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The Relationship between Oral Bacterial Biofilm and Titanium Dioxide Nanoparticles (TiO_2 NPs): A New Approach to Dental Caries Prevention/Review

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Annotation: Dental caries remains one of the most prevalent chronic diseases worldwide, primarily driven by biofilm-forming bacteria oral bacteria. These microorganisms exhibit strong resistance to conventional antimicrobial agents due to their capacity to form complex biofilm matrices, which hinder antibiotic penetration and promote horizontal gene transfer of resistance traits. Recent advances in nanotechnology have introduced titanium dioxide nanoparticles (TiO_2NPs) as promising antimicrobial agents with unique physicochemical properties, including high surface-to-volume ratio, photo catalytic activity, and the ability to generate reactive oxygen species (ROS). This study investigates the antimicrobial potential of TiO_2NPs against bacterial isolates from human dental caries. Characterization of the synthesized TiO_2NPs was performed using field emission scanning electron microscopy

(FE-SEM) and X-ray diffraction (XRD), confirming Nano scale crystalline structures with average particle sizes between 42–50 nm. Experimental findings demonstrated that TiO_2NPs significantly inhibited bacterial growth and disrupted biofilm formation through ROS-mediated membrane damage, protein and DNA degradation, and interference with extracellular polymeric substance (EPS) synthesis. Furthermore, TiO_2NPs treatment reduced acidogenicity within the biofilm microenvironment, mitigating enamel demineralization. These results highlight TiO_2NPs as an effective nanomaterial with considerable potential for dental applications, including antimicrobial coatings and preventive strategies against biofilm-associated oral diseases.

Keywords: Titanium dioxide nanoparticles, oral bacteria, dental caries, biofilm, antimicrobial activity.

Introduction

The human oral cavity's incredibly complex and rich salivary environment offers a specially designed home for over 700 species of commensal (aerobic/anaerobic) bacteria (1) colonize the oral cavity and create biofilms to guarantee their continued existence. Furthermore, well-known biofilm per sisters such as lactobacilli and streptococci coexist as mutual symbioses in biofilms (2). Furthermore, it's frequently hypothesized that the oral micro biota—which includes viruses, yeasts, and bacteria (2). Encourage the production of proteins, nucleic acids, and polymeric substances heterogeneous extracellular (EPS) to aid in the creation of biofilms (3). Bacterial adhesion and subsequent biofilm production are the causes of the two most common dental illnesses, periodontitis and caries. By developing drug resistance, multilayered bacterial biofilm matrices are essential for counteracting the antimicrobial actions of different chemical agents (4). Compared to planktonic cells, which can occasionally be more than 1000 time stronger against several antibiotics (4). Up to 60–90% of people suffer from periodontal disease and tooth decay as a result of the digestion of dietary sucrose and carbohydrates, which produces a highly acidic milieu on tooth surfaces during carcinogenic biofilm buildup (5). The clinical management of oral biofilms and related issues have serious negative economic effects as well. For instance, in the US, the total cost of treating illnesses linked to oral biofilms has been calculated to be over USD 81 billion annually (6) Because of their potential antibacterial and anti- adhesive properties, a range of metal nanoparticles (NPs are currently being investigated extensively for the clinical therapy of biofilm-induced carcinogenesis. The metal oxides under investigation include Nano scale Due to

the generation of reactive oxygen species (ROS), disruption/penetrating of cell membranes, glutathione depletion, and harmful oxidative stress amplifying effects, titanium dioxide (TiO_2NPs) has a well-established antibacterial impact (9).

Furthermore, although perspectives on inflammation caused by(10). Additionally, TiO_2NPs are typically applied at low concentrations and are generally regarded as biocompatible, while reactions to inflammation caused by cytokine release are controversial (11). A wide variety of cell types, including bacteria, fungi, and mammalian cells, can interact with TiO_2NPs more effectively than their micro/bulk-sized counterparts because of their higher surface-to-volume ratios(12). Novel persistent biofilms have long been thought to thrive in the oral cavity because of its open and dynamic nature, as well as the presence of very complex mixes of biofilm microorganisms caused by host sensitivity and poor oral hygiene(13).Novel persistent biofilms have long been thought to thrive in the oral cavity because of its open and dynamic nature, as well as the presence of very complex mixes of biofilm microorganisms caused by host sensitivity and poor oral hygiene(14). Periodontitis, an inflammatory condition, is caused by biochemical conditions that encourage the growth of harmful bacteria in the oral cavity. This condition can also increase the risk of other systemic diseases, including endocarditis and colorectal cancer (15). Thus, elucidating the role of microbial communities in human health and systemic diseases is both urgent and crucial (16).

The development of oral bacterial resistance to conventional antibiotics, which is not exclusive to human patients undergoing antibiotic treatments, further complicates the issue (17).

Stages of Oral Biofilm Formation Antoine Van Leeuwenhoek's enormous contribution to the invention and realization of microbiology and biofilm will always be remembered in their annals. He attempted to examine plaque from his own teeth using his crude light microscope (18), and he saw germs that darted before his eyes moved. He eventually named them animalcules, which at the time meant "little animals." Since then, his research and experiment have revolutionized the field of oral biofilms and opened the door to a deeper comprehension of microbial biofilms. interactions and factors pertaining to bacteria, surfaces, and nutrients. Bacteria generate biofilm in a similar manner regardless of the ecosystem they live in, according to Donlan and Costerton on Bacterial, surface, and nutrient-related variables and interactions combine to generate biofilms on various biotic or abiotic surfaces in a dynamic and sequential process. Regardless of the environment they live in, bacteria create biofilm in a similar manner, according to Donlan and Costerton. (19).

On the surfaces of mucosa and teeth, oral biofilms are physiologically and structurally ordered collections of microbial populations embedded in an extracellular matrix of exopolymers. (20). One way to represent the life of an oral biofilm is as a developing cycle. Thus, the process of biofilm formation in the oral cavity involves cyclical stages that necessitate: a) Planktonic bacteria can attach themselves reversibly to conditioned solid surfaces, like the surfaces of teeth; b) an exopolysaccharide matrix that resembles glue is synthesized; c) permanently of the cells that attached to the surface; d) biofilm structure of the matured that production ; e) ordered structure dispersion ; and f) new habitats are looked . (21,22). The protein known as acquired enamel pellicle (AEP) has the ability to erode and create "caves" on tooth surfaces. Bacteria such as streptococci that are linked to acid genic enamel convert dietary carbohydrates into organic acids in tooth caries. When the pH of the tooth surface is low, the enamel demineralizes and develops cavities, which can subsequently spread into the dentin. Thus, more aciduric species, such as Streptococcus mutans, are selected for by repeated acidification, further lowering the pH. Carious lesions arise when the environment's constant acidity upsets the mineral balance of the exposed tooth structures, such as the enamel and/or dentin (23). Acidic environment also imposes environmental stress on the microbial community causing acid-sensitive species to perish and acid uric micro biota to flourish(24). Figure1 An illustration of how bacterial biofilms grow on the surfaces of teeth: Microbial cells first deposit onto a conditioned tooth surface (pellicle), then they attach to the substratum reversibly, colonize and attach irreversibly, aggregate and expand,

mature, and finally disperse and detach under controlled conditions. Microbes secrete an extracellular polymeric material (EPS) that resembles cement and is crucial for adhesion. When different proteins obtained from oral fluid selectively stick to tooth enamel surfaces, EPS works as a biological glue that improves the adherence of films with distinct composition and characteristics. (25).

As a result, when one microorganism is present, it makes ecological niches for other microorganisms, which helps them survive in the new, favorable environment. Microbes create many metabolic products, including acids, on the surfaces of teeth in addition to adhering and growing (Figure 1 and Figure 2). Excavator considered by oral cavity through the acid produced of oral cavity 26).

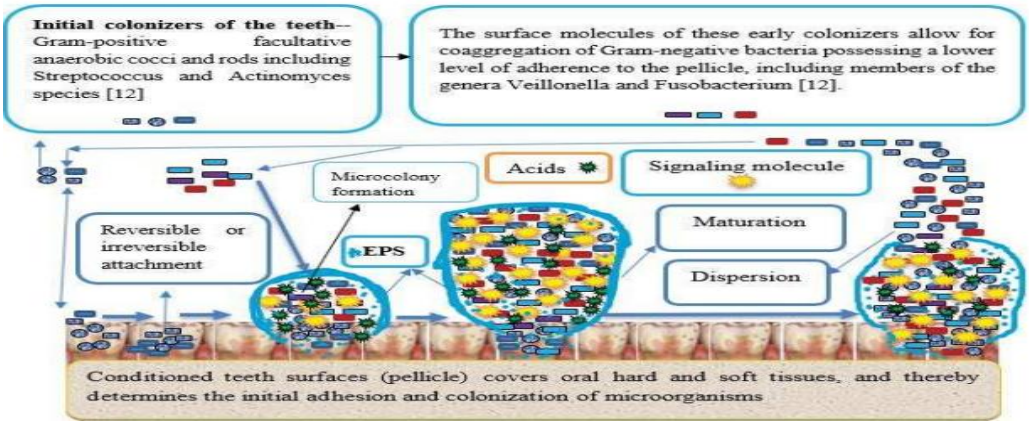

Figure 1: shown the surfaces of teeth with formation of bacterial biofilm

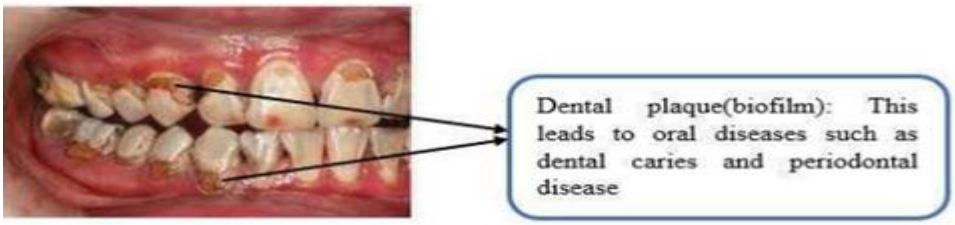

Figure 2: Dental plaque formation

Resistance Mechanism of Biofilm

Bacteria that grow in biofilms have a very high level of antibiotic resistance. Because of their stable structural features and the close proximity of the cells of bacteria within the biofilm, which creates an ideal environment for horizontal gene transfer, antibiotic-resistant genes may proliferate among the biofilm's inhabitants. The subsequent physiological and structural traits of bacteria that form biofilms contribute to their progressive development of antibiotic resistance, according to the available literature (27)**.**

Capsule

An essential part of the biofilm is the protective capsule, which can vary overall thickness between 0.2 to 1.0 μm for Gram-positive as well as Gram-negative bacteria. The capsule promotes biofilm adhesion and cohesion to a solid surface by utilizing Van der Waal, bonds of hydrogen, and electrostatic charges, which in turn promotes biofilm growth. The biofilm capsule is composed of carbohydrates and glycoproteins, substances that are affected by different environmental conditions. antibiotics that the bacteria create. The MDR bacteria's enzymes called exoenzymes break down antimicrobial drugs that are trapped in the adsorption sites of the glycocalyx matrix. Antimicrobial drugs inevitably become ineffective against the microorganisms in the biofilm. (28,29)

Plasmids/Enzyme-mediated Resistance

Bacteria that produce biofilms often contain extra chromosomal genetic units known as plasmids. Plasmids usually contain genes that are encoded for increased virulence through proteins and enzymes, leading to resistance to a range of contaminants and antibiotics. Occasionally, these plasmids have many genes for resistance that are especially resistant to most commonly used antibiotics, such as aminoglycosides, beta-lactams, fluoroquinolones, and macrolides. These genes are usually rearranged by an intrinsic recombination mechanism, like that present in integrons and transposons. The closely packed oral biofilm makes it easier for these resistant genes to spread horizontally because all of the plasmids involved are conjugative. Every bacterium eventually becomes multidrug resistant (30).

Management of Oral Biofilm

Consider the following methods for managing and eliminating oral biofilms effectively.

Antimicrobial Material

Since the majority of oral illnesses start with bacterial adhesion and biofilm formation, antimicrobial materials are essential. Numerous attempts have been undertaken to develop dental materials with antimicrobial qualities, with the goals of contact killing, antimicrobial agent release, and multifunctionality (31)

Antiplaque/Antimicrobials

Surfactants like sodium lauryl sulphate and essential oils like clove oil and eugenol have proven to be good antiplaque agents. To regulate and eliminate oral biofilms and dental plaque, antimicrobial agents such bisbiguanides, metal ions, phenols, and quaternary ammonium compounds have been effectively added to toothpaste and mouthwash, coupled with antiplaque agents..(32)

Multifunctional Mechanisms

Nanoparticles

The use of nanoparticles to remove oral biofilm has great potential. With their strong antibacterial properties, nanoparticles can be utilized to target particular microorganisms that create biofilms without interfering with the oral cavity's regular microflora. Nevertheless, their use is costly and cannot be routinely carried out in dental offices. Nanoparticle administration is extremely precise and can have serious adverse consequences. Additionally, in order to get the intended outcomes, the biomimetic characteristics of nanoparticles must be extremely accurate and target-specific(33).

TiO_2 Nanoparticles

Transition metal oxide nanomaterials have been widely employed for potential applications in applicable science and technology due to their special qualities, such as a large specific area, chemical resistance, and electrical activity at the nanoscale. Due to their exceptional performance in solar energy cells, medical equipment, quantum dots as sensors, photocatalysis, UV protection, and antimicrobial (dental caries represents one of the most common chronic diseases in the world), TiO2 and other transition-year nanoparticles made from metal oxide have been thoroughly studied in recent years (34). Bacillus circulans, Pseudomonas., and Peptostreptocci are the pathogens that induce decalcification of enamel, dentin, and cement as well as the breakdown of organic materials. The current study used TiO2NPs nanoparticles, which have high antibacterial efficacy against a wider range of bacterial strains, because microorganisms are becoming resistant to most antimicrobial treatments (35).

One of the most crucial aspects of creating such nanoparticles is controlling their size, shape, and crystallinity. Several synthesis techniques have been developed to accomplish this; some of the most researched ones include the ultrasound-chemical process, sol-gel technique, the use of lasers, the electrochemical method, precipitation using chemicals, and treatment with surfactants (36).

The three main crystalline forms of titanium dioxide (TiO2)—rutile, anatase, and brookite—as well as other structures like cotinine, which has been produced at high pressure, are seen in Figure 1. While anatase and brookite respectively are metastable substances and can be heated to become rutile, rutile constitutes the stable form. The most common step in the synthesis of TiO2 sol-gel is anatase.

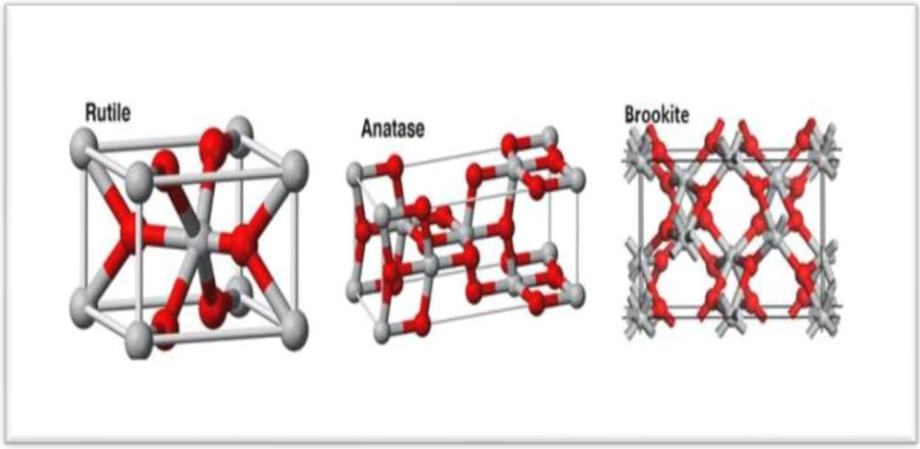

Figure 1. TiO_2 structures: Rutile, Anatase, and Brookite.

The Field Emission Scanning Electron Microscope (FE-SEM)

TiO2 nanoparticles have a homogenous, regular cubic, nano-sheets morphologies and nanoparticles sizes vary from 40-70 nm, according to a field scanning electron microscopy (FESEM) of TiO_2 / PVP shown in(figure 2), and the average size of the nanoparticles is around 42-50.97 nm

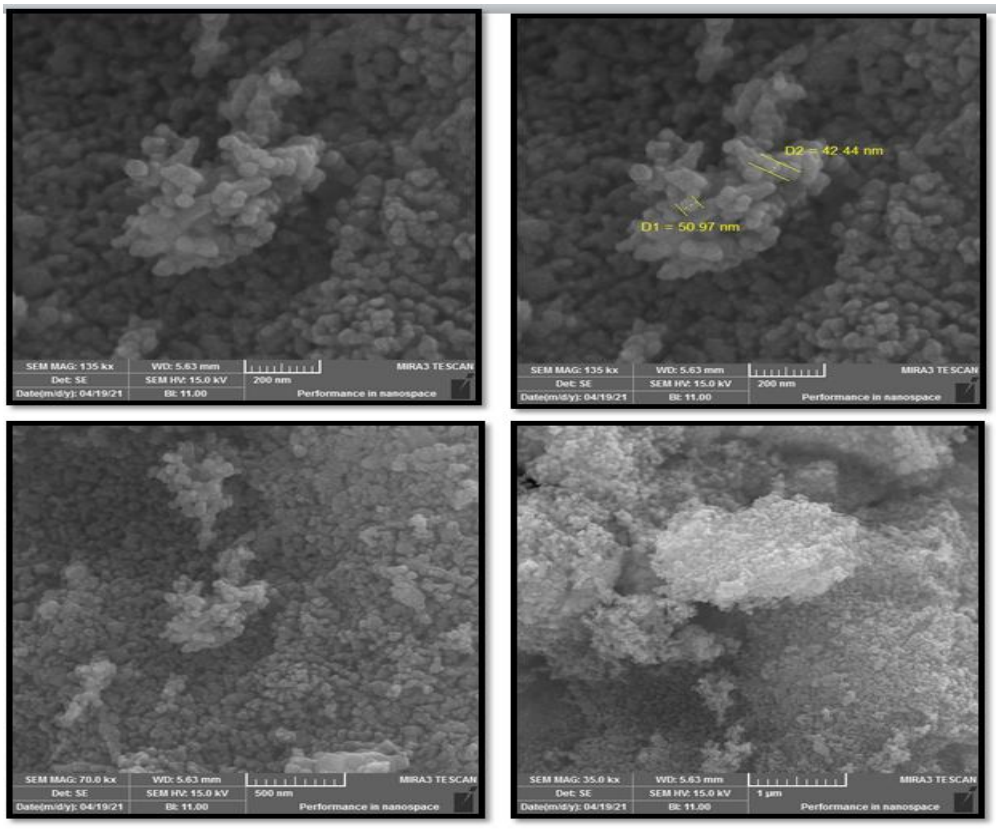

Figure 2. FE-SEM images of TiO_2 NPs

X-Ray Diffraction Analysis

Inorganic materials' phase purity and crystallinity are assessed using XRD (38). (Figure 3) shows the XRD spectrum of TiO_2 NPs electrochemically generated in a cell solution (200 mL) containing 10 mL PVP (10 g/100 mL) as a stabilizer and 10 mL KCl (10 g/100 mL) as an electrolyte at room temperature with a voltage range of (10-30) volts and a current density of 80 mA/cm2. The produced TiO_2 NPs were analyzed using a shimadzu 6000 diffract meter fitted with a Cu-Kα1 of (1.540598 A) under 50 kV and 40 mA in the range of 2 theta from 10 to 80. The XRD pattern revealed a wide(39).

The XRD patterns of TiO_2 indicate that all diffraction peaks correlate well with the TiO_2 standard diffraction data (JCPDS) (40). The XRD data of samples obtained with PLAL matched the standard anatase pattern (JCPDS No.: 00-021-1272), according to standard diffraction data during the same assay. The XRD diffractions peaks around 2θ = 25.41, 37.94 °, 48.05 °, 54.13 °, 55.39 °,70.24 ° and 75.14 ° which correspond to the planes (101), (004), (200), (105), (211), (220) and (215). These reflections peaks correspond to the anatase phase of TiO_2 according to (JCPDS 21-1272) (41,42) .This phase has a tetragonal structure (43).

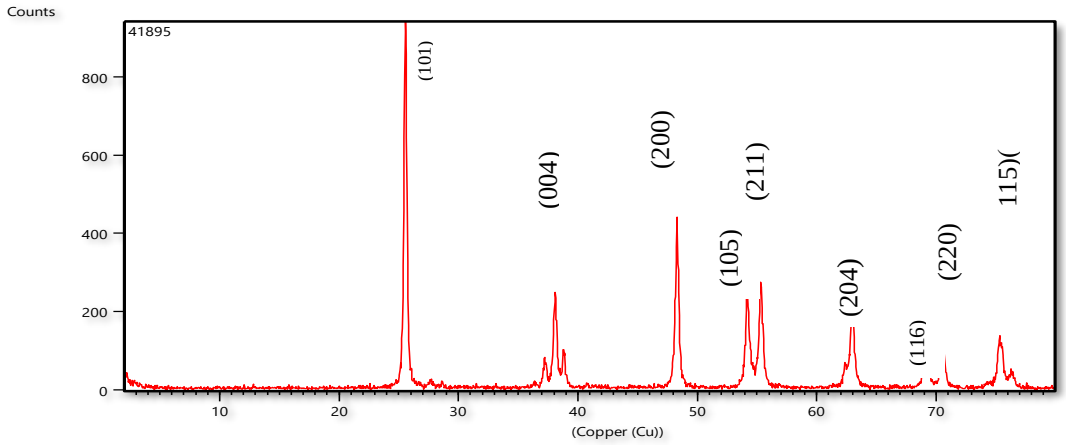

Figure 3. X-ray diffraction pattern of TiO_2 NPs.

Mechanisms: How TiO_2 Affects *Streptococcus mutans* (44)

1-Light activation (hν)

✓ UV or strong visible light strikes TiO_2 nanoparticles (often anatase or anatase-rich mixes).

2-Charge separation

✓ TiO_2 → generates electron–hole pairs (e^- in the conduction band, h^+ in the valence band).

3-ROS formation at the TiO_2 surface

✓ $e^- + O_2 \rightarrow O_2^{\bullet -} \rightarrow$ (disproportionation) $\rightarrow H_2O_2$

✓ $h^+ + H_2O / OH^- \rightarrow \bullet OH$

✓ Net result: a burst of ROS ($^{\bullet}OH$, $O_2^{\bullet -}$, H_2O_2) at the particle–biofilm interface.

4-Direct bacterial damage (oral bacteria)

✓ Membrane lipid peroxidation → pores/leakage, loss of membrane potential.

✓ Protein and DNA damage → enzyme inactivation, replication stress.

✓ Cytoplasmic leakage → rapid loss of viability.

5-Biofilm/EPS interference

- ROS and contact at the surface inhibit glucosyltransferase-driven EPS (insoluble glucans), so initial adhesion and micro colony maturation are impaired.
- Co-adhesion with other plaque bacteria is reduced; detachment increases.

6-Metabolic/cariogenic impact

- Damaged cells show lower glycolytic flux and less lactic acid output → smaller pH drop in the biofilm microenvironment → less enamel demineralization.

7-Surface effects on dental materials (if TiO_2 is a coating/filler)

- Light makes TiO_2-coated surfaces more hydrophilic, reducing bacterial sticking and making plaque easier to shear off.
- Smoother, low-energy surfaces further impede colonization.

8-In the dark (weaker but possible)

- Close contact with TiO_2 can still disrupt membranes; doped TiO_2 (e.g., Ag- or N-doped) can extend activity under room/visible light.

Outcome: Thinner, less virulent oral bacteria biofilm → reduced acidification and lower caries risk.

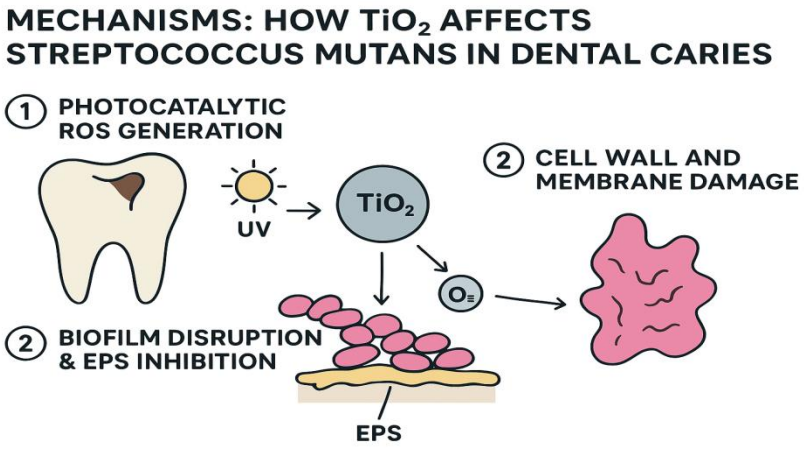

Figure 4. shown the mechanism of titanium dioxide effect on the oral bacteria in dental caries

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