*Research Article*

# Assessment of the microhardness of artificial enamel caries after treatment with a combination of silver nanoparticles and sodium fluoride solution

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**Abstract:** Background: New technologies have developed to allow newly created enamel caries to be remineralized. Silver nanoparticle (NP) products have a strong inhibitory and bactericidal effect. Silver ions may penetrate carious lesions, which may precipitate and cause enamel to harden. Objectives: This study evaluates and compares the effectiveness of the application of silver NPs with sodium fluoride solution on the microhardness of artificially induced demineralization on the enamel surface in comparison with sodium fluoride alone. Materials and methods: Thirteen upper first premolars that were extracted for orthodontic therapy were used. Each tooth was cleaned, inspected, polished, and painted with acid-resistant nail polish to create a circular window with a diameter of 2×2mm. Then it was soaked in a demineralizing solution for four days after that the teeth were divided in to three groups (A: untreated, B: treated with silver NPs and 5% sodium fluoride and C: treated with 5% sodium fluoride) groups were immersed for 4 minutes, and all stages were conducted under room temperature. A digital Vickers microhardness tester with a diamond indenter with 500 g of load applied for 30 s directed vertically to the enamel surface was used to measure microhardness. The Vickers microhardness test results show that the solution of sodium fluoride and silver NPs has higher microhardness ratings (mean surface microhardness [SMH] 286.837) than sodium fluoride alone (mean SMH 191.530) with significant P value = 0.00001. Conclusion: Using silver Silver nanoparticles with sodium fluoride is better than using sodium fluoride alone in increasing surface microhardness; thus, it is more promising in remineralizing enamel caries.

**Keywords:** Dental caries, Noninvasive dentistry, Nanoparticles, Silver Nanoparticles.

## Introduction

More than a third of the population worldwide have dental caries [1,2]. The rotatory phrases of demineralization and remineralization describe this dynamic process. Caries development or reversal depends on the balance between defensive agents e.g., remineralizing ions, and sufficient saliva and antibacterial agents that affect remineralization and the pathological agents of demineralization e.g., fermentable carbohydrates, cariogenic bacteria, and salivary dysfunction [3]. The major objective of the minimally invasive dentistry revolution is to start the remineralization of new enamel caries lesions. Various new remineralization techniques have been developed to encourage the remineralization of deeper lesions, decreasing the potential risk associated with oral care products that contain high amounts of fluoride and facilitating caries management over a lifetime. Fluoride remains to be the most crucial agent used in caries management strategies [4,5]. In addition, some nanoparticles (NPs) support antimicrobial and remineralizing traits which have magnificent control to prevent biofilm formation, slow down demineralization, remineralize tooth structure, and fight caries-related microorganisms. Nanotechnology offers new approaches for the therapeutic prevention of oral diseases, particularly dental caries, and periodontal disease [6,7].

Given their physicochemical properties, silver NPs have a long history in the biological and medical fields. Their byproducts have potent inhibitory and bactericidal effects with a broad spectrum of antibacterial activities that have been used for centuries to treat and prevent various diseases. They are also used in various dental specialties. [7] Silver ions may penetrate carious lesions, which may precipitate and cause enamel to harden. [8] This research would examine the capacity of silver NPs to boost enamel microhardness when combined with sodium fluoride instead of sodium fluoride alone.

Null hypothesis: The microhardness of artificially induced dental caries of outer enamel surfaces after the application of silver NP with sodium fluoride does not differ from that treated with sodium fluoride alone.

## Materials and Methods

After approval from the scientific committee of the College of Dentistry of Baghdad University (480, 19-1-2022) a total of 30 maxillary first premolars extracted for orthodontic purposes from patients between the ages of 15-20 were obtained from several private dental offices. The investigation had two components. The first stage involved preparing and testing the extracted materials, and the second stage involved preparing the tooth samples and measuring the Vickers microhardness.

Sample preparation

Using G power 3.1.9.7, the sample size is 21 samples, adding 10% as an error rate to compensate for the dropout in follow up so it will be 24 samples, so 30 samples is enough for this study(microhardness), and it will be more than calculated by G power.

Any tooth that had a visible fracture or crack was discarded. Every tooth was thoroughly cleaned with a cumin scaler; prophylaxis was performed with pumice and a rubber cup, and the teeth were kept for two weeks in a 0.1% thymol solution (de-ionized water with thymol crystals) at room temperature [9]. Then, with a contra-angle, slow-speed handpiece, the buccal surface was polished with Sof-Lex Disks (3M ESPE, USA), working from coarse to medium to fine to extremely fine to obtain a flat, smooth surface. Each tooth was painted with an acid-resistant nail varnish except for a 2×2 mm (diameter) window of exposed enamel on the buccal surface.

Preparation of demineralizing solution: -

A demineralising solution was prepared by using 2.2 mM sodium dihydrogen orthophosphate dehydrate (NaH2PO4), 2.2 mM calcium chloride (CaCl2) and acetic acid with a concentration of 0.05 M; then the pH was adjusted to 4.4 by adding 1M potassium hydroxide Then for over four consecutive days (96 hours), each tooth was immersed separately in the demineralizing solution to produce fictitious caries-like lesions on the enamel surface (10), After that, the samples were carefully cleansed, separated into three groups, and stored in artificial saliva for 7 days changing it each day at room temperature.

Preparation of silver NPs with sodium fluoride solution: -

A solution of 5% NaF was prepared. The solution had a basic pH. Then 0.7% silver NP (4000 ppm AgNPs) powder was mixed with the solution, and a homogenous mixture was obtained. The three groups are:

Group A: untreated (demineralization followed by soaking in artificial saliva as control group negative).

Group B: demineralization followed by soaking in Silver NP powder with 5 % NaF (22,600 ppm fluoride with 4000 ppm AgNPs) (11), for 4 minutes, then stored in artificial saliva (for one day at room temperature).

Group C: demineralization followed by soaking in 5% sodium fluoride solution for 4 minutes and stored in artificial saliva (for one day at room temperature), as control positive.

Until the surface microhardness test, each sample was kept by immersing each group separately in artificial saliva that was changed every day (9). To assess surface microhardness test at baseline (sound enamel surface), after the demineralization procedure, and after remineralization, thirty teeth (ten teeth from each treatment group) were employed. The tests were carried out in the metal checking laboratory of the Department of Metallurgy and Manufacturing Engineering, University of Technology, using a digital Vickers microhardness tester with a diamond indenter. After fixing the tooth with a metal mold measurement were taken using a five-hundred-gram load that was directed vertically toward the enamel surface for thirty seconds. All measurements were obtained using the same calibrated apparatus and examiner at room temperature. Each specimen's hardness value was calculated by averaging three indentations made during each study. (12)

Statistical analysis

Statistical Package for Social Science was used for data description, interpretation, and presentation (SPSS version 21, Chicago, Illinois, USA) $P \leq 0.05$ denotes significance, whereas $P>0.05$ indicates non significance.

**Results**

Normality test of the standardized residual of phases among groups

Results from the Shapiro-Wilk test at $P > 0.05$ reveal that the Standardized Residual of Microhardness in all phases is normally distributed among groups.

Multiple pairwise comparisons of surface microhardness among phases by groups:

Table 1 compares the phases of each group:

1- The baseline phase and each of the surface microhardness (SMH) test's demineralization and reminerlization phases for all groups differ significantly from one another p=0.00000 (the baseline records of SMH is higher).

2- In group A, the SMH test's demineralization and remineralization phases do not significantly differ from one another (p=0.93870).

3- In groups B and C, the demineralization and remineralization phases of the SMH test differ significantly from one another (SMH is higher in the remineralization phase); p for B, p = 0.00000; for C, p = 0.01072.

**Table 1:** Multiple Pairwise Comparisons of SMH(HV) among phases by groups.

| Groups | Phases (I) | Phases (J) | Mean difference | p value |
|---|---|---|---|---|
| **Group A** | **Baseline** | Dem. | 174.21 | 0.0001 |
| | | Rem. | 173.31 | 0.0001 |
| | **Demin.** | Rem. | -0.900 | 0.9387 |
| **Group B** | **Baseline** | Dem. | 163.71 | 0.0001 |
| | | Rem. | 35.00 | 0.0332 |
| | **Demin.** | Rem. | -128.71 | 0.0001 |
| **Group C** | **Baseline** | Dem | 137.11 | 0.0001 |
| | | Rem. | 104.91 | 0.0001 |
| | **Demin.** | Rem. | -32.20 | 0.0107 |

NS=not significant at p>0.05., *=significant at p<0.05.

Descriptive and statistical test of SMH among groups and phases

Table 2 compares the enamel microhardness between each phase of the three groups and between each group's baseline, demineralization, and remineralization phases (Figure 1).

1-Surface microhardness varies significantly between the three phases of each group (p ≤ 0.05), with baseline records showing the highest SMH and the demineralization phase showing the lowest.

2- No significant difference in surface microhardness in the baseline phase is found between the three groups (p=0.398NS).

3- No significant difference in surface microhardness in the demineralization phase between the three groups (p=0.153 NS)

4- Among the three groups, a statistically significant variation in surface microhardness during the remineralization phase (p 0.05) is found, with group B having the highest SMH and group A having the lowest.

**Table 2:** Descriptive and statistical test of (SMH)(HV) among groups and phases

| Groups | | Baseline | Demineralization | Remineralization | F | P value |
|---|---|---|---|---|---|---|
| **Group A** | **Mean** | 310.540 | 136.327 | 137.227 | 65.072 | 0.0001* |
| | **±SD** | 50.692 | 37.637 | 37.311 | | |
| **Group B** | **Mean** | 321.840 | 158.127 | 286.837 | 94.958 | 0.0001* |
| | **±SD** | 34.825 | 14.397 | 38.106 | | |
| **Group C** | **Mean** | 296.440 | 159.328 | 191.530 | 39.137 | 0.0001* |
| | **±SD** | 36.182 | 29.547 | 38.343 | | |
| | **F** | 0.954 | 2.014 | 39.884 | | |
| | **P value** | 0.398 NS | 0.153 NS | 0.0001* | | |

NS=not significant at p>0.05., *=significant at p<0.05.

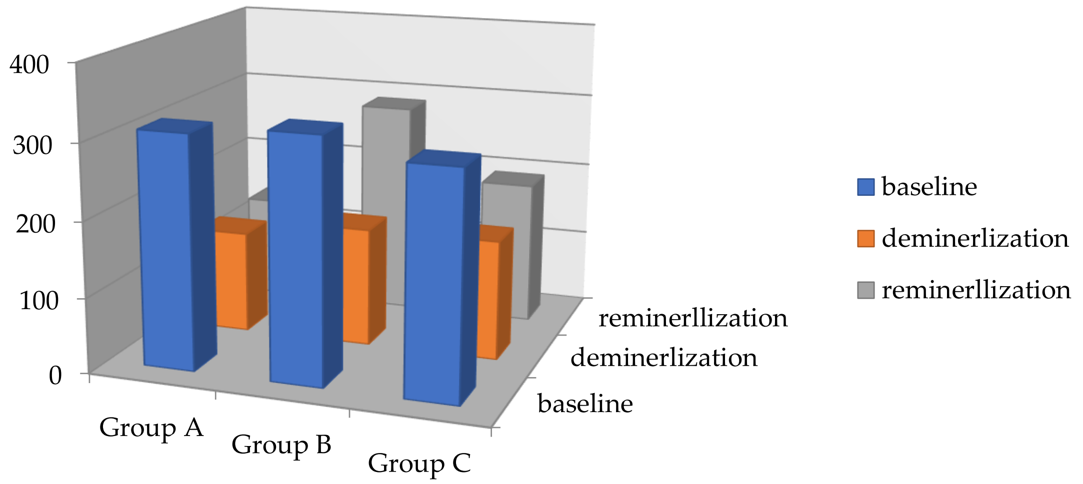


**Figure 1:** Difference in surface microhardness between the three phases of each group.

Multiple pairwise comparisons of SMH among groups in the third phase

Figure 2 displays the variation in SMH between groups during the remineralization phase. In SMH, group A differs significantly from the other groups as it has a lower SMH than other groups. A significant SMH difference is found between Groups B and C (p =0.0001).

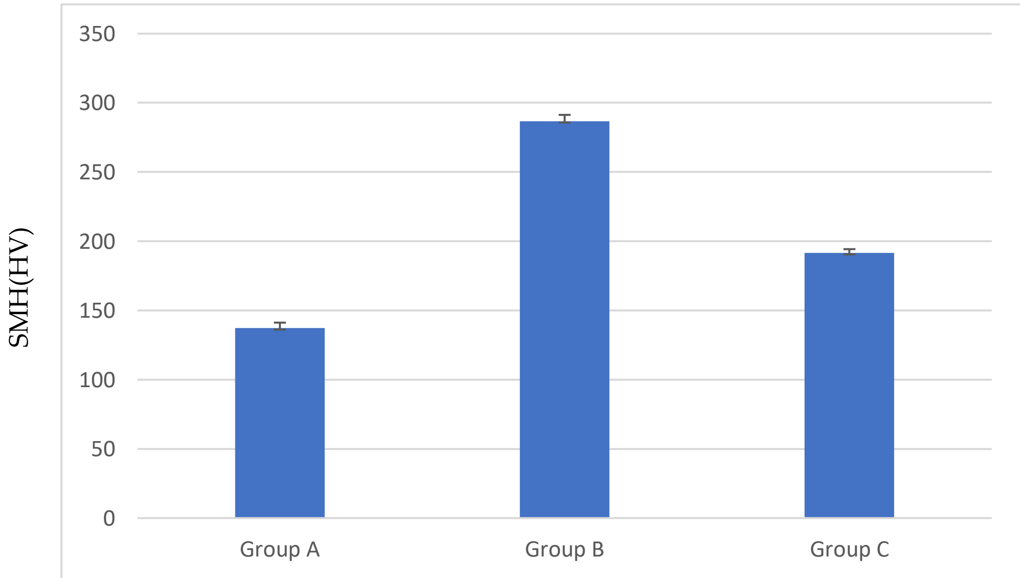


**Figure 2:** Difference in SMH(HV) in remineralization phase between the three groups

## Discussion

For the treatment of initial lesions, numerous techniques were developed to bridge the gap between preventive and restorative therapies [13, 14]. In this in vitro revision, the effects of sodium fluoride solution combined with zinc oxide NPs on permanent teeth were examined, and they were contrasted with sodium fluoride solution alone using a microhardness tester.

Previously used in earlier investigations, a demineralization method was used for the in vitro development of artificial enamel caries [10,15]. To create artificial caries-like lesions on the enamel surface, each sample was immersed in the demineralizing solution for 4 consecutive days (96 hours), after which it was carefully cleaned and preserved in deionized water. Different techniques are used to provide proof of mineral addition or loss. SMH analyses, which are quick, fast, and simple to gauge in a non-destructive pattern, are most frequently utilized. Its mode of operation relies on calculating the amount of material surfaces that collide with plastic deformation from a reference source. To reduce trial variance, measurements might be collected often from the same sample over a period [16], and the Vickers approach, which has previously produced fruitful outcomes, was used [17,18,19].

For sound enamel, the microhardness was measured before demineralization and after application of the chosen agents. More minerals are present in the enamel surface than the enamel subsurface. To reduce the typical variance of enamel surface among the samples that might react differently, polished teeth were used for the demineralizing solution [20].

Samples were set in a metal mold. The samples were prepared in this manner because any tilting or uneven surface could result in a protracted indentation, which can reduce hardness results. Therefore, in this test, a flat specimen surface must be accomplished by using a contra-angle, slow-speed handpiece, and the buccal surface was polished with Sof-Lex Disks (3M ESPE, USA), working from coarse to medium to fine to extremely fine [21,22].

Silver NPs have a long history of use in the biological and medical fields. Their byproducts have potent inhibitory and bactericidal effects with a broad spectrum of antibacterial activities that have been used for centuries to treat and prevent various diseases. They are also used in various dental specialties. [7] Carious lesions may be penetrated by silver ions, which may precipitate and cause enamel to harden [8] thereby decreasing the potential risk associated with oral care products that contain high amounts of fluoride and facilitating caries management over a lifetime. Fluoride remains the most essential agent used in caries management strategies [5].

In accordance with the results of this study, the null hypothesis was rejected. Given that an acidic environment causes outward migration of minerals, the results of this study showed a statistically significant decline in the microhardness of enamel surface after demineralization for all groups that indicate enamel caries. Mostly calcium and phosphate, leaving behind microscopic holes and losing solidity [23].

Group A (control negative), the SMH test's baseline phase (ranging from 270 - 360 VHN for normal enamel) and each of the demineralization and remineralization phases differ significantly (the SMH test's baseline records are higher), but the microhardness does not differ significantly between the two phases. This result is consistent with the research by Somani et al. The remineralizing potential of various agents on dental erosion (2014), it also agrees with the study by Yahya, who investigated the effectiveness of self-assembling peptide on enamel remineralization both alone and in combination with fluoride compared to fluoride-based delivery systems and casein phosphopeptide-amorphous calcium phosphate fluoride. (2020) [15,24], the results showed that the control group displayed the lowest levels of remineralization., which may be due to the absence of fluoride ions from the artificial saliva used in the study [25].

Group B (silver NPs with sodium fluoride), the SMH results showed a higher record in the baseline test, which significantly decreased after the demineralization phase. However, it also increased noticeably after remineralization with silver NPs with 5% sodium fluoride.

Carious lesions may be penetrated by silver ions, which may precipitate and cause enamel to harden. Dental doctors often apply sodium fluoride varnish to develop lesions and remineralize them. However, when 5% of nanosilver is added to sodium fluoride varnish, caries lesions in the remaining teeth are inhibited by 77% without developing a metallic taste or uncomfortable ulcerations (8), which is consistent with the study by Scarpelli et al. in 2017 (25), The theory put to the test is supported by the study's findings. Compared with the results of Cardoso et al., Ag-Nano demonstrated the capacity to remineralize 14.63 percent of enamel.

Moreover, Butrón et al. (2020) have confirmed that the results of laboratory and clinical research have demonstrated that silver NP compounds are superior to other preventive and treatment options for dental caries [26,27].

Group C (NaF), the SMH is highest at the baseline period, significantly decreases after demineralization, and increases once again after remineralization because fluoride promotes the formation of fluorapatite (Ca10(PO4)6F2) in the presence of Ca2+ and PO4 3- produced during enamel demineralization, but stays below SMH following the treatment with group B.

Groups' Comparison, the SMH results are the same for all groups in the baseline and demineralization phases, but they vary in the remineralization phase, with Group A having the lowest results because no fluoride with artificial saliva exists, followed by Group C, which contains sodium fluoride that aids in the precipitation of fluorapatite on the enamel surface and group B, which has the highest results with silver NP ions precipitating and making the strongest bonds to the outer enamel surface. As a result, using silver NP with sodium fluoride increases the efficiency of caries remineralization more than using sodium fluoride ions alone and produces a more homogeneous surface.

Even though remineralization occurred, none of the solutions used were able to increase enamel microhardness to the original values, and a statistically significant difference was found when comparing microhardness in the remineralization stage with that of the baseline values. This result may be due to the method of application used in this study. Modulation in the procedure or using a combination with other materials may further increase microhardness values, but this may require further studies.

**Conclusion**

The use of silver NPs in combined with 5% sodium fluoride is better than utilizing sodium fluoride alone in terms of enhancing surface microhardness because they are more effective together in increasing the microhardness of enamel cavities. Research recommendations stated that the effect of the incorporation of silver NPs with different fluoride-based delivery systems may be estimated.

**Conflict of interest**

The authors have no conflicts of interest to declare.

## Author contributions

Authors contributed equally to the research.


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## Informed consent

Informed consent was obtained from all individuals (or their guardians) who participated in this study.

## Ethical Approval

The research ethical committee at scientific research by ethical approval of environmental and health and higher education and scientific research ministries in Iraq (480, 19-1-2022).

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**تقييم الصلابة المجهرية لتسوس المينا الصناعي بعد المعالجة بمزيج من جسيمات الفضة النانوية ومحلول فلوريد الصوديوم**

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**المستخلص**

الخلفية :تم تطوير تقنيات جديدة تُمكّن من إعادة تمعدن التسوس الحديث في المينا. تُظهر منتجات جسيمات الفضة النانوية (NP) تأثيرًا قويًا مثبطًا وبكتيريًا قاتلًا. قد تخترق أيونات الفضة الآفات النخرية، مما يؤدي إلى ترسيبها وتصلب المينا.الأهداف :تهدف هذه الدراسة إلى تقييم ومقارنة فعالية استخدام جسيمات الفضة النانوية مع محلول فلوريد الصوديوم على الصلابة المجهرية لسطح المينا المتعرض لإزالة التمعدن المُحدثة صناعيًا، وذلك مقارنة باستخدام فلوريد الصوديوم فقط. المواد والطرق :تم استخدام ثلاثة عشر ضرسًا أول علويًا تم قلعها لأغراض علاج تقويمي. تم تنظيف كل سن، وفحصه، وتلميعه، وطلاؤه بطلاء أظافر مقاوم للأحماض لإنشاء نافذة دائرية بقطر 2×2 مم. ثم تم نقع الأسنان في محلول مزيل للتمعدن لمدة أربعة أيام، وبعد ذلك قُسِمت الأسنان إلى ثلاث مجموعات: المجموعة :A غير معالجة،المجموعة :B معالجة بجسيمات الفضة النانوية وفلوريد الصوديوم بتركيز 5%،المجموعة :C معالجة بفلوريد الصوديوم بتركيز 5% فقط.تم غمر جميع المجموعات لمدة 4 دقائق، وأُجريت جميع المراحل في درجة حرارة الغرفة.تم استخدام جهاز اختبار الصلابة المجهرية من نوع فيكرز الرقمي، مزود برأس ماسي بوزن 500 غرام ولمدة تطبيق 30 ثانية، موجه عموديًا نحو سطح المينا لقياس الصلابة المجهرية.أظهرت نتائج اختبار فيكرز للصلابة المجهرية أن محلول فلوريد الصوديوم مع جسيمات الفضة النانوية أظهر معدلات صلابة مجهرية أعلى) متوسط الصلابة السطحية (286.837 = [SMH] مقارنة بفلوريد الصوديوم وحده) متوسط(SMH = 191.530 ، بقيمة معنوية مهمة.(P = 0.00001) .الاستنتاج :إن استخدام جسيمات الفضة النانوية مع فلوريد الصوديوم يُعد أكثر فعالية من استخدام فلوريد الصوديوم وحده في زيادة الصلابة السطحية للمينا؛ وبالتالي، فهو يُعد واعدًا أكثر في إعادة تمعدن تسوسات المينا.