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ORIGINAL ARTICLE

Penetration Potential of a Silver Diamine Fluoride Solution on Dentin Surfaces. An Ex Vivo Study

Ines Willershausen [1], Daniel Schulte [2], Adriano Azaripour [1], Veronica Weyer [3], Benjamin Briseño [1], Brita Willershausen [1]

[1] *Department of Operative Dentistry, University Medical Centre, Johannes Gutenberg University, Mainz, Germany*
[2] *Technische Universität Darmstadt, Institute of Applied Geosciences, Darmstadt, Germany*
[3] *Institute of Medical Biostatistics, Epidemiology and Informatics, University Medical Centre, Johannes Gutenberg University, Mainz, Germany*

SUMMARY

Background: **Occurrence of open dentinal tubules as a cause of dental hypersensitivity is a very common phenomenon in patients. The aim of this *in vitro* study was to assess the effect of a silver diamine fluoride solution ($Ag(NH_3)_2 F$) on human dentin samples.**
Methods: **A total of five fully retained wisdom teeth were selected for this study. The crowns of the teeth were separated from the roots and the occlusal enamel surface was removed. All dentin samples were treated for 60 seconds with phosphoric acid (36%) and rinsed thoroughly to remove the smear layer. Then the desensitizing agent (Riva Star, SDI; 38% $Ag(NH_3)_2 F$) was placed according to the manufacturer's instruction. Three dentin samples were prepared for element analysis using an electron beam microprobe analyzer (JEOL JXA 8900RL). The Ag concentrations in the dentin samples were measured in depths ranging from 5 to 40 µm. The other two dentin samples were vertically fractured and accordingly prepared for visualization with SEM (Zeiss DSM).**
Results: **The application of the desensitizing agent on the dentin areas demonstrated an increased Ag concentration (JEOL JXA 8900RL). On the dentin surface an Ag concentration of 1.7 weight % (± 0.7) was measured, but at a depth of 20 µm only 0.3 weight % (± 0.1) were detected. In depths greater than 40 µm the Ag concentration was below the detection limit. The SEM results showed that deposits could be found in a covering on the dentin layer and in the dentinal tubules to a depth of 20 µm.**
Conclusions: **In this ex vivo study, the effect of silver diamine fluoride on dentin surfaces could be demonstrated. The desensitizing agent formed a film on the dentin surface and in some dentinal tubules deposits were detected. These findings can explain a certain desensitizing effect, but a direct translation to *in vivo* conditions can only be done with caution.**
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Correspondence:
Prof. Dr. Brita Willershausen
Department of Operative Dentistry
University Medical Center of
The Johannes Gutenberg University
Augustusplatz 2
D-55131 Mainz, Germany
Phone: +49 6131-177246
Fax: +49 6131-173406
Email: willersh@uni-mainz.de

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dentin surface, silver diamine fluoride, electron beam microprobe analyzer, SEM

INTRODUCTION

An increasing tendency of dentin hypersensitivity has been observed over the past years, especially in industrialized countries. The characteristic pain symptom of dentin hypersensitivity is primarily characterized by a short pain, which can occur after stimuli such as cold, warm, acidic substances, evaporative or osmotic pressure have been applied to the exposed dentin of the tooth [1]. Tooth wear, a wrong tooth brushing method and gingival recession after periodontal surgery are also considered as factors which could cause dentin hypersensitivity [2]. According to an epidemiologic survey, sensitive dentin areas are caused through gingival recession in the adult population of industrialized countries with a frequency of 60 to 90% [3]. The reason for this increasing dentin hypersensitivity tendency, which could also cause severe pain, is multifactorial and has been explained through different hypotheses [4,5]. Open dentinal tubules are discussed as being the primary cause of dentin hypersensitivity (Figure 1). However, their size can differ considerably according to the patient age and habits [6,7]. Furthermore, hydrodynamic changes within the open dentinal tubules are considered as intra-dental nerve activity cause [8]. A considerable number of desensitizing products, either for home or professional use, can be employed as a therapeutical agent [9]. The most common ones are topically applied products with active ingredients, which are mostly applied through a dental professional or hygienist. The most commonly employed solutions are sodium and stannous fluorides, potassium nitrate as well as sodium monofluorophosphate [10]. The therapeutic efficacy of fluoride with silver has been reported in different dentin hypersensitivity as well as caries prevention clinical studies [11-15]. This chemical compound has also been reported to be an effective antimicrobial substance during endodontic treatment [14,16] as well as being capable of inhibiting multi-species cariogenic biofilm formation [15].

Suge et al. [17,18] reported that undesirable staining effects can be avoided, when ammonium hexafluorosilicate instead of AgF are employed and that the ammonium hexafluorosilicate solution was also capable of obliterating the dentinal tubules; thus, the authors are of the opinion that this solution is an adequate one for the treatment of sensitive dentin. Research has shown that the negative dentin discoloration effect when employing silver can be avoided through the incorporation of silver fluoride and potassium iodide. The authors also report an antibacterial effect of the solution [19].

The aim of this ex vivo study was to determine the effect of a silver diamine fluoride solution ($Ag(NH_3)_2F$) on human dentin samples and the silver content at different dentin depths.

MATERIALS AND METHODS

Desensitizing product

The two step patented silver fluoride system (RivaStar, SDI GmbH, Cologne, Germany), which according to the manufacturer is an effective biofilm inhibitor being able to reduce to a minimum the risk of silver staining, was investigated. Furthermore, the material is supposed to have a desensitizing effect and can be employed as a cavity toilet. The material has two liquid components: one of them contains silver diamine fluoride (38%, $Ag(NH_3)_2F$) and the other contains potassium iodide (32%, KJ). Both solutions were placed, following the manufacturer's instruction, on previously prepared dentin surfaces of five selected wisdom teeth. The silver diamine fluoride solution was additionally spread on an aluminum mount according to the manufacturer's instructions and prepared for SEM analysis (SE-detection and BSE detection).

Tooth selection and preparation

Five completely retained wisdom teeth from young adult male and female patients were collected. All patients were informed about the purpose of this investigation and gave their written consent. All tissues were thoroughly removed from the teeth and were kept in a 1% chloramine B hydrate solution at all times. The crowns of the teeth were separated from the roots and the occlusal enamel surface was removed. The obtained dentin areas were thoroughly cleaned and the smear layer was removed by application of 36% phosphoric acid and water rinse for 60 seconds each. The silver fluoride system solution was then applied to the surfaces according to the manufacturer's instructions.

Electron beam microprobe analysis

Silver concentrations as a marker for penetration of the solution into both the dentin and the main elements of dentin of three teeth were determined by means of an electron beam microprobe analyzer (JEOL JXA 8900 RL, JEOL, Eching, Germany). The teeth were sliced vertically into two halves in order to achieve samples with intact dentin. The tooth slices were embedded in epoxy resin and ground down with SiC-paper until the obtained dentin surfaces were completely exposed [20]. The dentin surfaces were subsequently polished with diamond polishing cloths of decreasing particle size (9, 3, 1 and 0.25 µm). Each specimen was mounted so that the dentin surface plane could be exposed to the electron beam microprobe analyzer. This enabled the depth-profiling of the elements contained in the prepared dentin surfaces. Along with silver and the main elements of dentin (Ca, P, and O) quantitative measurements also included Na, K, Ca, Mg, F, and Cl. An untreated dentin surface and a sample of the solution were included as controls. The quantitative analyses were run at 15 kV and 12 nA. The beam diameter was set to 20 µm in order to avoid destruction of the samples through beam energy dispersion. The electron beam microprobe counting time for all elements was 60 seconds (backgrounds: 30 seconds each). The samples were analyzed for the above-mentioned components along lines perpendicular to the treated dentin surfaces consisting of ten measurement points per line with 20 µm spacing. These depth-profiles were measured at 10 different areas on each dentin sample resulting in 100 analysis points per sample; thus, providing a solid statistical analysis.

Figure 1. Large areas of exposed dentin in a 45-year-old patient; the teeth are extremely sensitive to temperature and also react painfully to the consumption of sweet and sour foods.

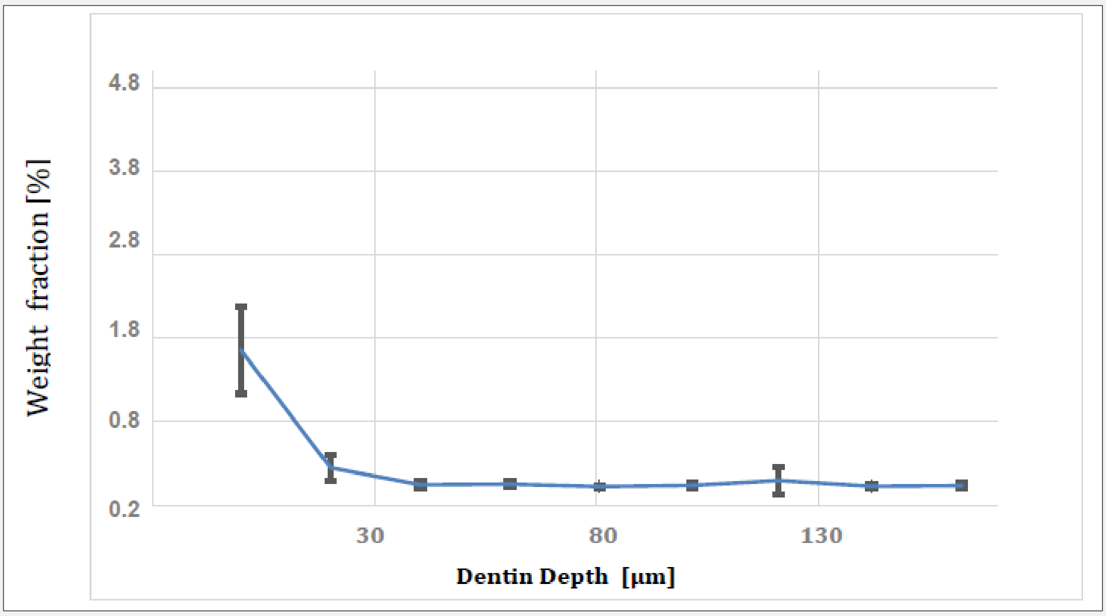

Figure 2. Determination of the Ag concentrations (n = 5; mean, SD) in dentin layers after application of a silver diamine fluoride solution (38%, $Ag(NH_3)_2F$). The dentin specimens were previously treated with phosphoric acid (36%) for 60 seconds.

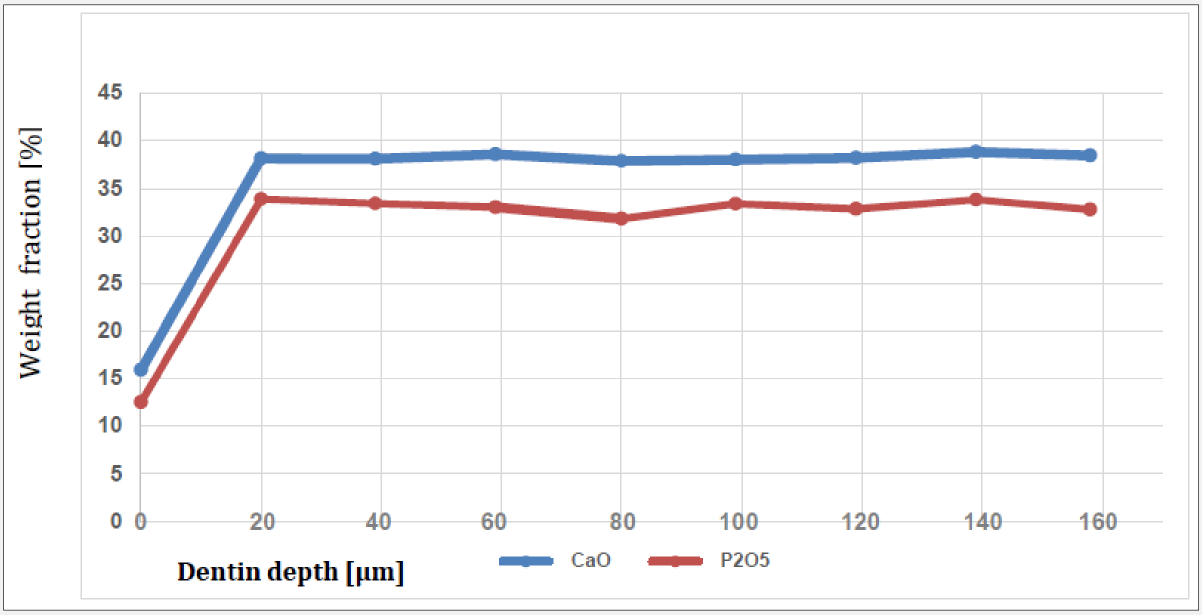

Figure 3. Mean determination of the essential elements CaO and P_2O_5 (n = 5) in different layers of dentin after prior treatment with phosphoric acid. A loss of minerals can be detected to a depth of 20 µm.

Scanning electron microscopy

Two previously prepared specimens were included in the surface investigation with a scanning electron microscope (Zeiss DSM, 962, Oberkochen, Germany). In order to attain a possible observation into the dentinal tubules, the teeth were fractured into two halves. The specimens were dried by means of ascending alcohol baths and kept dry. The scanning electron microscopy was carried out under a low pressure environment at 10 kV. Representative images of the dentin samples were taken at 2000 x magnification. The silver constituent of the desensitizing solution over the aluminum mount was dried and investigated with a 500 x magnification using the backscatter electron mode.

Statistics

Only descriptive analyses were made for the concentrations of the various elements, which are expressed as means ± SD (standard deviations).

RESULTS

In all samples, the results obtained with the electron beam microprobe analyzer showed an increased Ag content at the dentin surface with diminishing concentration to a depth of 20 to 40 µm. The Ag samples dentin surface content showed a relatively high mean value of 1.7% (weight %) with a range of 1.3 to 2.4 (weight %) (Figure 2). The resulting comparatively high standard deviation of the measurements reflects the influence of the difference in individual tooth dentin morphology. An Ag content mean value of 0.3 (weight %) could be established at a depth of 20 µm. In depths greater than 40 µm, the Ag content was below the detection limits.

Figure 3 reveals the dentin content of CaO and P_2O_5. A clear loss of Ca and P up to a 20 µm dentin depth caused by the conditioning with phosphoric acid can be observed. Such loss is congruent with the assimilation of the silver solution.

The SEM representation (Figure 4) of the dentin surfaces after preparation with the $Ag(NH_3)_2$ F solution showed a precipitate on the dentin surfaces and confirmed the relatively high Ag dentin content results obtained with the electron beam microprobe analyzer. Ag containing particles were detected occasionally in the dentinal tubules of the fractured samples at a depth of up to 30 µm from the dentin surface. The results of the $Ag(NH_3)_2$ F solution on the aluminum mount with the BSE and SE-detector showed a silver particle diameter size of 2 to 15 µm (Figure 5). The resulting highly diversified silver particle size can explain the significant silver layer accumulation at the dentin surface as well as its relatively low penetration potential into the dentinal tubules.

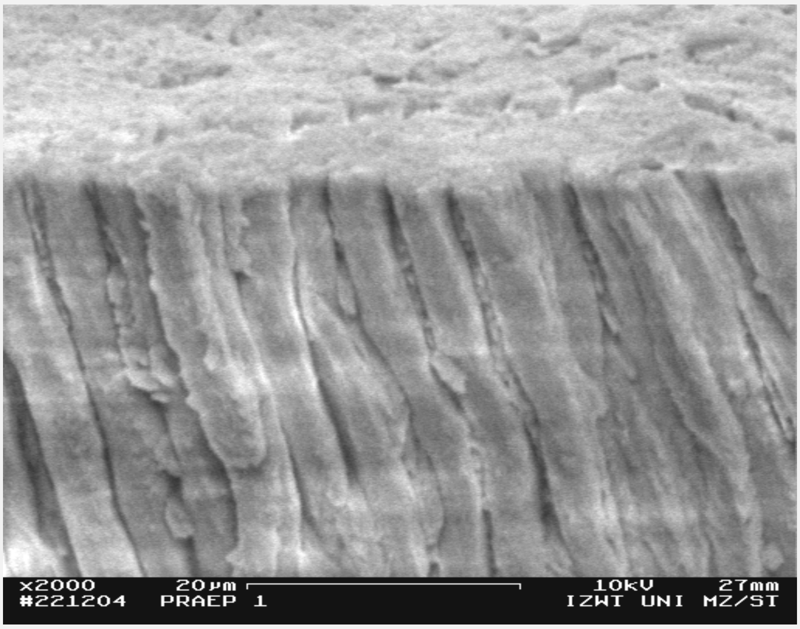

Figure 4. The scanning electron micrograph shows the fractured crown dentin surface after etching and application of the silver diamine fluoride solution. A thick layer of silver deposits is visible on the dentin surface. In the underlying dentinal tubules smaller silver deposits can be seen to a depth of 30 µm.

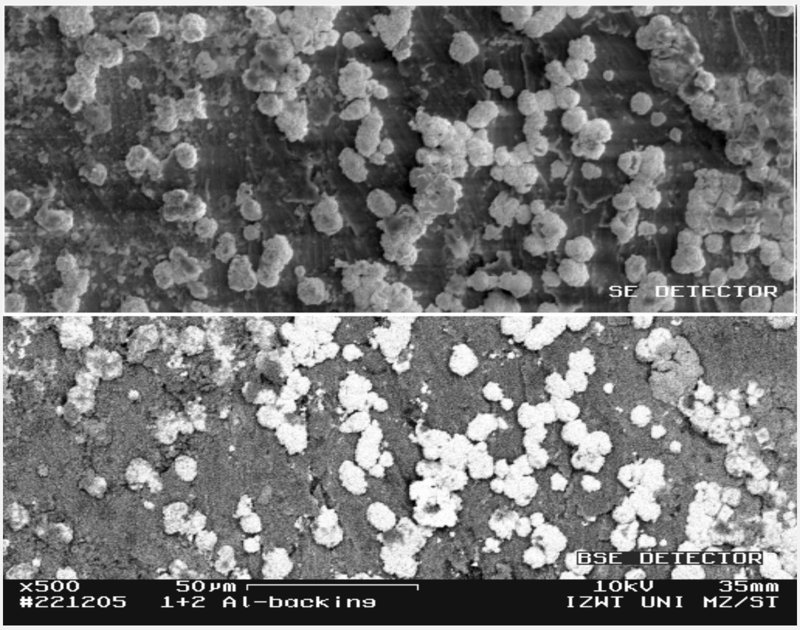

Figure 5. Scanning electron micrograph of the silver diamine fluoride solution, which was directly applied to the aluminum mount. Using the SE and BSE detector it can be seen that the silver particles differ in size.

DISCUSSION

One of the main reasons of dentin hypersensitivity is uncovered dentin areas, of which the majority show exposed dentinal tubules. The aim of treatment for dentin hypersensitivity, which mostly results from more or less pulp irritation, is to reduce pain through a short or long-term obliteration of the dentinal tubules.

Conventional products to be employed during personal hygiene or professionally, produce a short-term reduction of the dentin hypersensitivity and have to be employed routinely [9,10,21]. It has been reported that an effective reduction of dentin hypersensitivity can be achieved by the employment of diamine silver fluoride/ potassium iodide solution [22]. Craig et al. [13] compared an oxalic acid-based product with a silver diamine fluoride solution in a split mouth study and were able to demonstrate that the silver containing solution led to a significantly higher pain reduction after one week. Our results also support that silver diamine fluoride solutions seem to be appropriate to reduce pain caused by dentin hypersensitivity. However, Merika et al. [23], in a clinical study, were able to demonstrate that an oxalic acid based product as well as a 5% sodium fluoride solution were able to reduce dentin hypersensitivity effectively after four weeks of treatment.

The silver antibacterial and pain perception reduction peculiar effects have been known for a long time [24, 25]. Furthermore, it has been reported that silver diamine fluoride solutions also have a re-mineralizing effect, due to the fluoride concentrations, and are free of adverse effects [26,27]. Their antibacterial properties have also been discussed for their use during endodontic treatment [14,16]. However, possible black pigmentations caused during their application have restricted their use [28]. Knight et al. [29] reported that changes of tooth coloration can be avoided through the combined use of silver fluoride and potassium iodide.

CONCLUSION

The obliteration of exposed dentinal tubules has been shown to be responsible for pain reduction caused by dentin hypersensitivity; thus, the aim of this study was to obtain a quantitative measurement of the penetration potential of silver particles into the dentinal tubules by means of an ex vivo study. The results demonstrate that silver was able to penetrate up to 20 μm into the dentinal tubules after a dentinal tubule entrance exposure was achieved through previous etching of the dentin surface. These results are supported by the SEM measurements obtained.

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Declaration of Interest:
The authors declare that they have no conflicts of interest.

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