Research Article

Effect of surface preparation and light curing on penetration of silver particles from 38% silver diamine fluoride in dentin of primary teeth: An in vitro evaluation

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ABSTRACT: Purpose: To evaluate the effect of light cure, as well as various dentin surface treatment approaches, on the penetration depth of silver precipitating from 38% silver diamine fluoride into primary dentin tubules. **Methods:** The occlusal dentin surfaces of 42 non-carious primary molars were exposed and then sectioned into halves bucco-lingually. The halves from each tooth pair were randomly split in two mega-groups, and each mega-group was divided randomly as follows into six experimental groups: prepared by either carbide bur (G1, G2), ceramic bur (G3, G4), or erbium laser (G5, G6). SDF was then applied to all prepared surfaces, and finally even-numbered groups (G2, G4, G6) were light cured. One mega-group was assigned to quantitative evaluation of silver penetration depth along the axial wall, and the other mega-group was reserved for qualitative observation of relative silver distribution on the occlusal surface, both via scanning electron microscope. **Results:** No significant difference was observed in silver penetration depth between light cure and non-light cure groups ($P= 0.8908$). There was a statistically significant association between tooth preparation method and depth of silver penetration ($P< 0.000001$): laser-treated groups had significantly deeper silver penetration (1,148.9 µm G5, 1160.4 µm G6) than carbide bur ($P< 0.05$; 184.7 µm G1, 301.8 µm G2) or ceramic bur ($P< 0.05$; 184.1 µm G3, 131.0 µm G4) groups. A significant difference ($P< 0.05$) was noted in percentage occlusal surface coverage of particles between laser (51.4% G5, 35.8% G6) and carbide groups (21.1% G1, 19.3% G2). Light cure had no significant effect on the depth of silver penetration from 38% SDF in the dentin of primary teeth. Laser preparation resulted in deeper silver penetration than carbide or ceramic bur. (*Am J Dent* 2021;34:44-48).

CLINICAL SIGNIFICANCE: Exposure of 38% silver diamine fluoride-treated dentin to light cure did not affect the depth of penetration of silver particles into the dentin tubules of primary teeth. Rather, tooth preparation approaches that reduce the smear layer, like laser ablation, resulted in the deepest penetration of silver into the tubules. Clinical application of these findings will depend on scenario and treatment aim.

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Introduction

The therapeutic silver diamine fluoride (SDF)[1] has garnered increasing attention in oral health care.[1] In particular, 38% SDF is a clear topical agent containing 24.4-28.8% (w/v) silver and 5.0-5.9% fluoride (minimal average pH 10) that has shown efficacy in arresting and preventing active dental caries; it is used "off-label" in caries management because the US Food & Drug Administration clearance is for dentin sensitivity.[2] The constituent components in SDF are silver, fluoride, and ammonia. Silver has antimicrobial properties, while fluoride helps to remineralize and prevent demineralization. Ammonia acts as a stabilizer.

A manufacturer recommendation (may or may not apply to other products) is to not light-cure 38% SDF-treated surfaces as it "may limit the free silver ions available in the tooth, and cause them to oxidize, which renders them useless or less effective.[3] Once SDF is exposed to light curing devices, an immediate color change related to the silver ions is noted on the tooth surface. Considering the antibacterial properties of silver, some practitioners advocate avoidance of SDF exposure to light curing in order to maximize potential penetration of free silver ions into the dentin tubules. By contrast, other clinicians light-cure SDF-treated tooth structure to visualize the extent of its application. However what effect, if any, does exposure to light cure have on the depth of penetration of silver ions into dentin tubules has not been published. In addition, to the authors' knowledge, there is no current published study that examines potential differences in the depth of penetration of silver ion particles in dentin primary molars prepared using a carbide, ceramic bur, or hard tissue cutting laser (Er,Cr:YSGG).

While carbide burs are efficient in removing caries, their main limitation is their inability to distinguish between infected and affected dentin, resulting in over excavating and removing dentin that could be remineralized.[4] On the other hand, through the specific design of the fluted cutting edges, ceramic burs (CeraBur[a]) selectively remove soft and infected carious dentin while leaving affected and remineralizable tooth structure.[4]

The introduction of hard tissue cutting lasers provides alternative ways of preparing a tooth cavity. The effect of the laser energy is coined ablation, where the laser energy heats up the water within and causes it to steam, expanding the tissue and causing a "mini-explosion" that breaks the tissue into small particles.[5] Erbium lasers including erbium, chromium-doped yttrium, scandium, gallium and garnet (Er,Cr:YSGG; 2,780 nm) interact with hard tissues containing water and hydroxylapatite where demineralized hard tissue is ablated, carious tissue is removed, and healthy tissue is left intact. Several studies evaluating the cavity preparation of hard tissues by Er,Cr:YSGG and Er:YAG lasers reveal that the hard tissues treated with erbium lasers leave an irregular, rough surface with no presence of a smear layer, and open dentin tubules.[6-8] The effect of different tooth preparation methods on the penetration of silver ions into dentin tubules is not known.

The current study evaluated the depth of penetration of silver ions from 38% SDF in the dentin of primary teeth under different conditions of light (light-cure vs. non light-cure) and surface treatment (carbide, ceramic bur, or hard tissue cutting laser (Er,Cr:YSGG). The null hypotheses for this pilot study were: (1) There will be no difference observed in the depth of penetration of silver originating from 38% SDF in light-cured teeth vs. non light-cured teeth.; (2) There will be no difference observed in the depth of penetration of silver originating from 38% SDF among the dentin surface treatment groups prepared with carbide bur, ceramic bur, or laser (Er,Cr:YSGG).

Materials and Methods

This study was exempted from the Institutional Review Board at The University of Texas Health Science Center at Houston (HSC-DB-10-0396). De-identified extracted non-carious primary first and second molars (n= 42) were collected at the University of Texas School of Dentistry post graduate pediatric dental clinic. All 42 non-carious primary molars were cleaned of debris, then stored in a 0.2% sodium azide solution in a refrigerator at 37°F until investigation.

Specimen preparation - The roots were separated at the cemento-enamel junction (CEJ) by a diamond bur on a high-speed handpiece under a copious amount of water. Pulp tissue was removed, and the pulp chamber was cleaned using hand instruments, etched with 35% phosphoric acid gel[b] and rinsed; then, a bonding agent (OptiBond Solo Plus[c]) was applied and light-cured according to the manufacturer's instructions. A microhybrid resin composite (Filtek Z250[d]) was placed in the pulp chamber and light-cured for 20 seconds to act as support and facilitate handling of the sample.

The teeth were sectioned approximately 1 mm below the cusps, in order to expose the dentin surface, using a diamond blade (Isomet Precision Sectioning Saw[e]) under continuous water irrigation. Samples were examined under magnification to confirm no remaining enamel on the occlusal surface. The exposed occlusal dentin surface was smoothed sequentially with 180-, 320-, and 600- grit silicon carbide (SiC) paper on a water-cooled lathe (Ecomet 6 Grinder Polisher[e]) to obtain a standardized surface. Next, samples were sectioned bucco-lingually into halves starting from the occlusal to the apical. The halves from each molar were first randomly separated into two mega-groups, so that a half from each molar was present in each mega-group (n= 42 halves per mega-group). The halves within each mega-group were randomly distributed into the treatment groups and prepared as described below.

The three dentin surface treatment groups were: carbide bur, ceramic bur, Er,Cr:YSGG laser; each dentin surface treatment group was further sub-divided into light-curing and non-light-curing groups, resulting in six groups of seven samples. In the carbide bur groups (G1, G2), carbide #4[f] was used in a slow speed handpiece (10,000 rpm). In the ceramic bur groups (G3, G4), a ceramic bur #4 (CeraBur[f]) was used in a slow speed handpiece (1,500 rpm). In both the carbide and ceramic bur groups, dentin surface treatment was rendered by gently roughening the occlusal surface by sweeping the bur in a linear fashion from one side to the other, for a total of four times over the occlusal surface for each sample.

In the laser groups (G5, G6), Er, Cr:YSGG laser (Waterlase MD[g]) was used at 2W, 30 Hz with 50% water and 70% air. A MD Turbo handpiece with a MX7 tip[g] (700-µm beam diameter) was positioned perpendicular to the sample in a non-contact mode at 3 mm from the tissue. This is the working distance recommended by the manufacturer where the cutting efficiency by the laser beam is ideal.[9-10] An endodontic file was securely attached to the head of the handpiece to ensure proper distance and angulation from the target tissue. The laser irradiation was adjusted for approximately 2 mm/second speed; in this way the operator created a uniform linear laser ablation of dentin, ensuring that one pulse would not hit the same point twice and would cover the entire occlusal surface of the sample.

After dentin surface treatment group preparation, a thin layer of nail polish was used to cover all surfaces except the occlusal surface of each sample and air-dried. Nail polish was used to protect the surfaces and prevent any potential leakage of SDF onto the sides. Each sample received 38% SDF (Advantage Arrest[h]) procured directly from the manufacturer and stored per manufacturer's instructions; it was applied using a microbrush for 1 minute, rinsed for 30 seconds with distilled water, and air-dried.[5] In the light-curing groups (G2, G4, and G6), the SDF-treated occlusal surfaces were light-cured for 20 seconds with a LED curing light (Valo[b] corded LED curing light). After treatment, the samples were stored in 0.2% sodium azide solution in a refrigerator.

The first mega-group (n=42; seven molar halves for each of the six treatment groups) was designated for visual evaluation of silver penetration depth. To achieve this, nail polish from the axial wall was removed using a 600-grit silicon carbide (SiC) paper on a water-cooled lathe (Ecomet 6) followed by the application of 37% phosphoric liquid etch to the axial wall for 10 seconds, to remove any smear layer. This step was needed to ensure that the dentin tubules from the axial wall were visible and silver penetration could be observed by the scanning electron microscope (SEM) imaging.

The second mega-group (n=42; seven molar halves for each of the six treatment groups) was designated for visualization of silver on the occlusal surfaces via SEM; no removal of axial nail polish was needed for these specimens. Additionally, two reference specimens to aid in SEM comparisons were identified. One polished molar half with no dentin surface preparation and no SDF application was designated to visualize untreated dentin. To provide a baseline for silver appearance under SEM, SDF was applied directly to one of the metal stubs that acts as a specimen mount for the SEM. More specific details of SEM evaluation are found in the next section.

Scanning electron microscopy (SEM) - For SEM observations, the specimens were subjected to a gradual dehydration process using multiple exchanges of aqueous solutions of 50%, 70%, 95%, and 100% laboratory-grade ethanol. Specimens were then loaded into an E3100[i] critical point dryer subjected to multiple exchanges of liquid carbon dioxide, slowly warmed to 40°C (above the carbon dioxide critical point), and dried through venting of the carbon dioxide gas. Dry specimens were mounted directly onto aluminum stubs using conductive carbon tape and received no additional preparation (e.g. sputter coating). The Zeiss EVO 10[l] with a LaB6 source was used to capture SEM images of the occlusal and axial dentin wall interface in variable pressure mode (50 Pa pressure) at 20 kV accelerating voltage, using a Cascade Current Detector (C2D) and high-definition backscatter electron detector (HDBSD). The sensitivity of the HDBSD detector to atomic number was used to identify the brightest particles in each image as silver particles, which have a higher atomic number (47) than any of the other anticipated elements (Ca, P, C, O, N) in the specimens. This assignment was confirmed by energy dispersive spectroscopy (EDS) using Zeiss SmartEDX[l] to assign 10 regions of interest (ROIs), corresponding to bright spots (5) and distant dark spots (5) as controls. Spectra were collected using an EVO Element EDS Analysis system with the EVO 10, on a specimen at 8.5 mm working distance, under variable pressure mode as before, with 20 kV beam accelerating voltage as before.

For the mega-group of specimens designated for evaluation of silver penetration depth, images of the axial wall were captured at 70×, 250×, and 650× magnification, using the C2D and BSD detectors. From the occlusal axial line angle, three points of data were gathered and categorized by location, one at the center of the sample, and one from each side. Data points were labeled and defined by the location in relation to the tooth: left, center, right. The data point at the center was gathered at the center of the sample, in between the two pulp horns. Images of the left and right sides of the sample were captured in a standardized manner. The data point measuring the greatest distance in length of silver ions from the occlusal line angle towards the pulp among the left, center, and right data points was then recorded and reported in the results section. The DEJ was used as a point of reference, left and right measurement points were determined at a uniform distance along the x-axis of 400 µm from the DEJ. The points were positioned in between the DEJ and adjacent pulp. All measurements were obtained using ImageJ,[k] and used to calculate the length and area measurement of SEM images.[11]

To evaluate the depth of penetration of silver ions, the classification of penetration was defined as the presence of silver ions distributed in a linear fashion that was noted to be within the dentin tubules. A line was drawn at the defined distance from the DEJ, perpendicular to the axial occlusal wall line angle (x-axis), and the presence of silver ions was measured at the maximum depth of penetration (y-axis). The greatest depth of the penetration was then calibrated using the SEM scale bar and measured by the ImageJ software at the 250× magnification scale and verified using the 650× magnification. For some samples in which the greatest depth of penetration extended beyond the parameter of the magnification, 100× magnification was used to capture the overall image, while multiple overlapping 250× magnification images with landmark identification was used to measure depth. Additionally, the two reference specimens (one for untreated dentin and one for SDF) were imaged at a magnification scale of 70×, 250×, and 650×.

For the mega-group of specimens designated for evaluation of silver on the occlusal surface, images of the occlusal surface were captured at 85×, 250×, and 650× magnification using both C2D and HDBSD detectors at the center of the occlusal table.

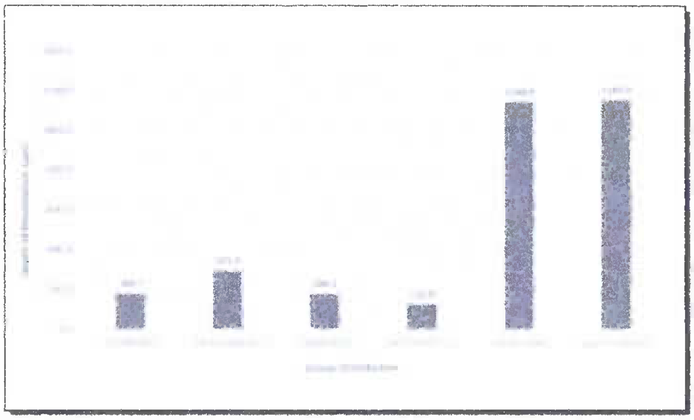

Fig. 1. Average silver penetration depth (µm) into primary tooth dentin by group.

The center of the occlusal surface was imaged with the axial occlusal line angle lined up to the border of the screen. ImageJ particle analysis and thresholding were used to identify the percentage of area coverage of radiopaque particles on the occlusal surface at 250× magnification HDBSD within the selected area and dimensions of 1,024 x 768 pixels. The topography of the occlusal surface was examined using C2D at 250× and 650× magnification.

Statistical analysis - Statistical analyses were performed using R statistical software (R Core Team 2018[l]).[12] A generalized linear model was utilized to analyze data under Gamma distribution. The maximum depth of penetration was analyzed in relation to the variables. The variables examined were: sample (sample 1-42), light-cure exposure (light-cure vs. no light-cure), and surface treatment (carbide, CeraBur, laser). The F-test and Tukey contrast test was used to determine if there was a statistically significant effect in the surface or light variables or any statistically significant effect noted between the surface and light variables. The statistical significance level was calculated at a 95% confidence interval, with $P \leq 0.05$.

Results

Depth of penetration - No statistically significant difference was detected in silver penetration depth between light-cured (G2, G4, & G6) vs. non-light-cured (G1, G3, G5) SDF groups ($P = 0.8908$). By contrast, surface treatment was linked to a statistically significant effect ($P < 0.000001$) on silver penetration depth, independent of light variable, indicating a statistically significant difference among samples prepared with carbide bur, ceramic bur, and laser (Fig. 1).

The depth of silver ion penetration values ranged from:

Carbide preparation: 20-428 µm (G1), 0-522 µm (G2).
Ceramic bur preparation: 5-525 µm (G3), 0-589 µm (G4).
Laser preparation: 202-1,626 µm (G5), 34-1,936 µm (G6).

There was a statistically significant difference in silver penetration depth noted between the laser preparation groups and carbide ($P < 0.05$) or ceramic bur ($P < 0.05$) preparation groups. The dentin surfaces treated with the erbium laser demonstrated the greatest depth of silver ion penetration in comparison to surfaces treated with carbide bur or ceramic bur (Fig. 2). No significant difference was found in silver penetration depth between carbide and ceramic bur groups ($P = 1.0000$).

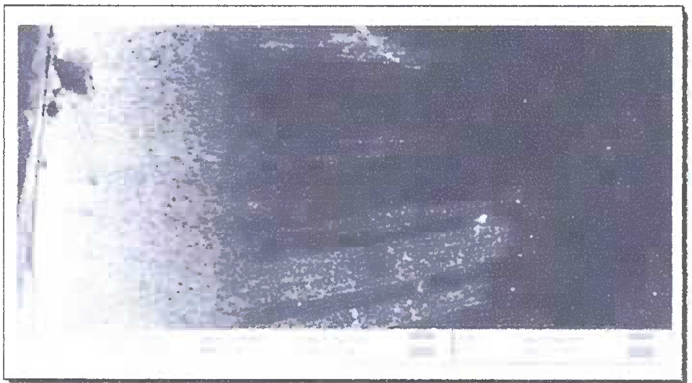

Fig. 2. Depth of silver ion penetration in surface treated with laser. Bright spots in the backscatter detector image correspond to silver deposits.

Silver on occlusal surfaces - The particles examined on the occlusal images resembled the shape, size, and radiopacity of silver ions noted on the SDF coated stub image (Fig. 3). The percentage of area coverage of radiopaque particles on the occlusal surface was 21.12% (G1) and 19.31% (G2) for carbide bur groups, 30.16% (G3) and 23.08% (G4) for ceramic bur groups, and 51.49% (G5) and 35.82% (G6) for laser groups. A significant difference was noted in percentage surface coverage of particles between laser and carbide groups ($P< 0.05$) (Fig. 4).

Discussion

This study evaluated the depth of penetration of silver ions from 38% SDF in the dentin of primary teeth under different conditions of light (light-cure vs. non light-cure) and surface treatment (carbide, ceramic bur, or hard tissue cutting laser (Er,Cr:YSGG). No statistically significant difference was detected in silver penetration depth between light-cured (G2, G4, & G6) vs. non-light-cured (G1, G3, G5) SDF groups ($P= 0.8908$); the first null hypothesis, that there would be no difference observed in the depth of penetration between light-cured teeth and non-light-cured teeth, was accepted.

Surface preparation did have a statistically significant effect ($P< 0.000001$) on silver penetration depth. Specimens prepared by laser demonstrated the greatest silver penetration depths on average (1,148.9 µm G5, 1,160.4 µm G6), significantly different from carbide ($P< 0.05$; 184.7 µm G1, 301.8 µm G2) and ceramic ($P< 0.05$; 184.1 µm G3, 131.0 µm G4) bur groups. Thus, the second null hypothesis, that there would be no difference observed in the depth of penetration of silver among the dentin surface treatment groups prepared with carbide bur, ceramic bur, or laser, is rejected.

The findings of this study suggested that exposure to light cure did not affect the depth of penetration of silver particles from SDF in primary tooth dentin; rather, surface preparation method of the dentin prior to SDF resulted in a significant effect on silver penetration. Laser-prepared dentin groups had an average maximum penetration depth of >1,100 µm, compared with 184 µm and 301 µm for carbide groups or 184 µm and 131 µm for ceramic bur groups. The silver ion penetration depth values in this study were greater than those of a 2018 study[13] (60 µm non-light-cured, 87 µm for light cured) in primary teeth with SDF applied to simulated enamel lesions, as well as a 2015 study[14] (20 µm) in coronal permanent dentin. Microhardness of dentin, which is affected by factors such as dentin depth and degree of demineralization, may have an effect on silver penetration; for example, silver particles from SDF have been reported at depths greater than 2,000 µm into carious dentin.[15,16] The diversity of these studies makes comparison challenging. The present study noticed significantly increased silver penetration depth (>1,100 µm) into sound primary dentin with laser preparation, versus traditional mechanical roughening with burs. A possible explanation for the statistically significant difference in surface treatment groups is the presence of smear layer in both the carbide and ceramic bur surface treatment groups, since no etchant was used prior to SDF application. In theory, the smear layer might have provided a physical barrier to silver entrance into the dentin tubules. By contrast, lasers do not generate a smear layer. A previous study[17] demonstrated that laser irradiation increased uptake of fluoride from SDF in dentin; perhaps a similar phenomenon occurred with silver in the tubules. The laser-treated groups also demonstrated the greatest percentage of occlusal surface area covered by silver particles; this may also be related to the absence of a smear layer but should be interpreted carefully until further investigation focuses on this.

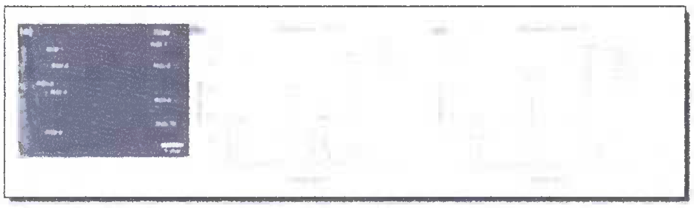

Fig. 3. EDS analysis of the bright particles confirmed that they contained high proportions of silver (Ag) (a, b), while complementary spectra deeper in the tooth showed no detectible Ag (c). This confirms that bright particles in image analysis were silver.

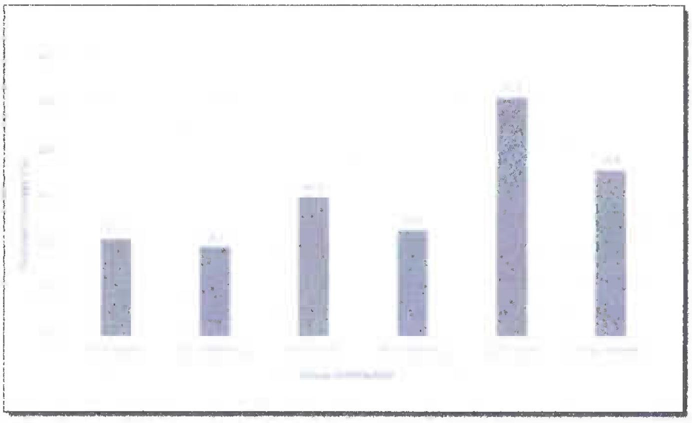

Fig. 4. Average percentage (%) of primary tooth dentin occlusal surface covered by silver particles by group.

SDF has antimicrobial properties.[18,19] Some clinicians try to "maximize" penetration of silver particles into dentin tubules, with the theory that possible bactericidal/bacteriostatic effects of the silver will aid in the long-term caries arrest/prevention process. Because exposure to light curing causes oxidation of the silver ions, this may limit available free silver; there is a hesitancy to light cure SDF-treated dentin surfaces.[3] Although the published data suggesting that SDF can exhibit an adverse effect on cariogenic bacteria in tubules is limited,[20] if the desire is to maximize silver penetration for this purpose, the results of this study suggest that light cure will not adversely affect the depth of penetration.

The results from this study suggest that dentin surface treatments that minimize a smear layer, like lasers, may help increase the depth of SDF silver penetration. The authors speculate that if acid etching had been used after mechanical preparation with carbide or ceramic burs, the depth of silver penetration in those groups would have increased as well. If true, this dynamic suggests a new clinical question: is there a safe limit to the depth of silver penetration? Limited reports[21,22] showed that in vital teeth the pulp complex can respond with protective tertiary dentin deposition, but further investigation is needed. In a deep preparation, if pulpal response is a concern, it may be beneficial to leave a smear layer before SDF application.

There were several limitations to this study. One limitation was the anatomical variances in the randomly allocated primary teeth such as the thickness of enamel and dentin, as well as the position of the pulp horns at different locations of the teeth (interproximal regions and center), which can in turn affect the size of the dentin tubules. Studies[23,24] have noted greater density and diameter of dentin tubules as proximity to the pulp increases. In addition, the authors were not expecting to observe a difference in the smear layer-eliminating surface treatment (laser) vs the smear-layer generating treatments (carbide and ceramic burs). In hindsight, it would have been ideal to have additional bur subgroups that utilized phosphoric acid etch to remove the smear layer prior to SDF application. It may be of interest to investigate the penetration of silver into the tubules of carious dentin; this is an area for further study. The present study focused on sound dentin in primary teeth to attempt to standardize specimen preparation as much as possible.

In summary, light curing 38% SDF-treated primary tooth dentin surfaces had no significant effect on the penetration depth of silver into the dentin tubules. Dentin surface preparation technique had the greater effect: laser ablation resulted in significantly deeper silver tubule penetration than either carbide or ceramic rotary burs.

a. Komet USA, Rock Hill, SC, USA.
b. Ultradent Products Inc., South Jordan, UT, USA.
c. Kerr, Brea, CA, USA.
d. 3M, St Paul, MN, USA.
e. Buehler Ltd., Lake Bluff, IL, USA.
f. Brasseler USA Dental, Savannah, GA, USA.
g. Biolase Technology Inc, Irvine CA, USA.
h. Elevate Oral Care, West Palm Beach, FL, USA.
i. Quorum Technologies Ltd, Laughton, East Sussex, UK.
j. Carl Zeiss Microscopy LLC., White Plains, NY, USA.
k. Rasband, WS. ImageJ, U.S. NIH, Bethesda, MD, USA.
l. R Core Team 2018, R Foundation for Statistical Computing, Vienna, Austria.

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