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Original research

# The antibacterial effects of vitamin $D_3$ against mutans streptococci: an in vitro study

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**Purpose**

This study aims to evaluate the antimicrobial effects of the cholecalciferol vitamin $D_3$ against *Streptococcus sobrinus* (*Strep. sobrinus*) and *Streptococcus mutans* (*Strep. mutans*) bacteria in vitro that is considered the main causative bacteria in dental caries development.

**Materials and Methods**

The antimicrobial effects of vitamin D3 were evaluated against *Strep. sobrinus* and *Strep mutans* using the agar disc diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of vitamin $D_3$ were determined using a microdilution method following the guidelines by the Clinical Laboratory Standards Institute (CLSI). Scanning electron microscope (SEM) was used to evaluate the morphological changes of bacterial cells following exposure to vitamin $D_3$.

**Results**

*Strep. sobrinus* was more sensitive to vitamin $D_3$ compared to *Strep. mutans* bacteria. The MIC values of vitamin $D_3$ against *Strep. sobrinus* and *Strep. mutans* were 60 µg/mL and 250 µg/mL respectively whereas the MBC values were 120 µg/mL and 500 µg/mL, respectively. Moreover, significant changes in the bacterial morphology were observed in treated bacterial cells with vitamin $D_3$ as compared to the untreated control bacteria using SEM.

**Conclusion**

These findings suggested that vitamin $D_3$ has excellent antimicrobial effects against *Strep. sobrinus* and *Strep. mutans* and may be considered as a promising compound in the prevention of dental caries in the future. Further research is recommended to elucidate the mechanism of vitamin $D_3$ on these bacteria.

***Keywords:*** *Vitamin $D_3$, Cholecalciferol, Streptococcus sobrinus, Streptococcus mutans, antibacterial effect*

## Introduction

Vitamin D is an essential component in the growth, maturation, and physiology of tissues and organs. It regulates the calcium-phosphorus metabolism and mineralization of bone tissue, including teeth (1). The lack of vitamin D during the tooth development period may lead to tooth developmental defects which makes the tooth more susceptible to bacteria attachment and colonization, and then eventually initiation of dental caries (2). Enamel hypoplasia is one of these developmental defects and is considered a significant risk factor for dental caries in children (3).

Dental caries and vitamin D deficiency are common health issues worldwide that are prevalent through all age groups and affect health and wellbeing (3). Dental caries has a negative impact on normal growth and on the quality of life of the affected individuals (4, 5). Recent studies

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have indicated a significant association between vitamin D deficiency and higher dental caries prevalence among children and adults (3, 6).

The potential role of vitamin D in inducing the innate immunity and improving the body's resistance against different pathogens is well documented (7). Theoretically the role of vitamin D in combating diseases is conceptualized by modulating the immune response of the infected host by production of antimicrobial peptides and inducing cell-specific receptors related to pathogen clearance (8). Almost all human cells have a specific vitamin D receptor (VDR), including B and T lymphocytes, macrophages, dendritic cells, and monocytes (7). Vitamin D boosts the expression of powerful antimicrobial peptides, such as cathelicidin and β defensin as well as cytokines response that exist in neutrophils, monocytes, and natural killer cells through its effects on the VDRs. Additionally, the level of vitamin D has a direct influence on macrophages, enhances oxidative burst of macrophages including maturation, production of cytokines and releases hydrogen peroxide. In addition, vitamin D assists neutrophil motility and phagocytic function (7).

Moreover, the efficacy of vitamin D against diseases is not only via modulation of the immune system but also via direct antimicrobial activities against different bacteria although little is known about the direct effects of vitamin D on bacteria such as *Mycobacteria* (8). The mechanism by which vitamin D inhibits *Mycobacterial* growth remains to be studied further. Vitamin D inhibits *Helicobacter pylori* growth (9) via the collapse and destabilization of the cell membrane structures and ultimately lysis of the bacterial cells (9). Vitamin D inhibits the growth of *Porphyromonas gingivalis* by decreasing the virulence factors of associated genes contributing in bacterial colonization, inactivation of host defence mechanisms, tissue destruction and nutrient acquisition (10). Besides that, it was indicated that vitamin D derivatives are bactericidal and possess lytic activity against *Strep. mutans* and target the bacitracin-associated efflux system (11).

Mutans streptococci mainly *Streptococcus mutans (Strep. mutans)* and *Streptococcus sobrinus (Strep. sobrinus)* are Gram positive and facultative anaerobic bacteria and are mainly found in the oral cavity. They are the main causative bacteria responsible for initiating dental caries (12); these bacteria can easily produce extracellular polysaccharides in large quantities from fermented carbohydrates and are strongly bound to teeth surfaces. They are able to survive in an acidic environment (13, 14). Therefore, eradicating such cariogenic bacteria would be considered a basic and essential step in preventing dental caries.

Recently, searching for novel antimicrobial agents is of great interest where overuse or misuse of antibiotics and antibacterial agents have led to antimicrobial resistance (15). Several antimicrobial agents such as chlorhexidine, triclosan and cetylpyridinium chloride are widely used as effective antibacterial agents against oral pathogens to reduce dental plaque and oral diseases including dental caries (16). However, side effects such as tooth discoloration and bacterial resistance still hinder their use (17, 18). Antibiotics is still an expensive option and misuse of them results in significant antibiotic resistance and contributes to increased health care costs (18). Using other alternative therapeutic products such as vitamin $D_3$ which is considered an inexpensive prophylactic option could be an essential step to discover a novel antimicrobial agent since the search for novel antimicrobial agents has been of great interest in the last few decades.

To the best of our knowledge, two previous studies by Grenier *et al.* (10) and Saputo *et al*. (11) have determined the antibacterial activities of vitamin D against *Strep. mutans*. However, in these studies (10, 11), different study methods and different vitamin D compounds (alfacalcidol, doxercalciferol, and calcitriol) were used. The antimicrobial activity of vitamin $D_3$ against *Strep. sobrinus* was very much lacking in the literature. Hence, this study may extend our knowledge about the antibacterial activity of another vitamin D compound which is cholecalciferol vitamin $D_3$ against the two most cariogenic bacteria that causes dental caries, namely *Strep. sobrinus* and *Strep. mutans* bacteria. Therefore, we hypothesized that vitamin $D_3$ might inhibit the growth of these bacteria which in turn may help in preventing dental caries. The objective of this study is to assess the antibacterial effects of cholecalciferol vitamin $D_3$ against *Strep. sobrinus* and *Strep. mutans* in vitro.

## Materials and Methods

*Preparation of vitamin $D_3$*

100 mg of analytical standard vitamin $D_3$ (Cholecalciferol) was obtained from Sigma Chemical (Sigma-Aldrich, Germany, Cat. No.: 47763) and was dissolved in 4mL of 95% ethanol to obtain 25mg/mL stock solution. This stock solution was then diluted in distilled water to obtain the working stocks and to reduce ethanol toxicity. The working stocks were aliquoted and kept at −80°C until used; once the working stocks were used, they were discarded.

*Bacterial strains and growth conditions*

Bacterial strain from the glycerol stock under −80°C was sub-cultured. The *Strep. sobrinus* DSM 20742 obtained from the German Collection of Microorganisms and Cell Cultures (Germany) and *Strep. mutans* (ATCC 25175 American Type Culture Collection, USA) were cultured on Brain heart infusion broth (BHI) and Brain heart infusion agar at 37°C under aerobic conditions for 18–24 hours. Microbiological media was obtained from Sigma-Aldrich (St. Louis, MO, USA and Oxoid Ltd, Basingstoke, UK) and prepared according to the manufacturer's instructions.

*Antibacterial susceptibility assay*

The antibacterial susceptibility of vitamin $D_3$ was investigated using the disc diffusion method on Mueller-Hinton agar plates (Sigma-Aldrich, St. Louis, MO, USA). Agar plates were inoculated with bacterial suspensions at a concentration of $1\times10^8$ CFU/mL. Then sterile blank discs (6-mm diameter) which were impregnated with 20 µL of (500, 1000, 2000, and 4000 µg/mL) cholecalciferol vitamin $D_3$ solutions were applied to give a final concentration of 10, 20, 40 and 80 µg/disc respectively, together with a positive (0.12% chlorhexidine) and negative control (2% ethanol). Preliminary experiments were carried out to test the effects of the sol-

vent (ethanol) on the tested bacteria which showed that at the dilution used, ethanol had no effect on bacterial growth. After 24 hours incubation at 37°C, the inhibition zones were observed and measured in millimetres.

*Minimum inhibitory concentration and minimum bactericidal concentration*

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth microdilution method following the National Committee for Clinical Laboratory Standards (19). In this study, MIC and MBC experiments of vitamin $D_3$ against *Strep. sobrinus* were carried out. *Strep. sobrinus* showed high sensitivity to vitamin $D_3$ at lower concentrations; however, these low concentrations did not work on *Strep. mutans*, hence higher concentrations of vitamin $D_3$ were used. The vitamin $D_3$ stock used for *Strep. sobrinus* was 240 µg/mL whereas 2000 µg/mL was used for *Strep. mutans*.

Serial dilutions of vitamin $D_3$ stocks were carried out in BHI broth in a sterile 96-well plate. Then, 100 µL of bacterial inoculum (a final concentration of $1\times10^6$ CFU/mL) was added to each well. These assays were tested in triplicates along with positive and negative controls. The positive controls contained bacterial cells in BHI broth to determine the bacteria growth throughout the experiment. The negative controls contained two-fold serial dilutions of the tested vitamin $D_3$ in BHI broth without any bacteria and served as primary negative control to determine the changes in absorbance due to the different vitamin $D_3$ concentrations. In addition, another negative control contained uninoculated BHI broth without vitamin $D_3$ to evaluate the sterility of the BHI broth (20). Then the plates were incubated aerobically at 37°C for 24 hours. The growth of bacteria was determined at OD 600 nm using a microplate Spectrophotometer (Infinite M200 Pro, Tecan). The MIC was assessed by subtracting the mean OD 600 values of the incubated test medium from the incubated primary negative control. The MIC was considered as the lowest concentration of tested vitamin $D_3$ at which the OD 600 absorbance falls below 0.05 with respect to the primary negative control (21). Three triplicate experiments were completed at different time intervals.

The MBC was determined by taking 10 µL aliquot from the clear wells and were plated on BHI plates and incubated at 37°C for 24 hours. The MBC was defined as the lowest concentration of tested vitamin $D_3$ that did not show any bacterial growth on BHI plates following the incubation period.

*Scanning electron microscope (SEM)*

In this experiment, the morphological changes were assessed for the untreated and treated *Strep. sobrinus* and *Strep. mutans* with vitamin $D_3$ application using SEM.

Briefly, overnight cultures of *Strep. sobrinus* and *Strep. mutans* were treated with cholecalciferol vitamin $D_3$ at MIC values and incubated for 18–24 hours at 37°C along with untreated bacteria cultures that serve as growth controls. The treated bacteria were fixed in 2.5% glutaraldehyde for 4–6 hours then washed with 0.1 M sodium phosphate buffer (pH 7.2) and post-fixed in 1% osmium tetroxide for 2 hours at 4°C. After washing again with 0.1 M sodium phosphate buffer, the samples were dehydrated using a series of alcohols. The specimens were coated with a thin layer of platinum and were observed under SEM.

*Statistical analysis*

The data was entered and analysed using Statistical Package for Social Sciences version 20.0 (SPSS Inc., Chicago, IL, USA). No data corrections were applied before the analysis. Bacterial measurement data under SEM were presented as the mean± standard deviations. The distribution of the data did not meet the requirements for normality and homogeneity of variance assumptions and therefore the length and width measurements between untreated and treated bacteria were determined by the nonparametric Mann–Whitney U test. The confidence interval was set to 95% and $p < 0.05$ was considered statistically significant.

## Results

*Antibacterial activity of vitamin $D_3$*

In this experiment, vitamin $D_3$ was investigated to evaluate its antibacterial activity against *Strep. sobrinus* and *Strep. mutans* using the disc diffusion method. The results revealed no inhibition zones for both bacteria against all tested concentrations of vitamin $D_3$.

*Minimum inhibitory concentration and minimum bactericidal concentration*

The MIC is considered the lowest vitamin $D_3$ concentration that inhibited bacterial growth, as measured at OD 600. The MBC is defined as the lowest concentration of tested vitamin $D_3$ that did not show any bacterial growth on BHI plates. The MIC values of vitamin $D_3$ against *Strep. sobrinus* and *Strep. mutans* were 60 µg/mL and 250 µg/mL, respectively, as shown in Figure 1 and 2. The MBC of vitamin $D_3$ against *Strep. sobrinus* and *Strep. mutans* were 120 µg/mL and 500 µg/mL, respectively.

*Scanning electron microscope*

SEM examination was conducted to investigate the possible changes in the morphology of *Strep. sobrinus* and *Strep.*

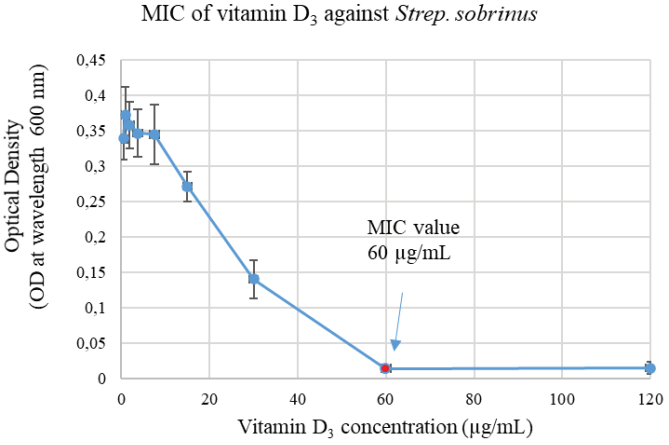


***Figure 1.*** *MIC value of vitamin $D_3$ against Strep. sobrinus.*

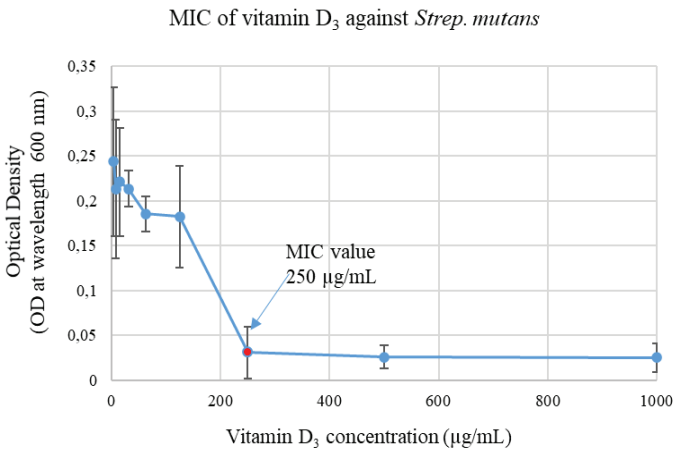


***Figure 2.*** *MIC value of vitamin $D_3$ against Strep. mutans.*

*mutans* bacteria in response to cholecalciferol vitamin $D_3$ application. The morphology of the tested bacteria was observed for the untreated and vitamin $D_3$ treated bacterial cells. The untreated *Strep. sobrinus* and *Strep. mutans* exhibited the typical streptococcal appearance as ovoidal (elongated) cells with smooth uniform shape and intact cell membranes (Fig.3a, c, e and Fig.4a, c, e). However, the treated *Strep. sobrinus* significantly appeared shorter and swollen compared to untreated *Strep. sobrinus* bacteria (Fig.3b, d) with mean length of 0.96±1.95 µm, 0.78±0.11 µm p=0.021 and mean width of 0.47±0.04 µm, 0.51±0.06 µm p=0.048 for non-treated and treated bacteria, respectively. On the other hand, the treated *Strep. mutans* cells did not exhibit any clear changes in their size compared to the untreated cells. Additionally, both treated *Strep. sobrinus* and *Strep. mutans* bacterial cells showed distinct surface alternations of formation of cell membrane blebs (Fig.3b, d) and (Fig.4b), cell membrane damage/rupture (Fig.3f) and (Fig.4f), cell membrane clumping (Fig.3f), intracellular material leakage (Fig.4b), wrinkled and rough cell membrane (Fig.3f). Furthermore, the bacterium-to-bacterium contact area appeared flattened and wider in the treated *Strep. mutans* (Fig.4b). Thus, the observed morphological alternations in both bacteria appear to be related to the damage in cell wall and cell membrane.

## Discussion

Vitamin D deficiency has been linked to the aetiology of many chronic diseases such as respiratory infections (22), asthma, allergic diseases (23), rheumatoid arthritis (24). Vitamin D supplements in asthmatic patients is associated with reduction of bacterial respiratory infections including *H. influenzae, S. pneumoniae, beta-haemolytic Streptococcus spp., S. aureus,* and *Chlamydia pneumoniae* (22).

Earlier studies have shown that young children and adults who had low serum vitamin D had higher dental caries occurrence compared to individuals with adequate serum vitamin D levels (3, 6). Vitamin D supplementation was associated with a 47% reduced risk of caries (25). In addition, serum vitamin D levels above 30–40 ng/mL may significantly reduce the risk of dental caries (26). It is unclear whether the circulating hormone vitamin D has exerted a direct antibacterial activity against oral bacteria that causes dental caries, or this is based on the findings that vitamin D regulates calcium and phosphate homeostasis that is essential for calcification, mineralization and maintenance of hard tissue, oral bone and teeth (2), or the fact that vitamin D regulates the expression of endogenous antimicrobial peptides which are human cathelicidin (LL-37) and defensins that have broad spectrum antimicrobial activities against many bacteria (8, 26). The results of this study showed that cholecalciferol vitamin $D_3$ was able to inhibit the normal growth of *Strep. sobrinus* and *Strep. mutans* and altered their normal cell morphology. Therefore, it suggests that cholecalciferol vitamin $D_3$ has a direct antibacterial action against these bacteria, which is totally different from its hormonal effects.

The antibacterial susceptibility of vitamin $D_3$ was investigated using the disc agar diffusion method. This method is one of the popular methods used to determine the antimicrobial effects of an agent (27). However, this test can be considered for materials which are soluble and capable of diffusing into the surrounding environment (28). This may explain why there was no zone of inhibition (ZOI) in the present study. It appears that the insolubility of the cholecalciferol vitamin $D_3$ may have hindered its diffusion to the surrounding agar surface and the inhibition zone.

In recent years, there was increasing attention towards the sunshine vitamin. Few studies had reported the antibacterial activities of vitamin D analogues including vitamin $D_3$ products against different bacteria including *Mycobacteria* (8), *Helicobacter pylori* (9) and *Streptococcus mutans* (10, 11). Varied MIC values were reported from previous studies depending on the applied methods, vitamin D compounds used and bacteria species. Hosoda and colleagues (9) have found that vitamin $D_3$ species (vitamin $D_3$; 25-hydroxyvitamin $D_3$; 1α,25-Dihydroxyvitamin $D_3$) at 5 µM concentration reduced the colony forming unit (CFU) count and exhibited the antibacterial action against *H. pylori*. A recent study found that the MIC for 1,25(OH)2$D_3$ ranging from 3.125 to 6.25 µg/mL inhibited the growth of oral *Porphyromonas gingivalis* (10). Another study indicated that 1,25(OH)2$D_3$ showed inhibition activities against *S. mutans* ATCC 35668 at MIC of 200 µg/mL, while MBC was > 400 µg/mL (10). In addition, a study by Saputo *et al*. (11) has determined the antibacterial activities of three vitamin D compounds, namely alfacalcidol, doxercalciferol, and calcitriol against *Strep. mutans*. They have concluded that vitamin D derivatives possess lytic activity against *Strep. mutans* at MIC of 16 µg/mL (11). In addition, the minimum biofilm inhibitory concentration of doxercalciferol and alfacalcidol was 64 µg/mL and 128 µg/mL, respectively; however, no biofilm formation inhibition was detected using calcitriol at any of these concentrations (11).

Both *Strep. sobrinus* and *Strep. mutans* are considered the most cariogenic bacteria causing dental caries; they are equally virulent in causing dental caries (12). Currently, chlorhexidine is considered the most effective oral antimicrobial agent due to its broad-spectrum action against Gram positive and Gram negative bacteria (29). Research has found that *Strep. sobrinus* has a higher resistance to chlorhexidine compared to *Strep. mutans*, and it may reappear earlier in saliva and plaque at higher levels than *Strep. mutans* after the application of chlorhexidine (30). However, in this study, we found that *Strep. sobrinus* is more sensitive to vitamin $D_3$ compared to *Strep. mutans,* as the MIC and MBC values of vitamin $D_3$ against *Strep. sobrinus* were lower than *Strep.*

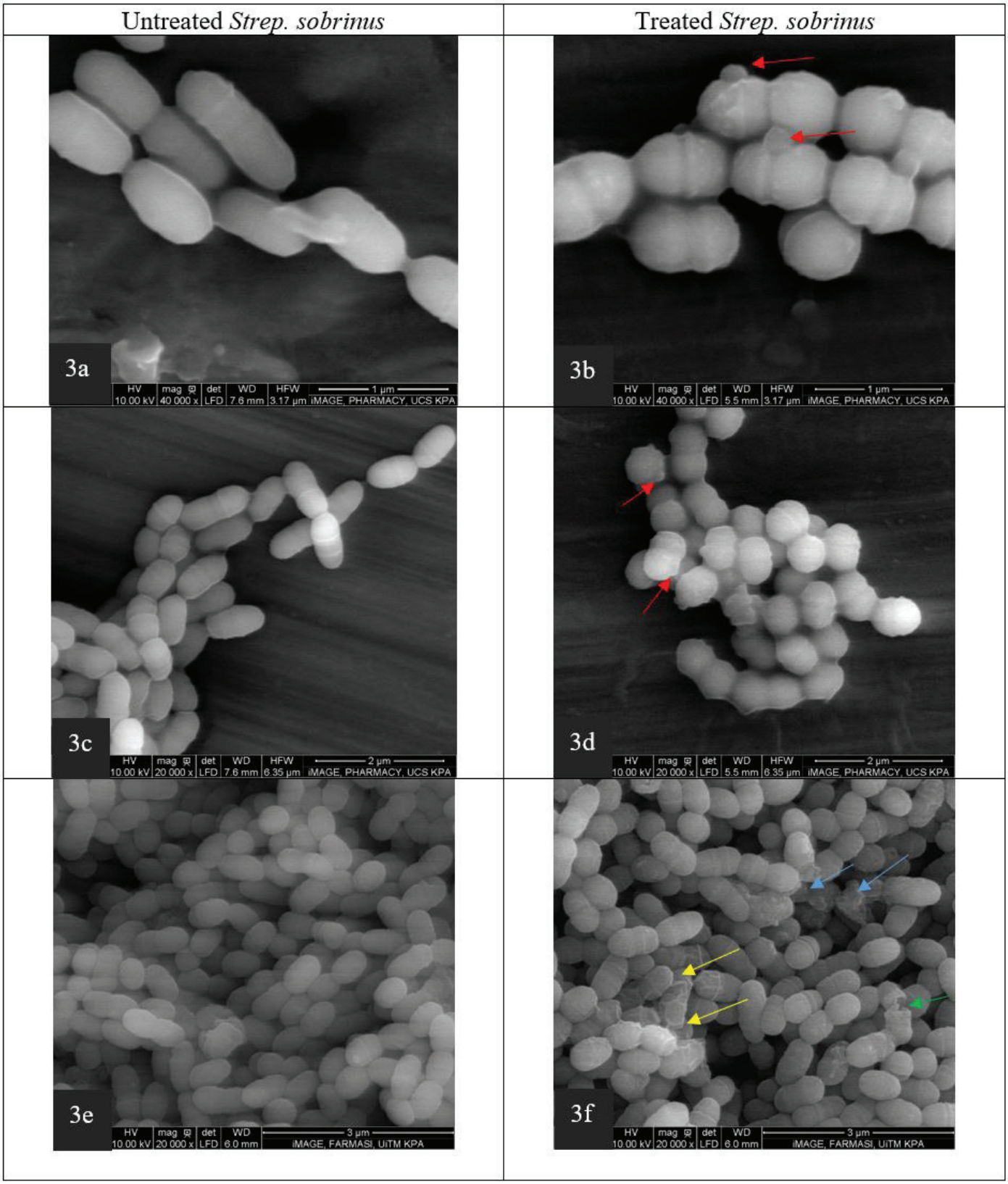


***Figure 3.*** *Scanning electron microscope of untreated Strep. sobrinus (3a,c,e). Strep. sobrinus treated with vitamin $D_3$ at MIC (3b,d,f) showing the formation of cell membrane blebs (red arrows) (3b,d), cell membrane damage/ruptured (green arrow) (3f), membrane clumping (blue arrows) (3f), wrinkled and rough cell membrane (yellow arrows) (3f).*

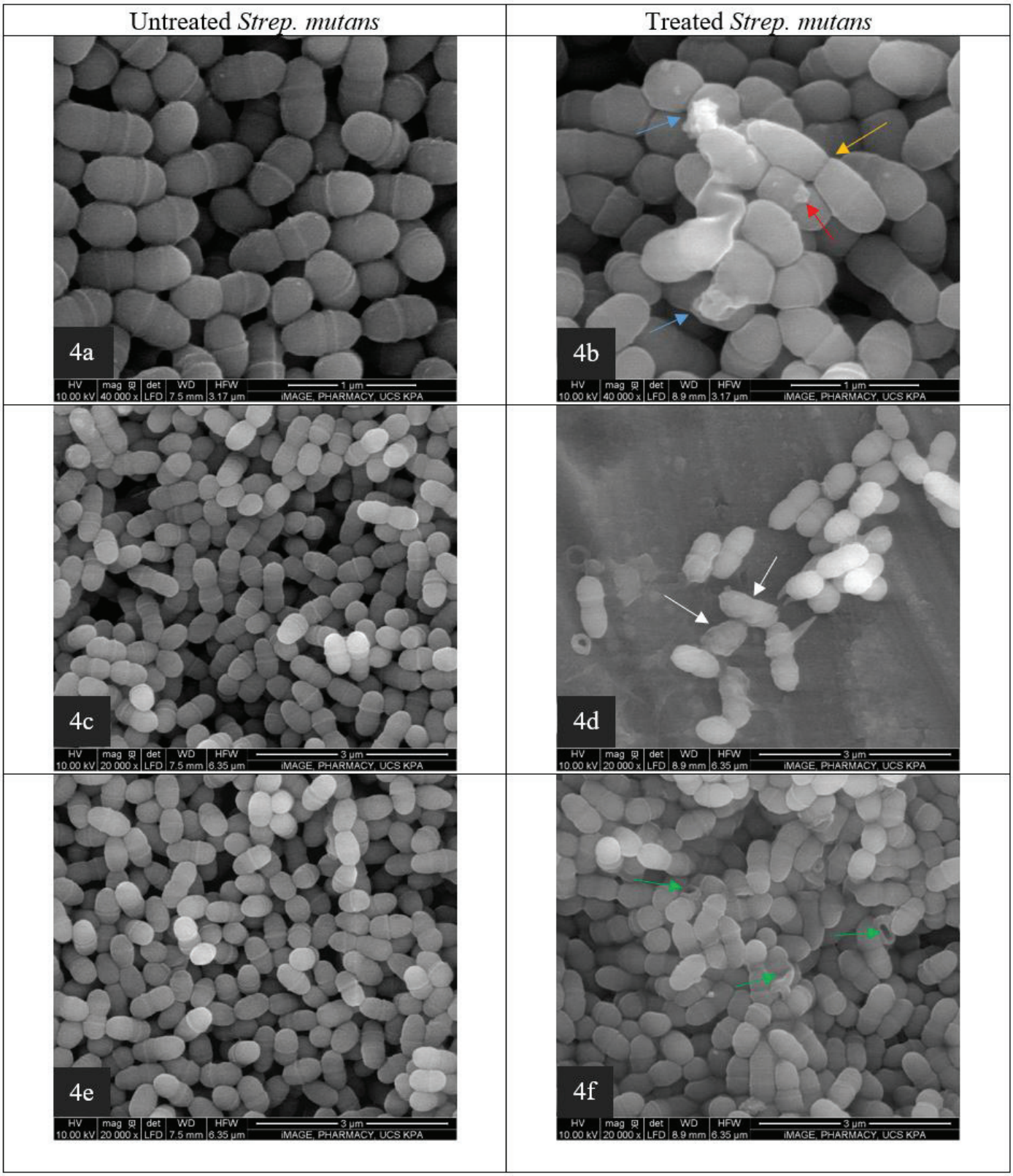


***Figure 4.*** *Scanning electron microscope of untreated Strep. mutans (4a,c,e). Strep. mutans treated with vitamin $D_3$ at MIC (4b,d,f) showing the formation of cell membrane blebs (red arrow) (4b), intracellular materials leakage (blue arrows) (4b), and the bacterium-to-bacterium contact area appeared flattened and wider (orange arrow) (4b). Bacterial cell distortion (white arrows) (4d) and cell membrane damage/ruptured (green arrows) (4f).*

*mutans*. Therefore, our findings indicate that vitamin $D_3$ has a potential promising antibacterial effect against cariogenic bacteria, mainly *Strep. sobrinus*.

Moreover, due to the absence of studies that evaluated the antibacterial activities of vitamin $D_3$ against oral bacteria, we were unable to compare our MIC and MBC values against the tested bacteria.

The microbial cell wall serves as a selective environmental barrier and contains determinants required for bacterial colonization and survival (31). The first barrier that an antimicrobial agent must overcome when interacting with its target is the bacterial cell wall (32). It was indicated that Gram positive bacteria were less sensitive to antibacterial agents compared to Gram negative bacteria because of the presence of a thicker peptidoglycan layer which acts as an additional barrier for the entry of antimicrobial agents inside the bacterial cells (33). From SEM results, it was demonstrated that treatment of *Strep. sobrinus* and *Strep. mutans* with cholecalciferol vitamin $D_3$ exhibited considerable morphological changes. Treated *Strep. sobrinus* cells appeared shorter compared to untreated cells (Fig.3b, d). It appeared that vitamin $D_3$ may impede the growth of *Strep. sobrinus*. Bacteria that grow in the presence of a compound which has antibacterial properties may experience environmental stress that could influence its ability to use nutrients efficiently and thereby slow down its normal growth (34). Morphological changes such as formation of blebs, wrinkled surfaces and cellular membrane damages were observed in the present study and the membrane damages are considered a key factor in the inactivation of bacteria (35). Such morphological changes in the surfaces of bacterial cells following the treatment with antimicrobial agent have been previously reported (35, 36, 37) and the results of the present study were consistent with them.

The SEM analysis in the present study proposed a possible mechanism for the antibacterial action of vitamin $D_3$. Vitamin $D_3$ attaches to the treated bacterial cell wall through interactions with the peptidoglycan of this Gram positive strain. The adherence of cholecalciferol vitamin $D_3$ to the cell wall caused disruption to the bacterial cell wall and membrane, making them shrink, become rough and increase the internal cellular pressure causing bleb-like formation and eventually causing cell membrane rupture and bacteria damage. Based on SEM findings, it is evident that vitamin $D_3$ is considered a membrane-active agent and is toxic to these oral bacteria and therefore affecting its normal growth.

To our knowledge this is the first study assessing the antibacterial activity of cholecalciferol vitamin $D_3$ against *Strep. sobrinus* and *Strep. mutans* bacteria in vitro. Cholecalciferol vitamin $D_3$ exhibited MIC and MBC as well as clear morphological alternations on both bacteria even though the exact mechanism by which vitamin $D_3$ inhibited *Strep. sobrinus* and *Strep. mutans* growth remains to be discovered. More studies to evaluate its effects on the bacterial membrane ultrastructure need to be considered.

## Conclusion

The findings of this study suggest that vitamin $D_3$ has a direct antimicrobial effect against mutans streptococci bacteria in vitro. It appears that vitamin $D_3$ is a membrane-active agent that affects bacterial cell wall and causes membrane disruption. It significantly altered the cellular structure of both the *Strep. sobrinus* and *Strep. mutans* cell walls and obviously hindered the normal growth of these bacteria. Therefore, vitamin $D_3$ could be considered as a promising compound that may be used in caries prevention. Further research is recommended to explicate the mechanism of antibacterial activity of vitamin $D_3$ on cariogenic oral bacteria.

***Türkçe Özet:*** *Vitamin D3'ün Mutans Streptokoklara Karşı Antibakteriyel Etkileri: Bir in vitro çalışma. Amaç: Bu çalışma, kolekalsiferol vitamin D3'ün diş çürüğü oluşumunda ana etken bakteri olarak kabul edilen Streptococcus sobrinus (Strep. Sobrinus) ve Streptococcus mutans (Strep. Mutans)'a karşı antimikrobiyal etkilerini in vitro olarak değerlendirmeyi amaçlamaktadır. Materyal ve Metod: Vitamin D3'ün Strep. sobrinus ve Strep. mutans'a karşı antimikrobiyal etkileri, agar disk difüzyon yöntemi kullanılarak değerlendirildi. Vitamin D3'ün minimum inhibitör konsantrasyonu (MIC) ve minimum bakterisit konsantrasyonu (MBC), Klinik Laboratuvar Standartları Enstitüsü (CLSI) yönergelerine göre mikrodilüsyon yöntemi kullanılarak belirlendi. Vitamin D3 uygulanmasını takiben bakteri hücrelerinin morfolojik değişikliklerini değerlendirmek için taramalı elektron mikroskobu (SEM) kullanıldı. Bulgular: Strep. sobrinus'un, Strep.mutans bakterilerine kıyasla vitamin D3'e daha duyarlı olduğu belirlendi. Vitamin D3'ün bakterilere karşı MIC değerleri; Strep. sobrinus ve Strep. mutans için sırasıyla 60 µg/mL ve 250 µg / mL iken MBC değerleri ise sırasıyla 120 µg/mL ve 500 µg/mL idi. Ayrıca vitamin D3 ile tedavi edilmiş bakteri hücrelerinin bakteriyel morfolojisinde, tedavi edilmemiş kontrol grubu bakterilerine kıyasla, SEM kullanılarak önemli değişiklikler gözlendi. Sonuç: Bu bulgular, vitamin D3'ün Strep. sobrinus ve Strep. mutans'a karşı üstün antimikrobiyal etkilere sahip olduğunu ve gelecekte diş çürüklerinin önlenmesinde umut verici bir ajan olarak düşünülebileceğini ileri sürdü. Vitamin D3'ün, bu bakteriler üzerindeki mekanizmasını aydınlatmak için daha fazla araştırma yapılması önerilmektedir. Anahtar Kelimeler:Vitamin D3, kolekalsiferol, Streptococcus sobrinus, Streptococcus mutans, antibakteriyel etki*

**Ethics Committee Approval:** Not required.

**Informed Consent:** Not required.

**Peer-review:** Externally peer-reviewed.

**Author contributions:** MMMA, ASH and HAT designed the study. MMMA and ASH participated in generating the data for the study. MMMA, SABN and NAEBE participated in gathering the data for the study. MMMA, HAT, SABN and NAEBE participated in the analysis of the data. MMMA and ASH wrote the majority of the original draft of the paper. MIAH, HAT and HBSGK participated in writing the paper. All authors approved the final version of this paper.

**Conflict of Interest:** Authors declared no conflict of interest.

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