedir.

**Anahtar Kelimeler:** Gümüş diamin florür; Sekonder çürük; Mikro bilgisayarlı tomografi; Sodyum florür; Titanyum tetraflorür

## INTRODUCTION

In recent years, due to increase of importance given to aesthetic appearance, composite resins are frequently used as dental restorative materials by patients and physicians. In resin materials, as with all polymers, a shrinkage occurs due to the conversion of monomers into the polymer chain. This reduction in volume is caused by the convergence of the monomer leads to polymerization stresses within the composite resin and on the tooth-restoration bonding surface.<sup>1</sup> If the tensile strength due to polymerization is greater than the bond strength, the bond between the composite resin and the dentin interface will fail. As a result, problems such as formation of microcavities, edge leakage, postoperative sensitivity, discoloration and formation of secondary caries may arise. In addition, microbial dental plaque deposition occurs more than amalgam restorations in composite resin restorations.<sup>2</sup> Apart from these, many factors such as adhesive systems and light sources may affect the success of composite resin restorations and indirectly cause secondary caries formation. New caries around old restorations is one of the most important reasons for replacing restorations.<sup>3</sup> When replacing old restorations, intact tooth tissues can be damaged. For this reason, it is of great importance to take preventive measures before secondary caries occurs.

Today, many preventive and protective methods against dental caries have been developed and continue to be developed. Secondary caries can be prevented by reducing the amount of bacteria between the tooth and the restorative material. For this, materials with antibacterial activity and potential to provide remineralization in dental tissues can be used.<sup>4</sup> Fluorides, one of the most widely used remineralization agents against dental caries, can be used alone or in combination with other agents. Solutions of compounds such as sodium fluoride (NaF), titanium tetrafluoride (TiF<sub>4</sub>) and silver diamine fluoride (SDF) at appropriate concentrations can be used topically to stop the progression of caries lesions and prevent caries formation.<sup>5</sup>

Silver diamine fluoride has been used as a therapeutic agent since 1970s to prevent dental caries and to stop initial caries.<sup>6</sup> Several studies have shown that the most effective concentration of SDF that prevents caries formation is 38% SDF solution.<sup>7</sup> There are few studies investigating the effect of silver diamine fluoride in preventing the development of secondary caries in composite resin restorations.

Micro-computed tomography (micro-CT) method, which allows 3D reconstruction, has recently been used to examine polymerization shrinkage, gap formation and microleakage.<sup>8</sup> In this study, secondary caries lesion volumes were evaluated by micro-computed tomography method.

The aim of this study was to evaluate the preventive effect of secondary caries formation of SDF in comparison with sodium fluoride and titanium tetrafluoride. The null hypothesis was there is no difference between the protective effect of silver diamine fluoride and other test materials against secondary caries formation.

## MATERIAL AND METHOD

The ethical committee approval required for our research was obtained from Gazi Faculty of Medicine Research Ethics Committee

(24074710-35 Issue: 23.10.2017). Written consent was not obtained because previously extracted teeth were used in our study.

### Materials selection and specimen preparation

In this study, 12 extracted human third molars without any caries, restoration, structural defects or damage were used. Extracted teeth were stored in 0.1% thymol solution until study time. The teeth were placed in cylindrical molds containing cold acrylic, leaving the crown areas out. Then, cavities of the same dimensions (4x2x2 mm) were prepared under water cooling on 4 different surfaces (mesial, distal, buccal, lingual) of the teeth.

In this study, samples were prepared for 4 groups on the same tooth in order to minimize the different results that may occur due to the structural differences of the dental tissues. 48 cavities were prepared on 12 teeth for 4 groups (n=12). The experimental groups the materials thereof and application protocols are shown in Table 1.

**Table 1.** Materials and application protocols of experimental groups.

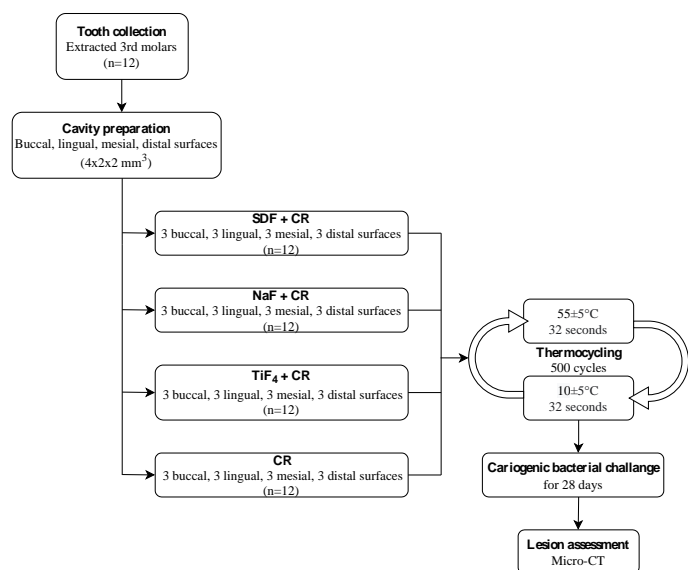
GROUP	MATERIAL	MANUFACTURER	PROTOCOL OF USE
SDF	38% SDF	FAGamin®, Tedequim S.R.L., Cordoba, ARGENTINE	After the product has been applied for 3 minutes rinsed with water for 30 seconds and air dried
NaF	2% NaF	Sigma Aldrich, St. Louis, MO, USA	After the product has been applied for 60 seconds dried with moisture absorbent papers
TiF <sub>4</sub>	2% TiF <sub>4</sub>	Sigma Aldrich, St. Louis, MO, USA	After the product has been applied for 60 seconds rinsed with water for 30 seconds
CG	Deionized water	Sigma Aldrich, St. Louis, MO, USA	After applying deionized water air dried

After the solutions were applied to all groups, 2-step self-etch adhesive system (Clearfil SE Bond 2, Kuraray, Tokyo, Japan) was applied to the cavities following the manufacturer's instructions. The cavities were then restored with composite resin (Filtek Z550, 3M ESPE, Seefeld, Germany). All restorations were polished using aluminium oxide coated discs (Sof-Lex; 3M ESPE, St. Paul, MN, USA). After that, all samples were subjected to thermal cycling 500 times in distilled water baths at 55 ± 5 °C and 10 ± 5 °C. Then, all teeth were sterilized with autoclave before the cariogenic bacterial challenge. The flow chart of the study is shown in Figure 1.

### Cariogenic bacterial challenge

The microorganisms used for the cariogenic challenge were *S. mutans* American Type Culture Collection 25175 and *L. acidophilus* American Type Culture Collection 4356. *S. mutans* strains were grown on tryptic soy agar medium and *L. acidophilus* strains were incubated on MRS agar medium until colony formation was visible (37 °C, microaerophilically). The bacterial suspension was prepared according to McFarland 0,5 (1.5x10<sup>8</sup> colony-forming units / ml). After the teeth were placed in the sample cups, 2 ml artificial saliva was added to form the pellicle and incubated for 1 hour at 37 °C. At the end of the period, artificial saliva was removed and bacteria suspensions were placed on the samples. Then, 5 ml of brain-heart infusion broth (BHI broth, Merc, Germany)

solution with 5% sucrose was added and the incubated at 37 °C . The teeth were maintained in this bacterial solution for 28 days; the medium was refreshed every 48 hours. During the incubation period, Gram stain test of the used medium was performed to check for contaminants.



**Figure 1.** Flow chart of the study.

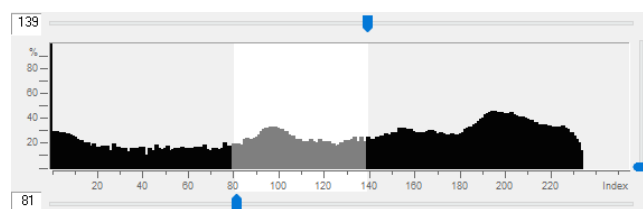
#### Evaluation of lesions by micro-computed tomography (micro-CT)

The volumes of secondary caries lesions were evaluated by Micro-CT method. The teeth were scanned using micro-CT (SkyScan 1275, Kontich, Belgium) to assess volumes of carious lesions (Figure 2). The X-ray source was operated at a voltage of 80 kV and a current of 125  $\mu$ A. A total of 1800 cross-sectional images of approximately 9 microns were taken using a 1 mm aluminum filter. The teeth were rotated by 0.20° each time and scanned 360°. Each scan lasted approximately for 35 minutes. Sectional images of the samples were converted from TIFF (Tagged Image File Format) format to BMP (Bit Map Picture) format. The sections were reconstructed by eliminating image impurities and radiological artifacts in the software NRecon (Version 1.7.4.2. SkyScan, Kontich, Belgium) and the image was made available for mathematical analysis. A high-capacity workstation (Dell Precision Tower 7910, Dell Company, Austin, Texas, USA) was used for the analysis of the images. Positional errors of all three planes of space were corrected by using SkyScan Dataviewer (version 1.5.6.2 64 bit, SkyScan, Kontich, Belgium) program of the processed images. The unnecessary regions were removed and the image sizes were reduced, thus enabling faster analysis. The new data series was uploaded to CTAn 18.4.0 (SkyScan, Kontich, Belgium) to limit the active examination area. First, the lesion site was separated from the surrounding intact tissues and air space using the regional disintegration function of the program.

As the second stage, the appropriate interval (theresholding) was determined according to the lesion density on the black and white image page (Figure 3). Volume calculations for the lesions were performed using the standard task list for each sample.



**Figure 2.** Micro-computed tomography image of the restoration margin after challenge with cariogenic bacteria.



**Figure 3.** Determination of the appropriate range for lesion density on the black and white image page.

#### Statistical analysis

Statistical analysis was performed using SPSS 21.0 V (IBM, Chicago, USA) statistical package program. After analysis of the normality of data distribution using the Shapiro-Wilk normality test, the results were analyzed by a one-way analysis of variance (ANOVA). The level of statistical significance was accepted as  $p < 0,05$ .

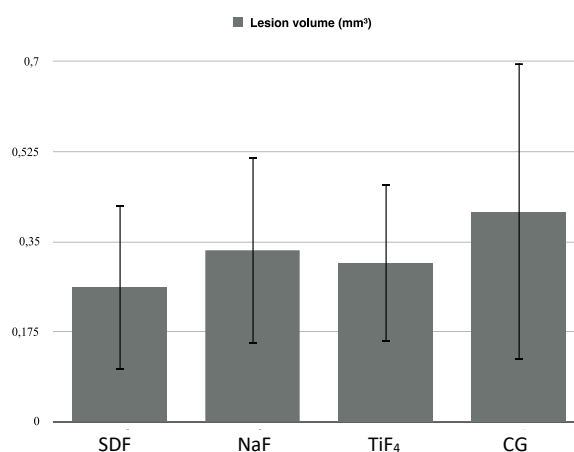
## RESULTS

The mean volume values, standard deviation values, minimum and maximum values of secondary caries lesions around the restoration of the study groups are shown in the Table 2. The mean lesion volume values for SDF, NaF, TiF<sub>4</sub> and control groups were calculated as 0.261 mm<sup>3</sup>, 0.333 mm<sup>3</sup>, 0.309 mm<sup>3</sup> and 0.409 mm<sup>3</sup>, respectively. When the volume of secondary caries lesion formed around the restoration was evaluated, the SDF group showed the lowest value. The SDF group was followed by TiF<sub>4</sub>, NaF and control groups (Figure 4). However, no statistically significant difference was found between the groups according to the results of one-way analysis of variance (ANOVA) ( $P > .05$ ).

**Table 2.** Mean lesion volumes, standard deviation, minimum and maximum values of test groups

Group	Mean of lesion volumes (mm <sup>3</sup> )	Minimum	Maximum	Standard Deviation
SDF	0,261 <sup>a</sup>	0,067	0,700	0,160
NaF	0,333 <sup>a</sup>	0,088	0,617	0,180
TiF <sub>4</sub>	0,309 <sup>a</sup>	0,119	0,613	0,153
CG	0,409 <sup>a</sup>	0,060	0,890	0,287

■ Distinct superscript letters indicate statistical significance ( $P < .05$ ).



**Figure 4.** Graphical display of secondary carious lesion volume mean and standard deviations of the groups.

## DISCUSSION

In current study, the effectiveness of SDF solution and NaF<sub>2</sub> and TiF<sub>4</sub> solutions in preventing secondary caries formation was evaluated. The null hypothesis was accepted because there was no statistically significant difference between the other solutions tested with SDF in terms of preventing secondary caries formation.

Previous studies rather focused on the effects of silver diamine fluoride on remineralizing initial caries lesions and preventing primary caries lesions. In addition, while there are many other studies with glass ionomer restorative material evaluating the protective effect of silver diamine fluoride on secondary caries lesions, there is only one study using composite resin. In addition, there is no study comparing the effects of silver diamine fluoride, sodium fluoride and titanium tetra fluoride to prevent secondary caries formation.

A recent study has reported that silver diamine fluoride (SDF), a current caries preventive agent, possesses antibacterial properties, hinders the formation of biofilm, halts the progression of cavities, and facilitates remineralization.<sup>9</sup> SDF, which has been used as a therapeutic agent since the 1970s, received approval from the United States Food and Drug Administration (FDA) in 2014 and was launched in 2015.<sup>10</sup> Silver diamine fluoride can be used in various concentrations such as 10%, 12%, 30%, and 38%. It is recommended to use 38% SDF solution to prevent caries and to stop existing carious lesions.<sup>11</sup> That's why we preferred the 38% concentration of SDF for this study.

Fluorides are widely recognized as a key factor responsible for the dramatic decrease in global prevalence of dental caries.<sup>12</sup> They primarily achieve caries control through their topical effects. The application of high-concentration fluoride products topically creates protective layers resembling CaF<sub>2</sub> precipitates on dental hard tissues.<sup>13</sup> Topical fluoride treatments promote inhibition of demineralization and an increase in remineralization in dental hard tissues. It has been reported that restorations with fluoride application to cavity walls before the placement of restorative materials experience less microleakage compared to restorations without such fluoride treatment.<sup>14</sup> In a study, it was reported that sodium fluoride was more successful in remineralizing early enamel caries compared to stannous fluoride and amine fluorides.<sup>15</sup> The most commonly used fluoride solution since the 1940s has been the 2% sodium fluoride (NaF) solution.<sup>16</sup>

Due to the low pH of TiF<sub>4</sub> solution, the penetration depth of fluoride ions is greater.<sup>17</sup> It has been reported that TiF<sub>4</sub> solution applied to dentin surfaces exhibits antibacterial effects against *S. mutans* and *L. casei*.<sup>18</sup> In a study investigating the optimal concentration of TiF<sub>4</sub> solution for remineralizing early dentin caries, the concentration of 2% TiF<sub>4</sub> solution was found to be more successful compared to 1%, 3%, and 4% TiF<sub>4</sub> solutions.<sup>19</sup> Therefore, we preferred the concentration of 2% TiF<sub>4</sub>. Basting et al. showed that the layer formed by applying TiF<sub>4</sub> to dentin was resistant to acids and remained intact even after 30 minutes of exposure to citric acid.<sup>20</sup> It has been reported that incorporation of TiF<sub>4</sub> into the hybrid layer prolongs the life of the hybrid layer and prevents the formation of secondary carious lesions.<sup>18,21</sup>

In this study, each of the remineralization solutions was applied using different protocols. When applying silver diamine fluoride SDF solution, two different methods were employed. In several studies, after waiting for sufficient time in SDF application, excess solution was rinsed away.<sup>35</sup> There are also different studies in which no washing process was performed after SDF application.<sup>23</sup> In our study, the 38% SDF solution was applied to cavity surfaces in accordance with the manufacturer's instructions. Specifically, 2-3 drops of the product were applied to the cavity surfaces using a microbrush. After waiting for 2 minutes, an active application was performed with the brush for 1 minute. Subsequently, the samples were washed and dried. The 2% NaF solution was applied to tooth surfaces in a manner similar to previous studies. It was left on

the surfaces for 60 seconds and then dried using sterile absorbent paper points.<sup>24</sup> The TiF<sub>4</sub> solution, following a procedure similar to prior studies, was actively applied to cavity surfaces using a microbrush for 60 seconds, followed by washing and drying.<sup>25</sup> The application of TiF<sub>4</sub> solutions to dentin surfaces before acid etching or application of acidic monomers is recommended.<sup>21</sup> Therefore, in this study, the dentin surfaces were treated with the TiF<sub>4</sub> solution first, followed by the application of the adhesive system.

The histopathological structures of caries-like lesions created in vitro are similar to caries lesions that occur in vivo.<sup>26</sup> Chemical and bacterial models can be used to create carious lesions in dental tissues in vitro. Chemical model is an easy and cheap system but it does not contain bacteria and it cannot mimic the mouth environment adequately. In contrast, the ecosystem that causes microbial dental plaque and caries development in bacterial model can be imitated in vitro.<sup>27</sup> In this study, a bacterial system containing *S. mutans* and *L. acidophilus* was preferred for the creation of in vitro secondary caries lesions. Bacterial challenge was carried out using two types of cariogenic bacteria strains. The microbiological experiments of the study continued for 28 days. Caries lesions were formed on coronal restorations by mimicking clinical conditions in vitro.

Restorations are constantly exposed to temperature and pH changes within the oral cavity. Due to the disparity in thermal expansion coefficients between dental tissues and restorative materials, thermal stresses can lead to the formation of micro-gaps at the tooth-restoration interface over time, consequently resulting in microleakage. Therefore, in order to simulate oral conditions, thermal and mechanical load cycles can be applied to samples. It has been shown that after 500 thermal cycles, there is a significant increase in microleakage at the enamel and dentin margins.<sup>28</sup> For these reasons, restorations from all groups in our secondary caries experiment underwent 500 thermal cycles in water baths at 5°C and 55°C before the microbiological test.

Both in vivo and in vitro methods are used in the diagnosis of secondary caries. The gold standard for in vitro caries detection is the histological evaluation of tooth sections taken from the relevant region.<sup>29</sup> However, the reliability of this method is reduced due to potential damage to the examined tissue during sample sectioning for histological examination. Furthermore, various factors in histological methods, such as the type of microscope used, the number of sections taken, section thickness, the direction of sectioning, and the type of stain used for detection, can influence the results.<sup>30</sup> Micro-CT imaging allows visualization of numerous sections without damaging dental tissues. Consequently, it has emerged as an alternative to histological methods in in vitro caries detection. In this study, the lesions created in vitro were evaluated using the micro-CT method. Micro-CT allows examination of samples from all groups on the same tooth without damaging dental structure. In addition, the ability to perform three-dimensional analysis of micro-CT images enabled the volumetric evaluation of the secondary caries lesions created. In this way, samples belonging to all groups can be prepared and evaluated on the same tooth.

In contrast to this study, Mei *et al.* (2016), evaluating the effectiveness of SDF in preventing secondary caries, showed that the application of SDF significantly increased the resistance of glass ionomer and composite resin restorations against secondary caries compared to the control group.<sup>31</sup> However, unlike our study, washing was not performed after applying SDF solution. In addition, although secondary caries lesions were evaluated with the micro-CT method in this study, the images were evaluated in 2-dimensions.

There are studies reporting that silver diamine fluoride reduces the bond strength of composite resin restorations to dentin<sup>32,33</sup>. It can be thought that this situation reduces the protective effectiveness of silver diamine fluoride against the formation of secondary caries due to increased microleakage.



Ishiguro *et al.* investigated the bacterial acid production, which causes caries formation at the tooth-bacteria interface, after applying 38 % SDF and 2% NaF solutions in aged and unaged samples.<sup>34</sup> While they found that both solutions were more successful in inhibiting bacterial acid production than the control group, they also indicated that 38% SDF solution is more successful than the 2% NaF solution. In aged specimens, there was no statistically significant difference in the inhibition of bacterial acid production between the 38% SDF, 2% NaF and control groups. In this study, it can be expressed that there was no difference between the control, NaF and SDF groups in terms of secondary carious lesion volumes due to the deterioration of the tooth-restoration connection and the decrease in the effectiveness of the solutions during the aging process.

In another study investigating the inhibitory effect of 2.5% TiF<sub>4</sub> solution on the formation of secondary caries, it was shown that less demineralization occurred in the TiF<sub>4</sub> treated group compared to the non-applied groups.<sup>35</sup> In this study, the volume of secondary caries lesion formed in the TiF<sub>4</sub> group was less than that of the control group. However, there is no statistically significant difference. It could be thought that this situation may resulted from the difference in the concentrations of the solutions used.

## CONCLUSION

Within the limitations of this in vitro study, silver diamine fluoride, sodium fluoride, and titanium tetrafluoride were not found to be successful in preventing secondary caries formation in composite resin restorations. Our study has some limitations as it was performed under in-vitro conditions. We believe that new studies are necessary to better understand the effectiveness of silver diamine fluoride compound in preventing secondary caries in composite resin restorations.

We think that some changes should be made in the solution content in order to increase the bond strength of silver diamine fluoride to dentin in the future. Thus, the effectiveness of preventing secondary caries formation can be increased by reducing microleakage caused by the reduction of the bond strength of composite resins to dentin.

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**Hakem Değerlendirmesi:** Dış bağımsız.

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**Author Contributions:** Concept - M.A.E., H.E.; Design-M.A.E., H.E., G.A.; Supervision-M.A.E., H.E., G.A.; Sources-M.A.E.; Data Collection and/or Processing- M.A.E.; Analysis and/or Interpretation-M.A.E., G.A., M.O.; Literature Review-M.A.E., H.E.; Written by-M.A.E.; Critical Review-M.A.E.

**Conflict of Interest:** The authors have no conflicts of interest to declare.

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