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The Assessment Of The Antibacterial Effect Of Diode Laser Versus Nanosilver Fluoride On Streptococcus Mutans Count Of Oral Biofilm Of Primary Teeth

Research Article

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Abstract

Aim: Streptococci are the most frequent cariogenic bacteria in tooth decay and are often present in the human oral cavity. The purpose of this study was toevaluate the antibacterial outcome of diode laser versus Nanosilver fluoride (NSF) on the count ofStreptococcus mutans inan oral biofilm of primary teeth in-vitro.

Materials and Methods: Seventy-two extracted human primary anterior teeth were prepared and equally split into four groups based on the test agent employed: group I: control group with two subdivisions (Negative control with no oral bacterial biofilm formation and Positive control with oral bacterial biofilm formation), group II: NSF, group III: diode laser 980 nm with 2 subdivisions(high power and low power) and group IV: NSF, then low power diode laser.A biofilm of Streptococcus mutans (ATCC 25175) was prepared and grown on the enamel surface of teeth. Therapy before and after, environmental scanning electron microscope (ESEM), and energy dispersive X-ray analysis (EDX) were used to analyze the surface morphology of the enamel surface. Following the medical treatments, the samples were put back in the glass tubes containing the 1ml phosphate buffer and incubated for 24 h. After that, the test tube was sonicated to separate the biofilm formed on the enamel surface of the sample, and the number of colony-forming units per milliliter in each group (CFU/ml dry biofilm weight) was determined.

Results: NSFand diode lasers (980nm) showed significant antibacterial effects on Streptococcus mutans biofilm, and reduce its number and vitality. Group II (NSF) and group IV (NSF and Low power diode laser) exhibited the uppermost reduction of bacterial count and viability, although no statistically significant difference was found between them where (p=0.654) followed by group III (diode laser group) without noticeable change between thehigh power diode and low power diode groups where (p=0.506), althoughthe low power diode gave better results than the high power diode.

Conclusion: The application of NSF, diode laser, or their mixture effectively reduces Streptococcus mutans bacterial colonies in the oral biofilm. NSF is the most effective agent against the viability and reduction of Streptococcus mutans followed by the diode laser, although there is no major change among high power and low power diodes. NSF and diode laser prevented the loss of minerals on the enamel surface of the specimen, and the content of calcium and phosphorus was retained and increased in all groups

Keywords: Antibacterial Effect; Streptococcus Mutans; Nanosilver; Fluoride; Diode Laser.

Introduction

Tooth decay is a microorganism infection of the teeth that causes regional disintegration and destruction to the mineralized architecture. This disease is considered to be one of the most common dental biofilm-related diseases. Although oral biofilm can be composed of a variety of bacteria, oral streptococcus is considered an important cause of dental caries. A more profound knowledge of the function of these bacteria in dental problems is required to minimize the incidence of the disease [1].

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The Streptococcus mutanscan quickly break down carbohydrates and produce a large amount of acid and induce tolerance to low pH environments [2].

Now a new material (Nanosilver fluoride) is used to combine the advantages of Nanosilver and fluoride in inhibiting cariogenic bacteria and preventing dental caries. Silver has intense antibacterial action and is effective against oral streptococci and periodontal infections, particularly when administered at nanoscale sizes [1]. Previous studies have described that as the particle size decreases, the antibacterial activity of silver nanoparticles (AgNPs) increases, and the toxicity and staining are reduced [3].

The biocidal effect of AgNPsand their wide range of activities, including bacteria, fungi, and viral agents, make them a great option for various medical applications. Based on particular ideas, AgNPs may accelerate cell lysis or obstruct cell transduction. In addition, it can inhibit bacterial adhesion to the surface and can be used as a beneficial antimicrobial ingredient in fluoride varnishes [1].

There is enough data to justify the use of AgNPsas antibacterial agent right now [3].

Besides the traditional anti-caries materials, lasers are also used-an alternative method for modifying the tooth surface and increasing its resistance to acids [4]. Dental caries has been suppressed using a variety of lasers with various test configurations. They cause structural and chemical changes in tooth enamel, making teeth more resistant to acid challenges [5]. Several studies have shown that diode lasers can have antibacterial effects on tooth enamel, dentin, and caries tissues, and at the same time,can cause minimal thermal damage to the remaining teeth [6].

The effect of laser irradiation on tooth enamel has been reported in several studies, either alone or in combination with fluoride. It has been proved that the combined application of laser and fluoride has a synergistic effect. The laser forms calcium fluoride on the surface and enhances the influence of fluoride on the enamel structure in its crystal structure [7]. The laser-fluoride combination was also investigated to see if the laser could boost fluoride's impact on the enamel architecture and raise the tooth structure's resistance to acidic demineralization [8].

Semiconductor diode lasers are one type of laser that is utilized. It is popular in clinical practice because of its advantages, including compact size, relatively inexpensive, and simplicity of use in the buccal mucosa. However, few studies have been done on the effects of diode lasers (with or without fluoride) on tooth enamel, particularly deciduous dental caries [8].

This study aims to evaluate the antibacterial effect of diode laser and Nanosilver fluoride alone or in combination on the count of *Streptococcus mutans* in the oral biofilm of primary teeth.

Materials and Methods

72 extracted human primary anterior teeth were selected, with no dental caries, developmental defects, or restorations. Before any treatment, the environmental scanning electron microscope (ESEM) with energy dispersive X-ray analysis (EDX) was used to analyze the enamel surface topography of the sample.

Teeth Preparation

Teeth were cleaned, polished, and stored in 0.1% Thymol solution [9]. The crowns were separated from the roots and then they were embedded in acrylic resin blocks, exposing only the buccal surface and labeled from the backside.

Biofilm Preparation

Streptococcus mutans (ATCC 25175) was used in the biofilm in this study. The biofilm is prepared as follows: Grown in Brain Heart Infusion (BHI) broth containing 1% (w/v) sucrose. They remain undisturbed for 24 hours, and we change the medium twice daily. The biofilm of *Streptococcus mutans* was formed on the enamel of the buccal surface of anterior teeth. It was placed in 2 mL of medium containing 1% sucrose in a 24-well cell culture plate, 5% CO_2, at 370 C. They were stored for three and five days, immersed in Phosphate Buffered Saline (PBS) three times at the end of each experimental period9. Using a metallic spatula, the biofilms were removed and placed in a falcon tube filled with PBS and sonicated for three 15s. The suspension was used for bacterial count and viability (colony forming units-CFU/ml of dry biofilm weight). On the first day of the biofilm formation, the adhesion test of *Streptococcus mutans* was carried out, and then the number of colonies was calculated [10].

Nanosilver Fluoride Varnish Preparation

In an aqueous solution, the AgNPs were formed by the chemical reduction of silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$). Chitosan biopolymer was a stabilizing agent and dissolved with $AgNO_3$ (1 mL, 0.11 M) in a 1% CH_3COOH solution and then blended until homogenous with a mechanical stirrer.We transferred the mix to a freezing bath, then we added newly made $NaBH_4$ (0.3 mL, 0.8 M), added a drop while mixing thoroughly. Removing the bottle from the freezing bath and then addingsodium fluoride NaF (10,147 ppm of fluorine). The stirring was maintained overnight [3].

In this study, a Quanta system diode laser was used. The radiation parameters indicate that the 980nm diode laser can produce chemical and morphological changes, thereby reducing the acid reactivity of tooth enamel.

Grouping of Samples

After the preparation is completed, the specimens were randomly allocated into four groups using closed envelope randomization according to the test agents used.

Group I (Control group): Included 24 teeth with no treatment This group was subdivided into two subdivisions.
-Control Negative: 12 teeth with no oral bacterial biofilm formation.
-Control Positive: 12 teeth with oral bacterial biofilm formation.

Group II (Nanosilver fluoride): included 12 teeth subjected to AgNPs fluoride varnish. For two minutes, the solution was in contact with the tooth surface. A micro brush was used to apply two drops of NSF solution to each specimen [11]. Evaluation is

carried out after 24 hours and stored in 2 ml of phosphate buffer [8].

Group III (Laser group): included 24 teeth irradiated with a diode laser (980 nm).

This group was subdivided into 2 subdivisions

A- High power diode laser included 12 teeth irradiated with high power diode.

The specimens were subjected to 2 irradiations. Using the following parameters: 980nm wavelength, 2W output power, 2 irradiations, each lasting 20 seconds, with a 60 seconds break, the tip diameter is 320 µm in sweeping motion and a continuous-wave laser generator [8]. The laser beam was irradiated across 1 mm of the specimens and kept at the same distance from them at all times [12].

B-Low power diode laser included 12 teeth irradiated with a low power diode laser.

The specimens were subjected to a 980 nm diode laser with a power of 200mW, applied in a continuous-wave laser with a distance of 0.5 cm and a tip diameter of 320 µm in a sweeping motion for 3 minutes [12].

Group IV (Nanosilver fluoride and diode laser): included 12 teeth subjected to two drops of Nanosilver fluoride varnish for 2 minutes, applied with a micro brush, then irradiated with low power diode laser 980nm with power 200 mW applied for 3 minutes at 0.5 cm distance, 320 µm tip diameter in continuous-wavelaser source and sweeping motion [12].

-Qualitative and quantitative assessment of surface morphology of enamel samples was done before and after treatment by using Scanning electron microscope Model Quanta 250 FEG (Field Emission Gun), an accelerating voltage 30 KV, magnification14x up to 1000000 and resolution for Gun.1n, attached with EDX Unit (an analytical technique used for the elemental analysis or chemical characterization of a sample). This was done at the National Research Center.

-Teeth were dried on filter papers, placed on aluminum stubs by carbon double-sided, and then placed in an automatic stage from 1 to 7 scales. Photomicrographs were taken under standardized working distance and magnification (1000X-5000X), [13], and percentage analysis of mineral content of calcium and phosphorus ions was calculated [14].

-After the treatment procedure, all samples were put back into the glass tube containing 1 ml of phosphate buffer and incubated for 24 hours. After that, the tube is sonicated to separate the biofilm formed on the sample's enamel surface, and the number of colony-forming units per milliliter (CFU/ml) was determined [10].

Statistical Analysis

Based on the previous paper by Savas et al., 2015, the minim expected difference between the studied groups is (3.9±3.3 CFU/mL ×103) [15]. Using power 80% and 5% significance level, we will need to study 12 teeth in each subgroup to be able to reject the null hypothesis that the means of the experimental and control groups are equal. The PS program calculated the sample size. Data were analyzed using "IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 21 (SPSS Inc., Chicago, IL)". The mean and standard deviation, or median and range, were used to describe numerical data. The "Kolmogrov-Smirnov" and "Shapiro-Wilk tests" were used to look for normality in the data. For normally distributed numeric variables, the "ANOVA" was used, whereas, for non-normally distributed numeric variables, the "Kruskal Wallis test" was used, followed by "post hoc testing." Statistical significance is defined as a p-value of less than or equal to 0.05.

Results

The following methods evaluate the antibacterial ability of the tested therapeutic agent:

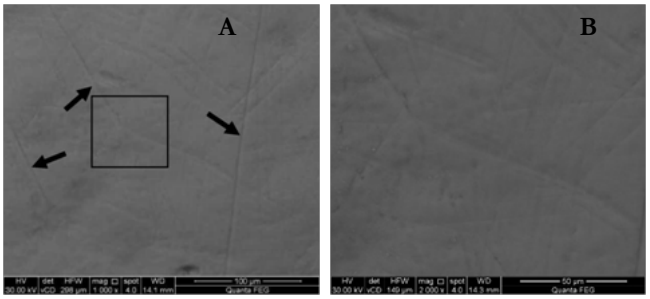
Environmental Scanning electron microscope

The ESEM pictures showed the quality of the tested tooth surfaces in different test periods andresearch groups. Photomicrographs were taken under magnification (1000X-5000X) [13].

A-Preoperative Assessment: The preoperative evaluation of all groups showed a relatively similar intact and smooth enamel surface, with few small scratches and few small pores. The enamel surface showed normal surface structure such as perikymata, and the normal rod ends are scattered on the smooth enamel surface (Black arrows) (Figure 1A and B).

B-Postoperative Assessment: -Group II (Nanosilver fluoride): The enamel surface of deciduous teeth is nearly entirely covered by a NSF layer, according to postoperative ESEM photomicrographs (Black arrows). (Figure 2A, 2B)

Figure (1) A: Preoperative ESEM photomicrographsof the enamel surface of deciduous teeth. (1000X). B: Preoperative ESEM photomicrographs of the enamel surface of deciduous teeth.(2000X).



-Group III: (Diode laser)

A-Postoperative ESEM photomicrographs of the enamel surface of deciduous teeth after high power diode laser application revealed a substantial increase in the number and accentuation of the keyhole enamel surface structure with somewhat uneven enamel surface (Black arrows). (Figure 2C, 2D)

B-Low power laser: After low power diode laser application, the postoperative ESEM photomicrographs of the enamel surface of deciduous teeth showed an apparent increase of surface irregularity of enamel (Black arrows). (Figure 2E, 2F)

-Group IV (Nano silver fluoride followed by a low-power diode laser): Postoperative ESEM photomicrographs of the enamel surface of deciduous teeth revealed that the enamel was clearly smooth and the enamel surface irregularity was minimal (Black arrows). (Figure 2G, 2H)

Energy Dispersive X-ray

In each test, the mean and standard deviation values were determined for each group. In the CFU findings, viable counts of antibacterial activity were converted to their log10 values. The "Kolmogorov-Smirnov" and "Shapiro-Wilk" tests were used to check for normality, and the results revealed a parametric (normal) distribution. To compare more than two groups in unrelated samples, a "one-way ANOVA" was employed, followed by a "Tukey post hoc test". The significance level was set at $P \leq 0.05$. Statistical analysis was performed with "IBM® SPSS® Statistics Version 20 for Windows".

Calcium results:- There were statistically significant differences between (negative control) and each experimental group and between (positive control) and each experimental group, where ($p<0.001$), and there were also statistically significant differences between all experimental groups, where ($p<0.001$).

-The calcium increased in all experimental groups. The Nanosilver fluoride showed the highest increase of calcium followed by the diode laser. However, there was no statistically significant difference between high power diode and low power diode groups where ($p=0.877$, then the lowest increase was shown in (Nanosilver fluoride and Low power diode) groups.

Phosphorus results:- There was no statistically disparity among (Negative control) and each experimental group and between (Positive control) and each experimental group where ($p>0.05$).

When the control and treatment groups (II, III, and IV) were compared, the treatment group had more phosphorus, without statistical disparity among them ($p=0.094$).

Bacterial count and viability: CFU results (Percentage of change):

There was a prominent change between (Negative control) and each experimental group and between (Positive control) and each experimental group where ($p<0.001$) and a statistically significant difference was also observed among all the experimental groups where ($p<0.001$).

In group I (control positive), the bacterial count and viability increased. In groups II, III, and IV, bacterial counts and viability decreased. Group II (Nanosilver fluoride) and group IV (Nanosilver fluoride and Low power diode laser) showed the highest reduction of bacterial count and viability, although no prominentchangewas found between them where ($p=0.654$) followed by group III (diode laser) with no prominentchangeamong the high and low power diode groups where ($p=0.506$), although the low power diode gave better results than the high power diode.

Figure 2. A: ESEM photomicrographs of the enamel surface of deciduous teeth of group II postoperatively (1000X) B: ESEM photomicrographs of the enamel surface of deciduous teeth of group II postoperatively (2000X), C: ESEM photomicrographs of the enamel surface of deciduous teeth of group III (High power diode) postoperatively (1000X), D: ESEM photomicrographs of the enamel surface of deciduous teeth of group III (High power diode) postoperatively (2000X) , E: ESEM photomicrographs of the enamel surface of deciduous teeth of group III (low power diode) postoperatively (1000X) , F: ESEM photomicrographs of the enamel surface of deciduous teeth of group III (low power diode) postoperatively (2000X) , G: ESEM photomicrographs of the enamel surface of deciduous teeth of group IV (Nanosilver Fluoride application followed by low power diode laser) postoperatively (1000X) , H: ESEM photomicrographs of the enamel surface of deciduous teeth of group IV (Nanosilver Fluoride application followed by low power diode laser) postoperatively (2000X).

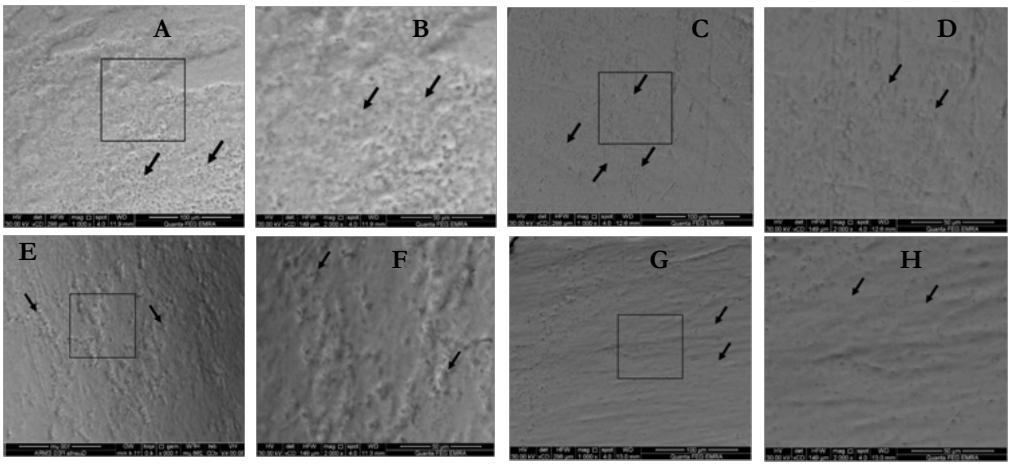


Figure 3. Bar chart representing percentage of change of Calcium in different groups.

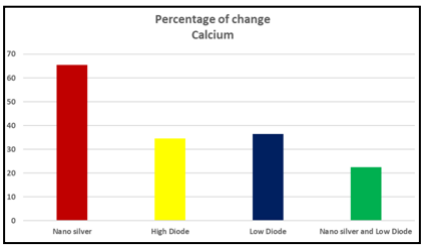


Figure 4. Bar chart representing Phosphorus for different group.

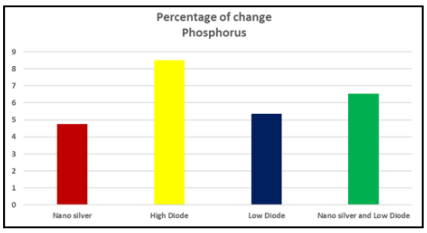


Figure 5. Bar chart representing antibacterial activity for different groups.

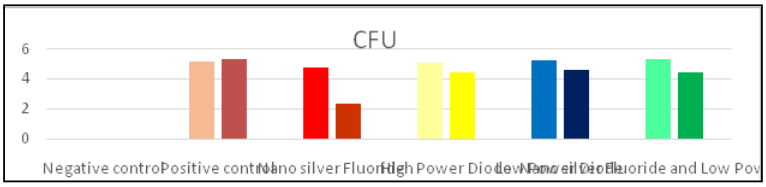
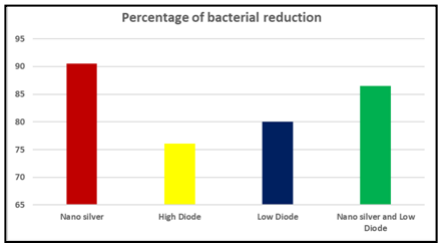


Figure 6. Bar chart representing percentage of bacterial reduction in different groups.



Discussion

In-vitro dental biofilms models have been frequently utilized in an attempt to simulate the human studies of tooth decay and investigate the cavity process under laboratory-controlled conditions. Even though the approach may not represent all aspects of caries development, it does allow us to conduct repeatable studies in a controlled environment [12]. This model is suitable for simulating the oral environment to evaluate different antibacterial agents, although it is complicated, expensive, and time-consuming [16].

The first step in the biofilm formation process is the initial adhesion and accumulation of bacteria, leading to demineralization of tooth enamel. Therefore, it is possible to reduce the occurrence of tooth decay by controlling the formation and accumulation of biofilms. The effect is owed to the capacity of *Streptococcus mutans* to utilize dietary carbohydrates, synthesize extracellular polysaccharides and its acidogenic and aciduric properties, it is considered the most cariogenic microorganism in dental biofilm. Although this oral biofilm does not completely replicate the complex microbial community in the oral cavity, previous research have demonstrated that the enhanced model of Streptococcus mutans biofilm formation is sufficiently effective in detecting biofilm alterations in the presence of antimicrobial drugs [15]. Therefore, in this study, our purpose is to evaluate and compare the effectiveness of diode laser and Nanosilver fluoride in controlling the growth of *Streptococcus mutans* in the oral biofilm of deciduous teeth.

Teeth were prepared and stored in 0.1% Thymol solution, one of the most accepted and used storage methods in in-vitro and in situ studies and it was proven that it maintains the integrity of the dental organ [9]. The NSF and diode laser were evaluated and compared to group I, the control group (control negative with no oral bacterial biofilm formation and control positive with oral bacterial biofilm formation and without any treatment). For standardization purposes, both antibacterial agents are tested after 24 hours [8].

Several studies have demonstrated that silver nanoparticle-containing materials achieved strong antibacterial effects against microorganisms' biofilms and the oral pathogenic species of streptococci [1]. The NSF containsAgNPs, chitosan that was used as a carrier to stabilize AgNPs, and fluoride [17]. It can replace old-style Ag compounds as an antimicrobial reagent for Streptococcus mutans and has the advantage of not staining teeth black [3]. Since scientists discovered that the reagent is safe, sodium borohydride was also added as a reducing agent for NSF compound [3].

AgNPs can dissolve cells and prevent their proliferation because they will adhere and accumulate on the surface of bacteria, causing cell membrane damage and structural changes, thereby mak-

ing bacteria more permeable. It can also prevent DNA replication, protein synthesis, and interfere with the energy transfer cycle of bacteria [12]. By creating a composite assembly with proteins and metals, chitosan chemically reacts with AgNPs and improves adhesion properties [3]. Hence there is a synergistic antibacterial action between the AgNPs, fluoride and chitosan components in the NSF preparation. Further study should be done on this potential synergistic effect [17].

Since AgNPs show greater antibacterial action against Streptococcus mutans when particle size is decreased, the spherical nanoparticles generated in this work are in the range of5.9 ± 3.8 nm.These results are similar to previous studies [3]. Two drops of NSF solution were applied to the tooth surface using a micro brush and kept on for two minutes [11].

This was supported by a study done by Targino AG et al., 2014, which stated that NSF formulation had an antibacterial effect against *Streptococcus mutans* and was efficient in demineralizing enamel and consequently preventing enamel caries [3]. In addition, consistent with our research, in 2017, Freire PLL et al. concluded that NSF reduced the number and viability of Streptococcus mutans biofilms in children [18]. In 2018, Teixeira JA et al.,[17], in 2020, Dina El-Desouky et al, [11], endorsed that and stated that NSF is effective as an anti-cariogenic material that can limit enamel demineralization caused by the cariogenic challenge.

All four previous studies agreed with our results, demonstrating that NSF has an excellent effect as an antibacterial agent and helps prevent dental caries. The search of the wed did not reveal any work that disagrees with this article.

Under proper irradiation parameters, the laser will cause structural and physiochemical changes to prevent dental caries. Only few investigations have looked at the effects of diode laser on deciduous teeth, and little study has been done on the influence of diode laser on dental architecture [8].

Various effects, particularly on enamel dissolution, can be detected depending on the temperature attained by laser irradiation. The melting and recrystallization of the surface after laser irradiation may reduce the solubility of hydroxyapatite and block the prismatic space. As a result, lasers are now being utilized to prevent enamel demineralization [19]. The diode laser exerts its antibacterial effect through thermal and photo-disruptive mechanisms. They cause sublethal damage to the cell that inhibits cell growth in the form of destruction of the cell wall integrity and denaturation of its wall protein. This will stop the cell from growing and cause it not to lyse immediately but after a period of exposure. On the other hand, due to the thermal changes of laser irradiation, denatured proteins may be produced, and this denaturation can be compensated by inducing cells to produce new proteins. The stress imposed on the cell to get rid of the denatured proteins could also cause its death [2].

A-High power diode laser

The irradiation technique used in this study was as cycles; irradiation twice, 20 seconds each time, with a 60-second break [8]. Two rounds of irradiation resulted in more bacterial decrease than a single round, suggesting that the bacterial layer was partially disrupted [20]. This is in line with the other scientists findings [21].

In this work, we discovered that 2 times diode laser irradiation (980 nm) with 2W output power in a continuous wave laser generator reduced the count and viability of Streptococcus mutans, indicating that it had an antibacterial impact on the Streptococcus mutans biofilm. This is consistent with the view of Lee BS et al. in 2006 that the combination of diode laser irradiation with 7W output power and distilled water cooling can kill 97.7% of CFU [22]. Matching with our results, Al-Habeeb A et al., 2013, Arslan I et al., 2019 and Vinothkumar TS et al., in 2020, showed that diode lasers showed significant antibacterial effects on *Streptococcus mutans* [23-25].

B-Low power diode laser

This study also evaluated the effect of low power diode lasers on Streptococcus mutans. This relationship can be derived from the early effect of low-power lasers by inhibiting the growth of biofilms, which may be due to the decomposition of these microorganisms due to exposure of these microorganisms to the laser [26].

In 1998, Moritz et al. reported that the diode laser has a bactericidal effect, and its application to Streptococcus mutans caused its reduction [27]. This fact is in agreement with Ahmed MI et al, in 2011, that stated that the diode laser had a lethal effect on *Streptococcus mutans* when a constant beam of coherent, continuous monochromatic light of diode laser 670 nm with a power of 30 mW is applied on it,[2], and in 2016, Farias SS et al, also assessed the effect of low-level laser (diode 660 nm)with 100 mW power output on *Streptococcus mutans* biofilm and proved its effects on the viability on the bacterial biofilm [28].

This is in agreement with our study. We found that the use of a 980 nm diode laser with a power output of 200 mW in continuous-wave laser source and sweeping motion for 3 minutes has an antibacterial effect, and reduces the number and survival rate of *Streptococcus mutans*. These findings are in line with the results of Saafan A et al. in 2018, who used diode lasers with the same parameters as our research. Their results showed a 94.27% reduction in Streptococcus mutans [12].

On the other hand, some researchers disagreed with our results. They considered that the antibacterial effect of diode laser alone is not enough to kill the *Streptococcus mutans* and better results would be obtained when conjugated with other methods. In 1999, Moritz et al.[29] and Lee BS et al in 2006,[22], observed the effect of diode laser irradiation alone and its effect when combined with the nanoparticles and they concluded that significantly better results were obtained with the combination between them. Few studies have investigated the antibacterial efficacy of diode laser and NSF on a mature *Streptococcus mutans* biofilm caries model and they demonstrated their antibacterial effect.

Some authors stated that tooth enamel could not absorb 980nm diode laser irradiation, so they recommend using pigmented varnish on the tissue surface to irradiate tooth enamel. This technique can improve the laser beam's reception on the surface, altering its chemical and morphological structure and improving acid resistance. They also discovered that applying fluoride varnish before irradiation can cause enamel hydroxyapatite to become fluoridated hydroxyapatite, resulting in a decrease in enamel dissolution and longer retention of fluoride ions on the surface than

non-irradiated enamel [19].

This matches our results because we found that NSF has the highest antibacterial result on cariogenic *Streptococcus mutans* biofilms, followed by the mixture of NSF and the low-power diode laser group, and their results are not significantly different. The efficiency of using diode lasers alone is low. Although low power diode gave better results, there was no significant difference between high power and low power diodes. In 2020, Sadony DM and Abozaid HE, agree with our result. They evaluated the antibacterial effectiveness of silver and gold nanoparticles in the presence or absence of a diode laser (970nm) with a power output of 2W and irradiation as cycles against *Streptococcus mutans*. They observed that when diode lasers were used in combination with AgNPs, the reduction in CFU was the greatest [20].

However, some authors disagree with our results. Nogueira RD et al., 2017, concluded that the addition of a fluoride varnish on the enamel surface before laser irradiation could increase the surface roughness and bacterial adhesion. They said that in the existence of fluoride varnish, the laser's ablation effect in hard tissues will increase. Therefore, the surface roughness is significantly increased, and the enamel is more sensitive to bacterial adhesion [19].

Because the differences between our findings and those of earlier studies might be partly explained by differences in laser settings and research conditions, more research into the various impacts of diode lasers on bacterial biofilms is required [8].

Environmental Scanning Electron microscope

Due to the difficulties of cleaning these regions, biofilm development and bacterial adherence are better developed and collected on rougher surfaces. Therefore, smoother surfaces or surfaces with almost no irregularities are less prone to caries lesions [19]. The ESEM pictures were used for qualitative analysis of the tested tooth surfaces during the different testing times.

-At baseline, the ESEM images of all groups showed a relatively intact enamel surface. These enamel surfaces did not show rodless enamel. This could be attributed to the fact that these teeth functioned before extraction, which might havecaused attrition of this rodless layer. Enamel surface showed a normal surface structure like rod end, perikymata, and varying degrees of irregularities in some areas.

-After using different antibacterial agents, the surface structure of tooth enamel shows different changes:

The NSF group showed that a layer of NSF almost completely covered the enamel surface. The decrease in the number of bacteria in this group may be that in addition to its antibacterial effect, there is also a layer that separates bacteria from tooth enamel. The high power diode laser group showed apparent increase of number and accentuation keyhole enamel surface structure with relatively irregular enamel surface. This resulted in removing the rodless enamel layer exposing the underlying normal structure (rod end keyhole). Hence, more irregularities that are less mineralized than surface enamel are observed which may explain why this group has a higher bacterial accumulation. The low power diode laser group showed an apparent increase in surface irregularities without removing the outer enamel surface. This may explain the superiority of the low-power diode laser on the bacterial count reduction over the high-power diode laser. This has been proved in group IV where NSF and low power diode laser are applied. The superiority in this group is due to the presence of a layer of NSF which prevented the laser beam from making surface irregularities and the unique effect of the low diode laser.

Energy Dispersive X-ray

Bacteria in biofilms are always metabolically active, leading to fluctuations in pH. When the pH value drops or rises, respectively, these fluctuations may cause a decrease or increase in the mineral content of the teeth. The cumulative result of this demineralization process may be a net loss of minerals, leading to the dissolution of tooth hard tissues and the formation of caries [30].

In this study, we measured each group's calcium and phosphorus content before and after treatment [14]. The results of the EDX measurements in our study showed that the calcium enamel content (wt. %) was preserved and increased after NSF and diode laser application in all groups ($p<0.001$). NSF shows the largest increase in calcium, followed by high power and low power diode lasers (there is no significant difference between them), and then the combination of NSF and low power diode lasers show the least calcium. The phosphorus results showed that a phosphorus enamel content (wt. %) was preserved and increased in all treatment groups. However, this increase was not statistically significant between the control and treatment groups and between the different treatment groups.

In agreement with our study, Abo ElSoud AA et al., 2020, [31] stated that there is a significant increase in calcium and phosphorus ions after NSF application in comparison to the control group and this may be due to the effects of Nanoparticles in increased deposition of these ions, which was also following Ata Mostafa, 2019 [32]. In 2021, Elkabbany HSM et al. pointed out that calcium and phosphorous ions increased in all treatment groups after diode laser irradiation, which is consistent with our results [14]. In 2016, Carrilloa LE et al. found that the therapeutic laser can improve the remineralization of the enamel surface in terms of calcium ions and a minor but substantial rise in phosphorus ions [33].

On the other hand, another study in 2006, Kato IT et al., evaluated the effect of a 960 nm diode laser on the solubility of calcium in tooth enamel. Additionally, they stated that the additional application of laser irradiation did not cause any significant increase or decrease in calcium solubility and that unless a fluoride agent is added to diode laser, it did not increase the calcium [34].

Because tooth decay is a complex illness, the optimistic results of this study are limited because it only looked at the bacterial component. As a result, additional biofilm research and clinical research are warranted [3].

Conclusion

In conclusion, the application of Nanosilver fluoride, diode laser, or their combination is an effective technique for microbial colony reduction of *Streptococcus mutans* in the oral biofilm. NSF

is the most effective agent against the viability and reduction of *Streptococcus mutans*, followed by the combination of NSF and the low power diode laser group, although their results are not significantly different. The efficiency of using diode lasers alone is low. Although low power diodes gave better results, there is no significant difference between high power and low power diodes. NSF and diode laser prevented the loss of minerals on the enamel surface of the specimen, and the content of calcium and phosphorus was retained and increased in all groups.

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