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# ANTIMICROBIAL ACTIVITY OF $TiO_2$ NANOPARTICLES AGAINST MICROBIAL ISOLATES CAUSING DENTAL PLAQUES

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**Abstract:** The mouth is a favorable habitat for a great variety of bacteria due to presence of nutrients, epithelial debris and secretions. Dental plaque is a soft deposit that accumulates on the teeth. The buildup of dental plaque on teeth is a normal physiologic process, occurring in both healthy mouths with cavities or gum disease. A study was carried out to isolate microorganisms from patients with dental problems. 40 samples were collected from Savitha dental clinic, Hyderabad, indicated the presence of both bacteria and fungi. The antimicrobial activity of Titanium dioxide nanoparticle was checked against all these organisms responsible for plaque formation and among these 40 dental isolates titanium dioxide nanoparticles showed antimicrobial activity against 18 isolates and comparatively high zone of inhibition against isolates like D2, D5, D14, D19, D23. The antimicrobial activity of Isopropanol as control, Titanium dioxide, and Titanium dioxide nanoparticle was comparatively analyzed and results shows that $TiO_2$ nanoparticles showed highest activity and the Minimum inhibitory concentration was found to be 15 mg /ml

**Key Words:** Antimicrobial activity, Dental plaques, Nanoparticles, Titanium dioxide.

## INTRODUCTION

Dental plaque is a biofilm which is pale yellow in colour that develops naturally on the teeth. A dental plaque is formed by colonizing bacteria trying to attach themselves to the tooth surface. Dental biofilm, more commonly referred to as dental plaque is composed of about thousand species of bacteria that take part in the complex ecosystems of the mouth [1]. Dental plaque starts when bacteria that are present in the mouth attach to teeth and begin multiplying. Plaque can form on teeth both above the gum line, where it is called supragingival plaque, or below the gum line on the roots of teeth, called sub gingival plaque [2]. The bacteria adhere to a clear sticky substance from saliva called glycoprotein, which binds immediately to the surface of freshly cleaned tooth. The combination of bacteria and glycoprotein on the tooth surface is called pellicle or biofilm [3]. By the time twenty four hours have passed, different types of microorganisms are living in pellicle as plaque thickens and builds up at measurable levels. The plaque is made up of colonies of microorganisms such as bacteria, yeast dumped together in a gel like organic material composed of bacterial byproducts including sugar, food debris and body tissue. The predominant bacteria found at distinct sites on tooth surface are *Streptococcus, Actinomyces, Neisseria, Veillonella* etc. [4].

The cells divide and generate a biofilm. At first, the biofilm is soft enough to come off by using the fingernail. However, it starts to harden within 48 hours, and in about 10 days the plaque becomes dental calculus (tartar) hard and difficult to remove [5]. Dental plaque can give rise to dental caries (tooth decay) the localized destruction of the tissues of the tooth by acid produced from the bacterial degradation of fermentable sugars and periodontal problems such as gingivitis and chronic periodontitis [6] [7].

Methods to inhibit biofilm growth on dental composites have been sought for several decades and the suitable solution for the present problem was the application of nanotechnology. Nanotechnology is a technology that deals with nanometer sized objects. Nanoparticles have a very high surface to volume ratio. This provides tremendous face in diffusion, especially at high temperatures.

Nanoparticles considered being of a size not greater than 100 nm and the exploitation of their unique attributes to combat infection has increased in the past decade. Nanoparticles have the ability to control formation of biofilms within the oral cavity as a function of their biocidal, anti-adhesive and delivery capabilities. Possible uses as a constituent of prosthetic device coatings, as topically applied agents and within dental materials are being explored. In particular nanoparticulate silver, copper, zinc, silicon and their oxides are considered to have antibacterial effects.

Dental implants are long term replacements that surgically place in the jawbone [8] [9]. Composed of titanium metal that fuses with the jawbone through

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a process called Osseo integration, titanium dioxide dental implants never slip or make embarrassing noises or advertise the fact that it is a false teeth. Titanium dioxide nanoparticle implants also have proved to have potential antibacterial action and also used in control of biofilms. These implants also are much economical compared to others like gold and silver.

## MATERIALS AND METHODS

***Sample collection***

The samples were collected from patients having signs and symptoms of dental caries from Savitha Dental clinic, Jawaharlal Technological University, Hyderabad. The swabs were inserted at the site of caries lesion and kept for few minutes and placed in a tube containing sterile nutrient broth. The swabs were collected in sterile nutrient broth solution before starting of antibiotics and transported to laboratory for processing.

***Growth media for isolation of organisms***

For isolation of different etiological agents causing dental plaques, specific isolation media were used, nutrient agar for bacteria, Czapekdox agar with Martin rose Bengal for fungal isolation and Glycerol yeast extract agar for *Actinomycetes.* A loopful of suspension is streaked onto different isolation media. The inoculated plates were incubated at their respective temperatures i.e Nutrient agar plates at 37°C for 24 hours, Czapekdox agar plates at RT for 5 to 7 days and Glycerol yeast agar for 3 to 4 days at room temperature.

***Synthesis of Titanium dioxide nanoparticles***

**Chemicals required:** 20 ml Titanium chloride is mixed with 60 ml of 0.1N Ammonium hydroxide and this mixture is stirred for 48 h at room temperature. Titanium dioxide nanoparticles formation is indicated by change in colour from purple to white colored solution. Solution was centrifuged and the precipitate was washed with distilled water and dried in isopropanol at RT. The samples were furthered subjected to antimicrobial activity and characterization by XRD.

***Analysis of antimicrobial activity of $TiO_2$ nanoparticle***

Nutrient agar plates were prepared and 0.1ml of culture was seeded and then the wells were bored. 50µl and 100µl of Titanium dioxide nanoparticles were added into the wells. In another set of plates 100µl of isopropanol as control and 100 µl of Titanium dioxide was added and the antimicrobial activity was checked by the zone of inhibition obtained. The diameter of each zone was measured in cm and compared.

***Minimum inhibitory concentration***

The minimum inhibitory concentration of $Tio_2$ nanoparticles against the samples D2, D5, D14, D19 and D23 was carried out. Different concentrations of $TiO_2$ such as 2mg, 4 mg, 6 mg, 8mg, 10 mg, 12mg and 15 mg/ml were taken and tested against the above samples. Control tubes were also maintained without the $TiO_2$ nanoparticle.

## RESULTS AND DISCUSSION

***Isolation of the Microorganisms***

Forty samples were collected from the dental clinic which was reported to be causative organisms of dental plaques. The swabs were placed on the tooth surface and then inoculated into nutrient broth. These samples were checked for the antimicrobial activity. Out of the 40 samples, Tio2 showed good activity against 18 samples. These samples were further cultured onto Nutrient agar, Czapekdox agar and Glycerol yeast extract agar. The organisms were further Gram stained to identify the morphology. There were of Gram positive and gram negative in nature. Spore forming bacilli were also observed. Fungi were also isolated and were identified as *Yeast, Alternaria, Aspergillus* as given in Table 1.

**Table 1:** Different isolates from dental plaques

| | | | |
|---|---|---|---|
| Gram +ve Bacteria | Cocci | | D2, D14 |
| | Bacilli | | D1, D9, D20, D 29, D30 |
| | Sporulating Bacilli | | D3, D4, D10, D24 |
| | Actinomycetes | | D5 |
| Gram –ve Bacteria | Bacilli | Short | D19 |
| | | Long | D12, D15 |
| Yeast | | | D11 |
| Fungi | Alternaria | | D23 |
| | Aspergillus | | D28 |

Out of the 18 samples, D23 and D28 were fungal cultures. D23 was identified as *Alternaria alternata* by its morphological features like conidiophore pale brown to olive brown, straight or flexuous individual conidiophores arise directly from substrate forming bushy heads consisting of 4-8 large catenate conidia chains. D28 was identified as *Aspergillus*, colonies of which were black as seen in Fig. 1. From the present study it shows that even fungi have been isolated from dental plaques.

***Analysis of the Antimicrobial activity***

The activity of Titanium dioxide, Titanium dioxide nanoparticles and isopropanol as control was tested on the 18 samples isolated and the following results were obtained as seen in table 2.

The results show that the effect of Titanium dioxide nanoparticles on dental plaques was better when compared to that of the Titanium dioxide and isopropanol. For example samples D3, D4, D5, D9, D10

D14, and D15 Titanium dioxide nanoparticles showed good antimicrobial activity whereas titanium dioxide has no effect against these organisms. The other samples D1, D2, D12, D19, D20 D24, Titanium dioxide nanoparticles have slightly higher activity compared to Titanium dioxide alone. In case of the fungi which have been isolated $TiO_2$ nanoparticles showed greater inhibition against *Aspergillus* where the oxide had no inhibition. Other fungi like Yeast and *Alternaria* TiO2 nanoparticles showed activity of 1.7 and 2.4 cm respectively. Thus the activity of $TiO_2$ nanoparticles tested for different bacterial and fungal strains and the zone of inhibition indicates the efficiency of these particles. The greater the zone of inhibition, greater the activity.

**Table 2:** Comparative Analysis of Anti-Microbial Activity of TiO2 Nano Particles and TiO2 on dental Samples

| S. No. | Samples | Gram Character | Titanium Dioxide Nano Particle (Cm) | | Titanium Dioxide (Cm) | |
|---|---|---|---|---|---|---|
| | | | 50µl | 100µl | Control (100µl) Isopropanol | TiO2 (100µl) |
| 1 | D1 | gram +ve bacilli | 1.1 | 2 | 1.2 | 1.5 |
| 2 | D2 | gram +ve cocci | 2.3 | 2.7 | 0 | 2.6 |
| 3 | D3 | gram +ve sporulating bacilli | 1.6 | 1.9 | 0 | 0 |
| 4 | D4 | gram +ve sporulating bacilli | 1.9 | 2 | 0 | 0 |
| 5 | D5 | actino mycetes | 1.8 | 2.9 | 0 | 0 |
| 6 | D9 | gram +ve bacilli | 1.2 | 1.4 | 0 | 0 |
| 7 | D10 | gram +ve sporulating bacilli in chains | 1.6 | 1.8 | 0 | 0 |
| 8 | D11 | yeast | 1 | 1.7 | 0 | 1.1 |
| 9 | D12 | gram -ve bacilli | 1.5 | 1.7 | 0 | 1.3 |
| 10 | D14 | gram +ve cocci in clusters | 1.9 | 2.1 | 0 | 0 |
| 11 | D15 | gram -ve bacilli | 1.5 | 1.6 | 0 | 0 |
| 12 | D19 | gram -ve bacilli | 1.2 | 2.5 | 1.2 | 1.9 |
| 13 | D20 | gram +ve bacilli | 1.1 | 1.8 | 1 | 1.3 |
| 14 | D23 | Alternaria | 1.1 | 2.4 | 1.2 | 1.2 |
| 15 | D24 | gram +ve sporulating bacilli | 0 | 1.1 | 0 | 1.1 |
| 16 | D28 | Aspergillus | 0 | 1.3 | 0 | 0 |
| 17 | D29 | gram +ve bacilli | 0 | 1.2 | 0 | 1.9 |
| 18 | D30 | gram +ve bacilli | 0 | 1.5 | 0 | 1.5 |

***Minimum Inhibitory Concentration***

The minimum inhibitory concentration of $TiO_2$ nanoparticles was carried out on D2, D5, D14, D19 and D23 and the following results were obtained. MIC of the D2 sample is 12 mg, D5 is 4 mg, D14 is 15 mg, D19 is 15 mg and D23 is 6 mg. D5 require lower concentrations of Tio2 nanoparticles for inhibition, D23 is inhibited by 6mg concentration whereas D14 and D19 are inhibited by 15mg concentration of the nanoparticle.

**Table 3:** Minimum inhibitory concentration

| Sample | Control | 2 mg | 4 mg | 6 mg | 8 mg | 10 mg | 12 mg | 15 mg |
|---|---|---|---|---|---|---|---|---|
| D2 | 0.06 | 0.04 | 0.04 | 0.04 | 0.03 | 0.03 | 0 | 0 |
| D5 | 0.04 | 0.01 | 0 | 0 | 0 | 0 | 0 | 0 |
| D14 | 0.18 | 0.06 | 0.06 | 0.12 | 0.08 | 0.05 | 0.03 | 0 |
| D19 | 0.13 | 0.08 | 0.07 | 0.07 | 0.06 | 0.05 | 0.03 | 0 |
| D23 | 0.08 | 0.04 | 0.03 | 0 | 0 | 0 | 0 | 0 |

## DISCUSSION

In this study, Antibacterial effect of $TiO_2$ nanoparicles against organisms causing dental plaques were carried out. Microorganisms are gaining resistance to most of the mouth washes and tooth pastes (16). Introducing the new antibacterial agents can control the mortality and morbidity rate of organisms causing dental diseases. It has been known that Nano-Materials exhibit strong inhibiting effects towards a broadened spectrum of bacterial strains. According to several studies, it's believed that the metal oxides carry the positive charge while the microorganisms carry negative charges; this causes electromagnetic attraction between microorganisms and the metal oxides which leads to oxidization and finally death of the microorganisms (17) Nano materials also could deactivate the cellular enzymes and DNA by coordinating to electron-donating groups, such as: Thiols, Carbohydrates, Amides, Indoles, Hydroxyls etc. They cause pits in bacterial cell walls, leading to increased permeability and cell death (18).

From the above study it is evident that Tio2 nanoparticles have greater activity in inhibiting the growth of microorganisms and thus can be used in controlling organisms causing dental plaques. These nanoparticles also have potential effect in controlling fungi like *Yeast, Alternaria* and *Aspergillus.*

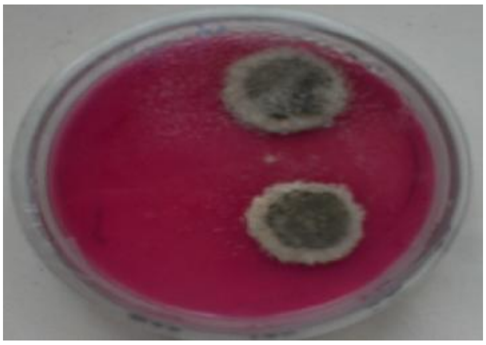

**Fig. 1:** Fungi like Aspergillus isolated from D28 sample cultured on Czapekdox agar media.

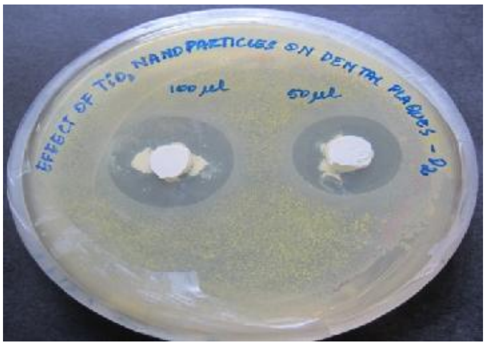


**Fig.2:** Analysis of the Antimicrobial activity of TiO2 Nano Particle on D2 sample showing that Tio2 is inhibiting the growth of the organism.


**Fig. 2.1:** Analysis of the Antimicrobial activity of TiO2 Nano Particle on D5 sample showing that Tio2 is inhibiting the growth of the organism


**Fig. 2.2:** Analysis of the Antimicrobial activity of TiO2 Nano Particle on D14 sample showing that Tio2 is inhbiting the growth of the organism

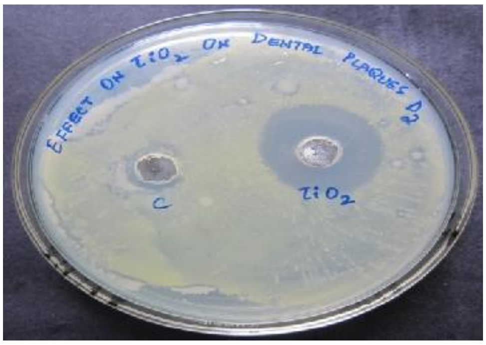


**Fig. 3:** Analysis of Anti-microbial activity of Titanium Dioxide on dental sample D2


**Fig. 3.1:** Analysis of Anti-microbial activity of Titanium Dioxide on dental Sample D5


**Fig.3.2:** Analysis of antimicrobial activity on titanium dioxide on dental sample D14

## CONCLUSION

Nanocrystalline semiconductor metal oxides have achieved a great importance in our industrial world today. They may be defined as metal oxides with crystal size between 1 and 100 nm. $TiO_2$ nanosize particles have attracted significant interest of materials scientists and physicists due to their special properties and have attained a great importance in several technological applications such as photo catalysis, sensors, solar cells and memory devices. During recent decades, $TiO_2$ powders have begun to appear in many applications, mainly due to their ability to confer whiteness and opacity on various products, such as paints, papers and cosmetics. Its high technological attractiveness originates from its light-scattering properties and very high refractive index, which mean that relatively low levels of the pigment are required to achieve a white, opaque coating. The range of light that is scattered depends on the particle size. Numerous technological improvements, based on nano-sized $TiO_2$, have been introduced that enable its use for antifogging and self-cleaning coatings on glass, for building facades, in confectionary, in the plastics industry, and so on. Furthermore, $TiO_2$ is accepted as a food and pharmaceutical additive. In the United States it is included in the Food and Drug Administration (FDA) Inactive Ingredients Guide for dental paste, oral capsules, suspensions, tablets, dermal preparations and in non-parenteral medicine. The future prospects from the study would help in identification of microorganisms causing dental caries by molecular characterization and this could also give a novel approach as to what concentration of Tio2 incorporated into dental implant will help in inhibiting microorganisms causing dental plaques as they have greater antimicrobial activity.

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