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ORIGINAL ARTICLE

Role of Silver Ions in Preventing Demineralisation of Human Enamel

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ABSTRACT

Aim: To determine the changes in Ca2+ release before and after the application of Riva Star, AgNO3 and to analyse the changes in surface roughness of the samples.

Methods: The Riva Star, AgNO3 were used to measure their effects on the demineralisation processes of the windowed human enamel in 50ml pH4.0 acetic solution under 370C. The sound human enamel samples were sliced and varnished using nail polish and 5mm^2 was left on the surface of enamel sample in order to make windowed-type enamel. The demineralised enamel samples were treated by these agents after 4hours of demineralisation, and later put back into the same acetic acid solutions for further 4hours of demineralisation to detect the changes.

Results: The results demonstrated that Riva Star (SDF/KI) treatment exerted the inhibition ability in demineralisation remarkably. However, when the silver nitrate was applied alone, it slightly accelerated the demineralisation firstly and then followed a straight line. The treatment of silver nitrate alone also caused black staining. Reducing agent such as potassium iodide following the treatment of silver nitrate can inhibit the demineralisation instantly without causing the initial acceleration in demineralisation and the final black staining.

Conclusions: Riva Star (SDF/KI) is an effective demineralisation inhibition agent for up to 48 hours following the topical application. The potassium iodide application following the treatment of silver nitrate can not only accelerate the inhibition performance, but also effectively avoid black staining.

Keywords: Silver ions, Demineralization, Caries, Riva Star, SDF, Surface roughness

INTRODUCTION

To this day, dental caries prevention and inhibition with silver compounds have been used for more than a century in permanent and primary dentitions. However, a major issue limiting the compounds' usage in clinical settings is the fact that they create black staining. Silver fluoride, silver nitrate, and silver diammine fluoride are the most regularly used silver compounds in dentistry (Peng et al., 2012).

In 1891, Stebbins suggested that amalgam restorations caused black discoloration on tooth surfaces where caries had stopped progressing. According to the research findings (Lansdown, 2006), a silver ion concentration of 10~40ppm was shown to be effective in killing the majority of infections. The use of silver compounds as anti-bacterials gained awareness in the 1970s due to their low toxicity and broad spectrum of metabolic inhibition (Peng et al., 2012).

Stebbins believed that the "black crust" deposition that occurred in sclerotic dentine was caused by the antibacterial property, which resulted in the favourable outcome. Following that, silver nitrate was regarded as an effective anti-caries agent and was used clinically until 1900. In 1917, Howe discovered ammoniacal silver nitrate, Ag (NH3)2NO3, which he dubbed "Howe's solution to heal dental cavities" because of the same results. Because of this, Howe's solution has been used for the next 50 years to help people keep their teeth clean and healthy (Rosenblatt et al., 2009). To treat deciduous teeth in the 1970s, silver fluoride (AgF) and stannous fluoride (SnF2) were used. The proliferation of Strep. Mutans and the metabolism of the biofilm were observed to be altered by the combination of these two. Silver ions have also been demonstrated to limit the growth of cariogenic bacteria and modify the production of biofilms (Mei et al., 2013). In 2014, the Food and Drug Administration (FDA) approved the use of silver ions as a desensitising agent. Silver ion is a new phenomenon, and hence there is no consensus on how to use it. Even though a new SDF implementation has been written, one is still used (Horst et al., 2016). When compared to NaF varnish, SDF is more effective in remineralizing tooth decay. (Duangthip et al., 2016; Wierichs et al., 2018).

Silver nitrate appears to be the first silver chemical used to treat caries. Some believe that silver nitrate has the ability to stop dental cavities by encouraging coagulation in the dentinal tubules and causing metallic silver precipitation on the tooth surface, thereby providing a barrier between the tooth and the surrounding environment. However, the uncertainty over the possibility of pulpal irritation has always been a source of concern. Several investigations found that after applying Howe's solution, the black particle diffused through primary, secondary, and carious dentine, reaching the pulp. As a result, it appears that silver nitrate does not consistently impair the vitality of the pulp since the penetration of silver nitrate does not appear to be self-limiting in nature (Peng et al., 2012).

The objective of the study was to determine the changes in Ca2+ release before and after the application of Riva Star, AgNO3 and to analyse the changes in surface roughness of the samples.

METHODOLOGY

The effects of application agents on demineralisation of human enamel and simultaneously measuring the surface roughness of the sample at four intervals using a profilometer.

Outcomes of application agents on demineralisation of human enamel and its surface analysis

The graphs from the enhancement studies indicated that a 4-hour period of demineralization was sufficient to identify the results. the effect of the treatment agent. Furthermore, windowed enamel was used in the following demineralisation experiments to facilitate faster demineralisation and to enhance the effect of the application agent. The measurement of the surface roughness of the enamel samples was carried out using a profilometer.

1. Before putting it in the demineralisation solution.

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2. After 4 hours of demineralisation, (0–4 hrs)
3. After treatment with the agent.
4. After demineralising the treated sample for the last 4 hrs. (4–8 hrs)

Treatment with Riva Star/AgNO3: Windowed enamel was immersed in 50 ml of demineralisation solution for a 4-hour demineralisation process as a control. Following that, the enamel sample **was** removed and treated with Riva Star according to the process outlined, before being reintroduced into the same solution for another 4 hours of demineralisation. Every minute, data were collected in the form of mV measurements for the electrodes.

Treatment with AgNO3: Windows-Enamel was submerged for four hours in a demineralization solution. The enamel sample was then treated for one minute with 0.5 mL of 3.16 M AgNO3 and allowed to dry for ten minutes before being reinserted into the test tube. The excess agent was absorbed by the tissue. Four hours later, a new demineralization solution was used to treat the sample. The same graph was used to compare data collected during the course of the 8-hour experiment.

Surface Analysis: The surface roughness of the enamel sample was measured in 4 steps simultaneously. Firstly, the sample was run for a surface scan before immersing it in the demineralization solution, and the sample was then scanned after 4 hrs of demineralization. Following that, the sample was scanned again for surface roughness after treatment with AgNO3. Lastly, the surface roughness of the sample was measured after the last 4 hrs of demineralization of the treated enamel sample, and the data was plotted.

Outcome of temperature on ISE reading: The data demonstrated that as the temperature climbed, the ISE values (mV) decreased, despite the fact that the CaCl2 content remained constant. Thus, temperature did actually have an effect on the ISE measurement. The silver and calcium electrode calibration operations were carried out using a beaker wrapped in paper film and foil. Both calibration graphs were linear, and appropriate equations were developed for converting the ISE data to concentration levels in the subsequent tests.

Determination and verification of Riva Star's impact and demineralization time: In comparison to the experimental group, the control group had a quicker calcium release. By examining the lower slope, it appeared as though the application of the Riva Star was efficient in suppressing apatite demineralisation. The fact that the slope value changed from 1E-06 to 4E-07 indicates that the treatment had an effect on the demineralisation process. Additionally, because the calcium release trend remained continuous throughout the trial, a shorter duration of demineralisation, such as 4 hours, should be sufficient to demonstrate the application agent's effectiveness.

RESULTS

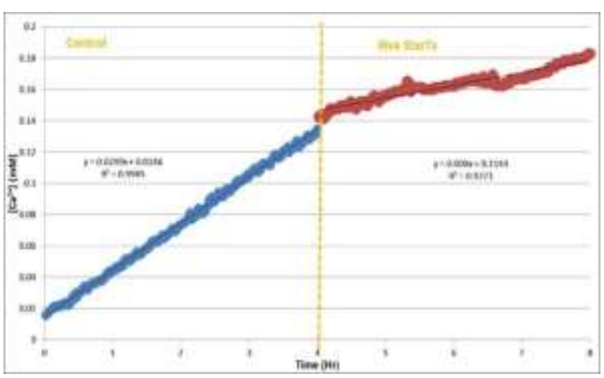


Figure 1. Calcium ion release inhibition before and after application of Riva Star (Silver ion), AgNo3 and AgNo3+KI

Calcium release inhibition

Treatment with Riva Star/AgNO3: After the Riva Star treatment, the slope decreased significantly, from 0.0299 to 0.008. The flattening trend revealed that calcium ion release appeared to have been nearly completely ceased following the operation. Additionally, there was a decrease in the inclination immediately following the Riva Star therapy, which might be attributed to the production of the CaF2 (Fig 1)

Surface roughness due to Riva Star

Surface Analysis and Rate of Ca2+ release: There was a profound rise in the surface roughness of windowed enamel after treating it with Riva Star. This sudden rise in the surface roughness might be due the formation of barrier which halts the release of calcium ions. This entire process mimics the course of erosion (Fig 2) (Table 1).

The comparison of all the experiments of demineralisation study in slopes rates before and percentage calcium inhibition after the treatment with application agents.

Difference between rates of Ca2+ release:

Control (0-4hrs) = 0.032 mm/hr

Riva Star Tx (4-8hrs) = 0.01mm/hr

Difference= 0.022mm/hr

Percentage Ca2+ inhibition= 68.75%

Treatment with AgNO3: The application of AgNO3 on the enamel resulted in 28% inhibition of Ca2+ release (Fig 2) (Table1)

Surface Analysis and Rate of Ca2+ release: There was a slight rise in the surface roughness of the windowed enamel after 4 hrs of demineralisation which is a normal phenomenon associated with the demineralisation. However, the surface roughness of the sample showed a consistent result before, after treatment with AgNO3 and after 8hrs of demineralisation. (Fig 2) (Table 1)

Difference between rates of Ca2+ release:

Control (0-4hrs) = 0.07mM/hr

AgNO3 Tx (4-8hrs) = 0.05 mM/hr

Difference= 0.02 mM/hr

Percentage Ca2+ inhibition= 28%

Treatment with AgNO3+KI: After treatment with AgNO3 (3.16 M) followed by KI (saturated solution), the slope of calcium release decreased from 0.0313 to 0.0289, indicating that the quicker deposition might allow the faster performance of the inhibitory effect. Due to the application agent's composition of silver iodide, the complete result of the enamel sample demonstrated that there was no black staining. After the application of KI, a slight drop in the concentration of silver ion was observed. This illustrated the effective reducing ability of the agent, which is responsible for the conversion of silver ions into silver iodide. Whereas the application of AgNO3+KI on the enamel resulted in 25% inhibition of Ca2+ release (Fig 2) (Table 1)

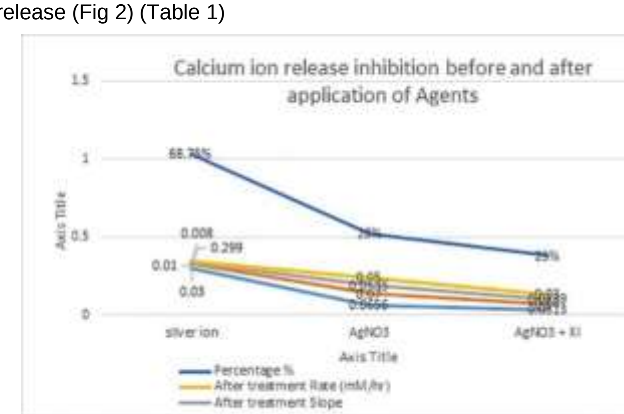
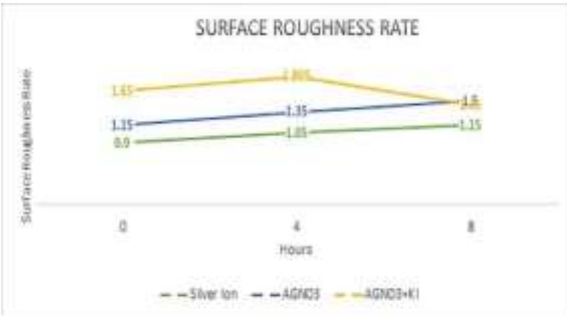


Figure2. Calcium ion release inhibition before and after application of Riva Star (Silver ion), AgNo3 and AgNo3+KI



Surface Analysis and Rate of Ca2+ release: There was a slight rise in the surface roughness of windowed enamel after the application of AgNO3 followed by application of KI (saturated solution). This sudden rise in the surface roughness indicates the formation of a barrier after treating enamel sample with AgNO3+KI. The establishment of the barrier might be due to the formation of silver iodide.

Difference between rates of Ca2+ release:

Control (0-4hrs) = 0.04mM/hr
AgNO3+KI Tx (4-8hrs) = 0.03mM/hr
Difference= 0.01mM/hr
Percentage Ca2+ Inhibition= 25% (Fig 2, Table 1)

Combined comparisons: Riva Star treatment (slope: from 0.0299 to 0.008; Rate: 0.01 mM/hr; % Ca2+ inhibition: 68.75%). Whereas the treatment of AgNO3 slightly accelerated the calcium firstly before it before it exerted the inhibition ability (slope: from 0.0656 to 0.0535; Rate: 0.05 mM/hr; % Ca2+ inhibition: 28%). While in the treatment of AgNO3 followed by application of KI also exerted the inhibition ability (slope: from 0.0313 to 0.0289; Rate: 0.03 mM/hr; % Ca2+ inhibition: 25%). Consequently, it was indicated that all the components in the Riva Star worked synergistically to stimulate its inhibition effect in release of calcium.

Determination and verification of the effect of Riva Star and period of demineralization: In order to verify the effectiveness of the Riva Star in inhibition of calcium release, windowed enamel was used to undergo the demineralisation process for 24 hours, subsequently, the windowed enamel was taken out and treated with Riva Star and was again dropped in demineralisation solution for another 24 hours. The obvious deviation between the control and Riva Star treated group, the lower slope in the Riva Star treated group was identified which indicated that Riva Star possesses profound ability to halt the calcium release. The demineralization inhibition was seen after the application of Riva Star for upto 24 hours. Since, the slope was almost consistent from 24 to 48 hours. Therefore, it was decided to limit the demineralization cycle to 4 hours each before and after application of inhibition agents.

Table 1: The comparison of all the experiments of demineralisation study in slopes rates before and percentage calcium inhibition after the treatment with application agents

Treatment	Before treatment Ca^{2+} Inhibition		After treatment Ca^{2+} Inhibition		%age
	Slope	Rate (mM/hr)	Slope	Rate (mM/hr)	
silver ion	0.299	0.03	0.008	0.01	68.7%
$AgNO_3$	0.0656	0.07	0.0535	0.05	28%
$AgNO_3$ + KI	0.0313	0.04	0.0289	0.03	25%

DISCUSSION

Outcomes of application agents on demineralisation of enamel and its surface roughness

Effect of Riva Star: The following tests employed windowed enamel samples to increase the effects of demineralisation and inhibitory agents. When the slope of calcium release flattened, it signified that the rate of demineralization slowed significantly, if not stopped entirely. This was demonstrated in Fig 1. Since Riva Star is a complex demineralisation inhibition agent, the constituents include fluoride, Silver, potassium iodide and ammonia. Therefore, it is necessary to understand the effect of individual ingredients of Riva Star on possibly the inhibition of Ca2+ release. Fluoride in the Riva Star can contribute to the inhibition of calcium release and inhibit enamel demineralization by reacting with Ca2+ of enamel HAP and forming insoluble CaF2 (Caslavska et al, 1975). Therefore, the Ca2+ which was being released before the treatment of Riva Star was now being halted by the action of fluoride and forms CaF2. Similarly, the presence of silver in the Riva Star also aids in the inhibition of enamel demineralisation by reacting with phosphate ions likewise and forms Ag3PO4. It was confirmed in a study conducted by Lou and his co-workers in 2011. It was concluded that CaF2 and Ag3PO4 are the major products of reaction of SDF with tooth tissue (Lou et al., 2011).

As we know that Ca2+ and PO4 ions are the vital components of HAP in enamel therefore their integrity is of key importance. Moreover, the potassium iodide which is also present in Riva star and act as reducing agent as well as prevents demineralisation (Chu et al., 2002). Hence, the KI was applied after the application of SDF, a white precipitated was seen subsequently. It is due to interaction of silver with the Iodide ion and forms silver iodide which appeared as barrier and thus halted the release of calcium and prevented the black staining. The current study's findings corroborated prior microbiological research demonstrating the significant demineralization inhibitory effects of SDF/KI-containing drugs. The use of a silver diamine fluoride/potassium iodide product is a dependable approach for improving the NaOCl-based gel's limited antibacterial activity. This application, however, may result in a delay in the formation of the white precipitate following application of the silver diamine fluoride/potassium iodide product. (2015) (Hamama et al). Knight and his co-workers in 2005 also confirmed that SDF/KI exhibited short effects on inhibition of dentine demineralization (Knight et al., 2005) (Table 1).

To analyse the surface changes before and after the treatment of demineralization inhibition agents the enamel samples were scanned for surface roughness using Proscan 2000 profilometer. From Fig 3 it was indicated that the surface roughness of enamel was consistently low before the application of Riva Star. However, a sudden rise was seen in the surface roughness of enamel. It is assumed to be due to the formation of a white precipitate, which forms a physical barrier following the treatment of enamel sample with Riva star. The establishment of the barrier is probably because of the formation of silver iodide. However, after the application of Riva Star when the enamel sample was immersed again into the demineralization solution for the next four hours, it was observed the Ramean value was higher as compared to the initial value and after 4 hours of demineralisation (Fig 3). So, it can be postulated that the value of surface roughness was higher than the surface roughness at the initial and first four hours of demineralization. It is possibly due to the presence of physical barrier even after demineralization, although the release of Ca2+ was inhibited. Therefore, the surface of the enamel gets rougher after the application of Riva star, which facilitates the inhibition of calcium release, since it is a natural phenomenon that whenever there is an acid attack on the enamel surface it will make the surface smoother. Abrasive wear on the softened degraded tooth surface may readily remove the superficially demineralized dental hard tissue, and mechanical impacts such as tooth brushing can do so. **(**Davis WB and Winter, 1980, Attin, 2006).

Effect of Silver Nitrate: A silver nitrate solution (3.16 M) was used to treat an enamel sample to see how it affected the effects of the silver component in the product. After the silver nitrate treatment, there was a minor rise in calcium release, but the inhibitory ability of silver was exercised, which came out to be 28%. (Fig 2). Micro-CT results from a prior demineralization investigation showed that the artificial carious lesion following silver nitrate administration was identical to that of the control group, therefore this might provide as support for those findings (Liu et al., 2012). The fact that the total mineral loss from the initial acceleration and subsequent slowing of the calcium release was comparable to that of the control group attests to the silver nitrate treatment's ineffectiveness.

It is critical to remember that the ISE measures the ion concentration in the solution, not on the enamel surface. The concentration of silver ion increased rapidly following the topical application of silver nitrate due to the exceptionally high concentration of the chemical utilised. Increasing the concentration of silver ions could result from the surface diffusion of silver ions into solution. Rather than reacting with HAP phosphate ions, Ag_3PO_4 was formed when $AgNO_3$ was near the enamel surface. Subsequently, the slope didn't change much, it might be due to the compact crystal structure of enamel, since the enamel is very compact as compared to porous dentine. Therefore, the silver ions were unable to penetrate much to react with the phosphate ions and exhibit prominent demineralisation inhibition effect. Fluoride ions entered the enamel to a depth of around 25 micrometres, whereas silver ions were mostly deposited on the surface and some penetrated as deep as 20 micrometres into the enamel following SDF treatment (Suzuki et al., 1974). It is reasonable to believe that once some of the silver ion began to transform into metallic silver at the beginning of the experiment, the inhibitory ability of silver began to exert its influence.

The deposition of metallic silver, which showed up as a black staining, may have established a barrier on the enamel surface, inhibiting the release of calcium ions just enough. Silver nitrate solution quickly interacted with HAP to generate Ag_3PO_4, which was presumably helped by the solution's acidity, which caused the HAP to dissolve and therefore precipitate the silver salt. In the presence of light, Ag_3PO_4 degrades to black metallic silver (Lou et al., 2011). Meanwhile, since the silver ions interacted with the calcium phosphate compounds of the enamel apatite, massive calcium ions were released due to the production of the soluble $Ca(NO_3)_2$ (Yamaga et al., 1972). Most of the time, the affected tissue turns white, and the change is short-lived (Llodra et al, 2005). Possible that the slight drop in Ca^{2+} release is because of the buildup of Ag_3PO_4. Since a result, the first quick rise in calcium ion concentration should be considered a pseudo-demineralisation, as the silver ion may have replaced Ca^{2+} to generate Ag_3PO_4. Similarly, when the windowed enamel was scanned for surface roughness before and after the treatment of $AgNO_3$. From Fig 2, it can be deduced that the surface roughness of the enamel didn't change much as compared to results of the sample treated with Riva Star. The Ramean value remained constant even after the application of Riva Star. Hence, it can be assumed that it would probably be due to the formation of metallic silver which formed a physical barrier on the surface and stained it black. Consequently, the surface roughness of enamel was consistent even after the treatment with $AgNO_3$.

CONCLUSIONS

Based on the limitations of the study we can conclude that Riva Star is an effective demineralisation inhibition agent for up to at least 48 hours following the topical application. The surface roughness of enamel has also increased following the application of Riva star, which is due to the formation of a white precipitate of AgI which acts as a physical barrier to halt the calcium release. The silver ion effect is a result of the creation of Ag_3PO_4 and the formation of the barrier. It is possible that the acceleration of demineralisation following silver ion application is due to the synthesis of Ag_3PO_4, which can encourage the development of soluble $Ca(NO_3)_2$.

Clinical relevance; Our study revealed that multiple clinical studies have established topical silver nitrate as an effective anti-caries medication. The black staining, which was the primary reason for limiting the use of silver compounds, has been successfully eliminated by following the silver treatment with potassium iodide. It should be used as a liner with "sandwich technology." Silver compounds should be preserved by avoiding exposure to heat and light, which can encourage the conversion of the silver ion to metallic silver.

Conflict of interest: Nothing to declare

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