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Research Paper

The Relationship between Oral Bacterial Biofilm and Titanium Dioxide Nanoparticles (TiO_2NPs): A New Approach to Dental Caries Prevention/review

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ABSTRACT

Dental caries remains one of the most prevalent chronic diseases worldwide, primarily driven by biofilm-forming 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. Characterisation of the synthesised TiO_2NPs was performed using field emission scanning electron microscopy (FE-SEM) and X-ray diffraction (XRD), confirming nanoscale crystalline structures with average particle sizes between 42 and 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 demineralisation. 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.

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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] to colonise 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 hypothesised that the oral microbiota, which includes viruses, yeasts, and bacteria [2]. Encourage the production of proteins, nucleic acids, and heterogeneous extracellular polymeric substances (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 times 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 has 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 nanoscale. Due to the generation of reactive oxygen species (ROS), disruption/penetration 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 realisation of microbiology and biofilm will always be remembered in the 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. He eventually named them animalcules, which at the time meant "little animals." Since then, his research and experiments have revolutionised the field of oral biofilms and opened the door to a deeper comprehension of microbial biofilms. Interactions and factors about bacteria, surfaces, and nutrients. Bacteria generate biofilm in a similar manner regardless of the ecosystem they live in, according to Donlan and Costerton. 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) cells are permanently attached to the surface; d) the production of a matured biofilm structure; e) the dispersion of an ordered structure; and f) looking for new habitats [21]. These stages of the cycle are determined by physical, biological and environmental factors. Generally, the formation of dental biofilm (or dental plaque) consists of several steps, which start with the formation of the acquired enamel pellicle, followed by the initial adhesion of planktonic bacteria to the pellicle layer by binding sites, subsequent maturation of the bacterial biofilm and, finally, the dispersion of biofilm with detachment of cells/clusters of cells [22]. The protein known as acquired enamel pellicle (AEP) can erode and create "caves" on tooth surfaces. Bacteria such as streptococci that are linked to acidogenic enamel convert dietary carbohydrates into organic acids in tooth caries. When the pH of the tooth surface is low, the enamel demineralises 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]. An 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, colonise 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, favourable environment. Microbes create many metabolic products, including acids, on the surfaces of teeth in addition to adhering and growing (Figures 1 and 2). Acids produced in the oral cavity can be considered as an "excavator" [26].

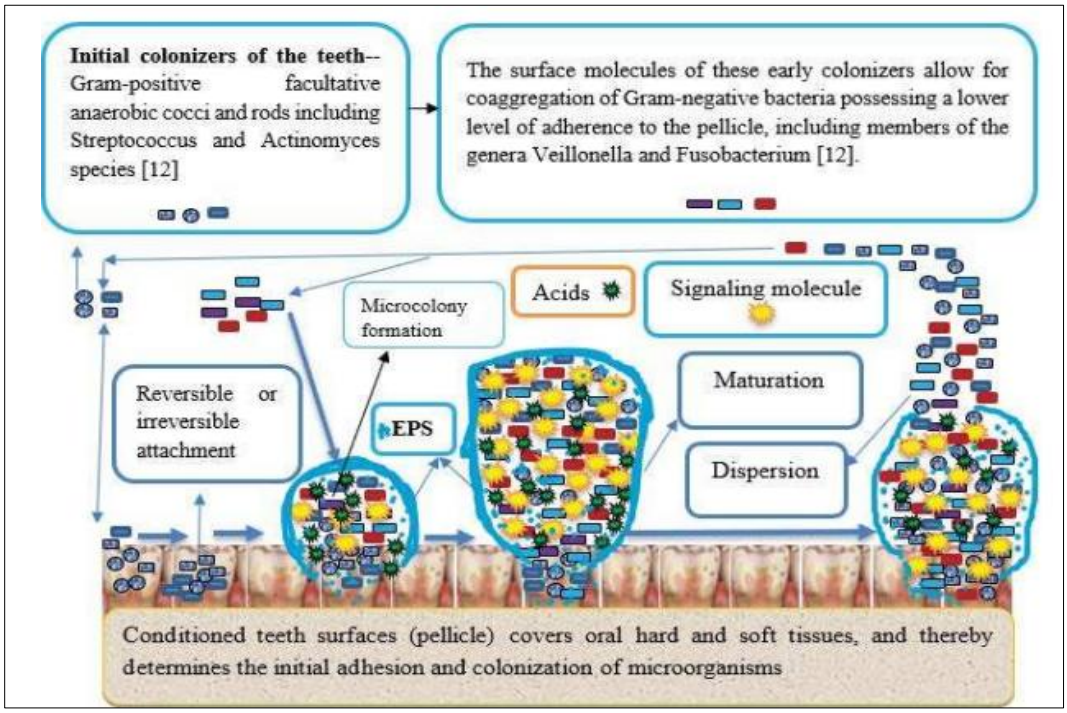

Fig 1: Schematic of the bacterial biofilm formation process on the tooth surfaces

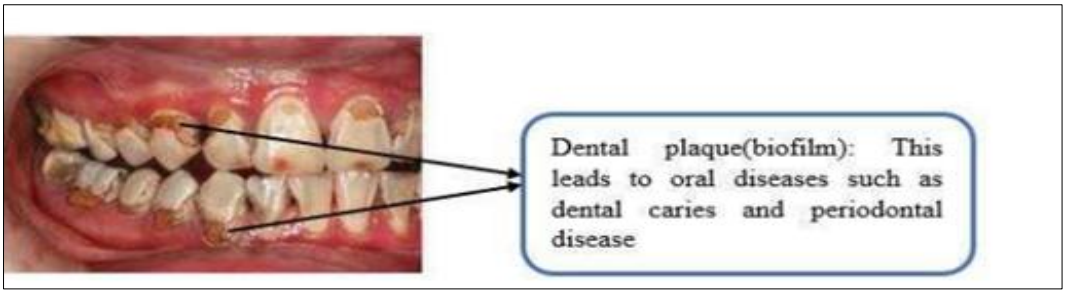

Fig 2: Dental plaque formation

Resistance Mechanism of Biofilm

Antibiotic resistance is extremely high in bacteria that live and thrive in a biofilm. Antibiotic-resistant genes may spread among the biofilm's residents due to their stable structural characteristics and the proximity of the bacterial cells within the biofilm, which provides an ideal setting for horizontal gene transfer. According to the literature that is currently accessible, the following physiological and structural characteristics of bacteria that form biofilms aid in their gradual development of antibiotic resistance [27].

Capsule

The capsule, which can range in thickness from 0.2 to 1.0 µm for both Gram-positive and Gram-negative bacteria, is a crucial component of the biofilm. Through the use of Van der Waals, hydrogen bonds, and electrostatic forces, the capsule facilitates biofilm adhesion and cohesiveness to a solid surface, hence aiding biofilm growth. Polysaccharides and glycoproteins that are impacted by various environmental circumstances make up the biofilm capsule. Antibiotics are produced by bacteria. Antimicrobial medications are entrapped in the glycocalyx matrix's adsorption sites, where the MDR bacteria's exoenzymes break them down. Antimicrobial medications so gradually lose their ability to fight the bacteria in the biofilm [28, 29].

Plasmids/Enzyme-mediated Resistance

Extra chromosomal genetic elements called plasmids are frequently present in bacteria that generate biofilms. Genes encoded for enhanced virulence through enzymes and proteins are typically found on plasmids, which results in resistance to a variety of heavy metals and antimicrobials. These plasmids occasionally contain several resistance genes that are particularly resistant to the majority of widely used antibiotics, including fluoroquinolones, beta-lactams, aminoglycosides, and macrolides. Typically, an innate recombination machinery, such as that found in integrons and transposons, helps rearrange these genes. Since all of these plasmids are conjugative, the tightly packed oral biofilm facilitates the horizontal transfer of these resistant genes. All of the bacteria gradually develop multidrug [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 as bisbiguanides, metal ions, phenols, and quaternary ammonium compounds have been effectively added to toothpaste and mouthwash, coupled with antiplaque agents [32].

Multifunctional Mechanisms

Nanoparticles

Removal of oral biofilm using nanoparticles has enormous potential. Nanoparticles have excellent antibacterial activity and can be used to target specific biofilm-forming microorganisms without disturbing the normal microflora of the oral cavity. However, their use is expensive and cannot be regularly practised in dental clinics. Administering nanoparticles requires great precision and may lead to severe side effects. Furthermore, the biomimetic properties of nanoparticles should be highly precise and target-specific to achieve the desired results [33].

TiO_2 Nanoparticles

Because of their unique properties, such as high specific surface area, chemical stability, and electrochemical activity at the Nano scale, transition metal oxide nanostructures have been extensively used for promising applications in applied science and technology. TiO2 and other transitional metal oxide nanoparticles have been studied extensively in recent years for their outstanding performance in solar cells, biomedical devices, quantum dots, sensors, photo catalysis, solar cells, UV protection [34], and antimicrobial (Dental caries is one of the most prevalent chronic diseases of people worldwide. The disease process may involve enamel, dentin and cement, causing decalcification of these tissues and disintegration of the organic substances. It is caused by Bacillus circulans, Pseudomonas spp., and Peptostreptocci. Microorganisms are gaining resistance to most of the antimicrobial agents, so the present work involved the application of TiO_2NPs nanoparticles, which have good antimicrobial activity gainst broadened spectrum of bacterial strains [35].

The control of particle size, morphology, and crystallinity is one of the most important factors in the synthesis of such nanoparticles, and numerous methods of synthesis have been established to achieve this goal; some of the most studied approaches include the sono-chemical process, sol-gel method, laser ablation, electrochemical method, chemical precipitation, and treatment with surfactants [36]. (Figure 1) shows the three major crystalline structures of titanium dioxide (TiO_2): rutile, anatase, and brookite, as well as other structures such as cotinine, which has been synthesised at high pressures: rutile (tetragonal), anatase (tetragonal), and brookite [37]. Rutile is the stable form, whereas anatase and brookite are metastable and can be converted to rutile by heating. Anatase is the step that is typically found in TiO_2 sol-gel synthesis.

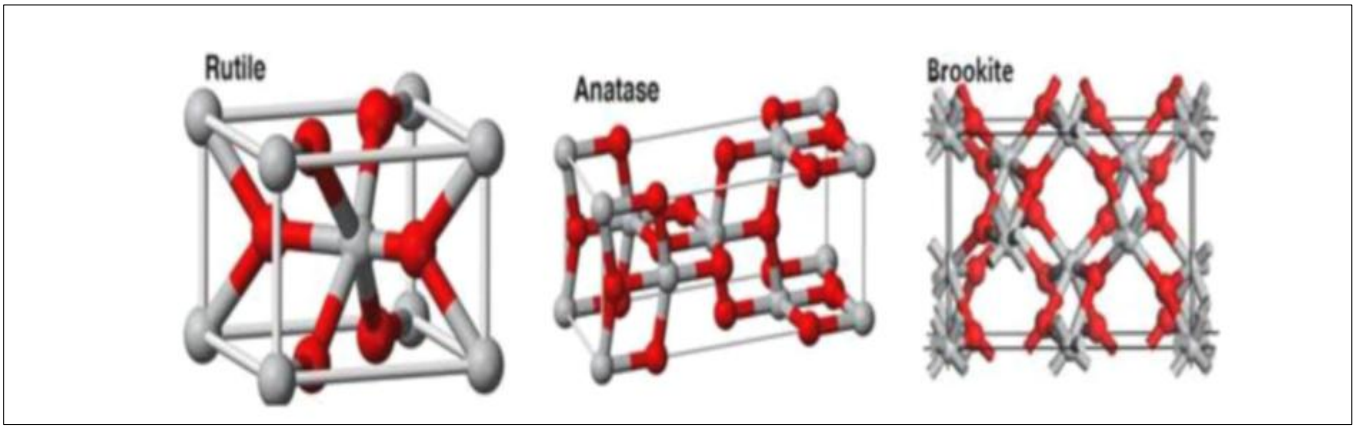

Fig 1: TiO_2 structures: Rutile, Anatase, and Brookite.

The Field Emission Scanning Electron Microscope (FE-SEM)

TiO2 nanoparticles have a homogenous, regular cubic, nano-sheets morphologies and nanoparticle 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

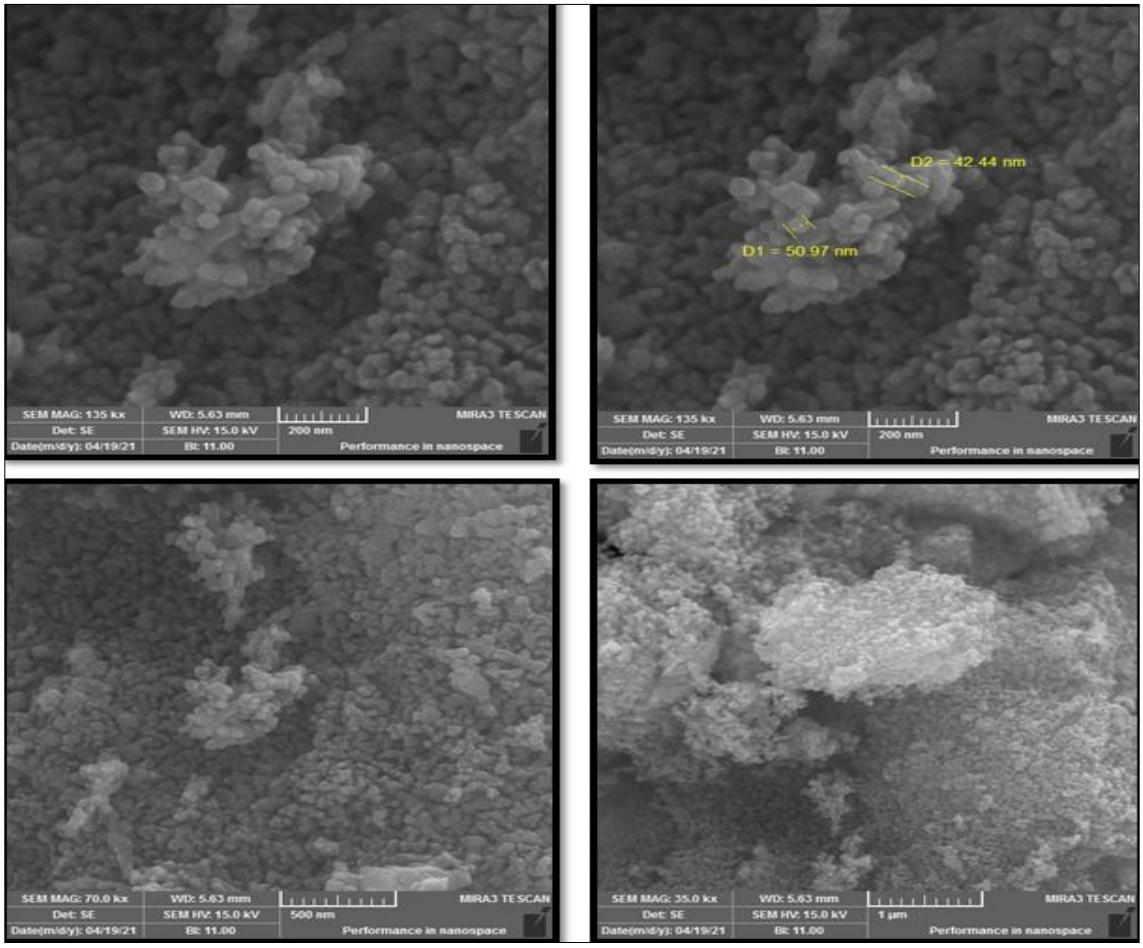

Fig 2: FE-SEM images of TiO_2 NPs

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 stabiliser 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 analysed 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 diffraction 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 correspond to the anatase phase of TiO_2 according to (JCPDS 21-1272) [41, 42]. This phase has a tetragonal structure [43].

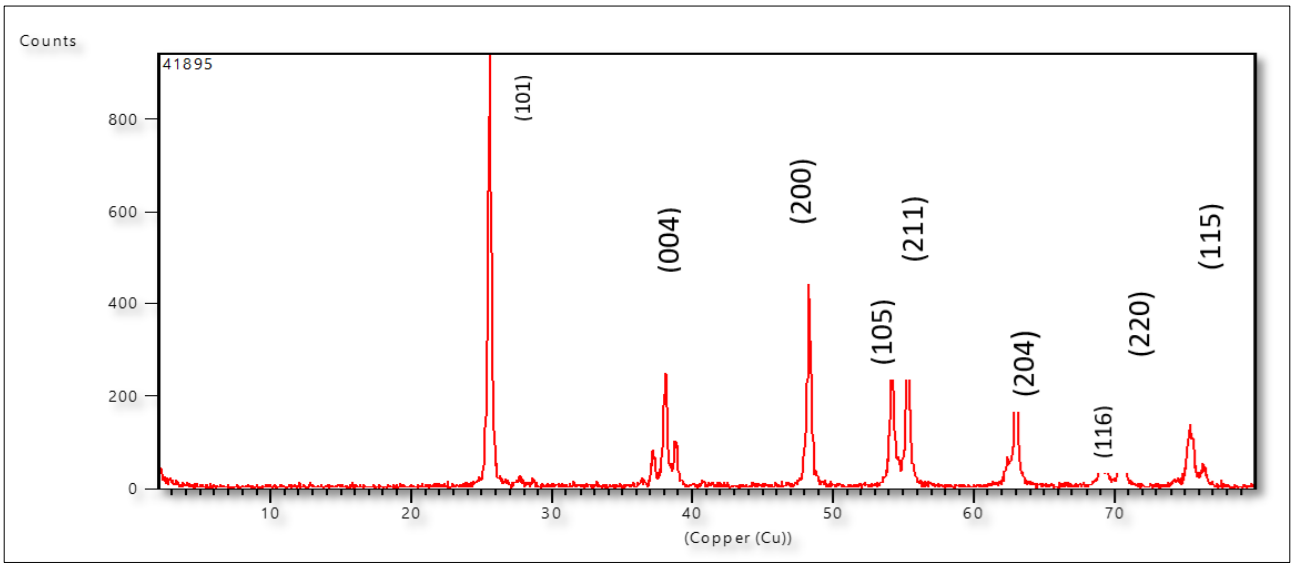

Fig 3: X-ray diffraction pattern of TiO_2 NPs.

Mechanisms: How TiO_2 Affects *Streptococcus mutans* [44]

1. **1-Light activation (hv)**
- 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. **3-ROS formation at the TiO_2 surface**
- $e^- + O_2 \rightarrow O_2^{\bullet-} \rightarrow$ (disproportionation) → H_2O_2
- $h^+ + H_2O$ / $OH^- \rightarrow$ •OH
- Net result: a burst of ROS (•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 demineralisation.
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 colonisation.
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.

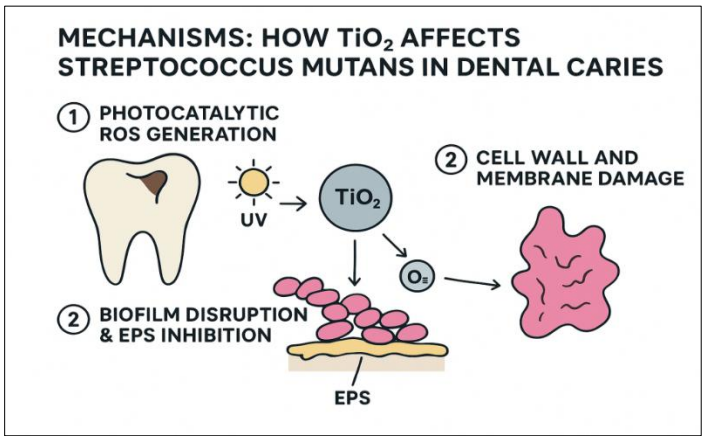

Fig 4: Shows the mechanism of titanium dioxide on the oral bacteria in dental caries

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