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Analysis of Antimicrobial Activity of Titaniumdioxide Nanoparticles on Aerobic and Anaerobic Dental Isolates

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ABSTRACT

Dental plaque is a biofilm or mass of bacteria that grows on surface within the mouth. It is a sticky colorless deposit at first, but when it forms tartar it is brown or pale yellow that is commonly found between the teeth, front of teeth, behind teeth, on chewing surface, along the gumline,. A study was carried out to isolate microorganisms from patients with dental problems. Total 8 dental plaque samples were collected from Savitha dental clinic, Hyderabad, which showed the presence of both aerobic and anaerobic bacteria. Titanium dioxide nanoparticle was synthesized and checked for its antimicrobial activity against dental plaque samples. TiO2 nanoparticles were then incorporated into some commercial toothpastes and checked against dental plaque causing organisms using commercial toothpaste as control. The activity was also checked using bulk TiO_2. TiO_2 nanoparticles incorporated into tooth paste showed enhanced activity compared to toothpaste alone. Antimicrobial activity of TiO2 synthesized by wet chemical method was compared against commercial TiO2 P23.It was observed that TiO2 nanoparticles synthesized by wet chemical method showed greater antimicrobial activity than commercial TiO_2 nanoparticles and bulk oxide. Astringent mouthwash was prepared and TiO2 nanoaprticles were incorporated into it and the activity was compared.

Keywords: Antimicrobial activity, Dental plaques, Nanoparticles, Titanium dioxide.

I. INTRODUCTION

Dental caries is one of the most common chronic infectious diseases in the world [1,2]. Bacterial plaque accumulated on dental surfaces and composed of native oral flora is the primary etiologic agent of dental caries. Cariogenic bacteria interact by various recognized ways including co-aggregation [3], metabolic exchange, cell to cell communication [4], and exchange of genetic material [5].Dental plaque can give rise to dental caries (tooth decay) – the localised destruction of the tissues of the tooth by acid produced from the bacterial degradation of fermentablesugar – and periodontal problems such as gingivitis and periodontitis .[6]Removal of dental biofilm is important as it may become acidic causing demineralization of the teeth (also known as caries) or harden into calculus (dental) (also known as tartar).[7] In the development of caries a major role play bacteria of the *Streptococcus* and *Lactobacillus* genera which are acidogenic and aciduric.[8]Method to inhibit biofilm growth on dental composites have been sought for several decades.Suitable solutions for the present problem is the application of nanotechnology. Nanotechnology is a technology that deals with nanometer sized objects .Nanoparticle size is not more than 100nm . One such nanoparticle is titanium dioxide. Generally TiO2 bulk is used in toothpaste as white pigment.It is non-carcinogenic. It has high refractive index which provide whiteness to the teeth. It is used in paints and sunscreen as well.

But TiO2nanoparticles are small in size and it has high surface area to volume ratio so it can be easily diffuse on to the surface and it has high refractive index which provide whitening to the teeth. It has photocatalytic properties which makes it antimicrobial agent [9].So TiO2 nanoparticles can be used in tooth paste to

enhanced its antimicrobial activity on dental plaques. According to nanowerk spotlight article (2012) roughly 50,000 tons of TiO2 nanoparticles were produced in 2010 and this amount is expected to grow to over 200000 tons by 2015.

II. METHODS AND MATERIAL

1. Sample collection

Total 8 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 and brain heart infusion broth before starting of antibiotics and transported to laboratory for processing. Samples were D4,D5 ,D12, D13,D15 ,ADC1,ADC4,ADC8.

1.1 Growth media for isolation of organisms

For isolation of different etiological agents causing dental plaques, specific isolation media were
used, nutrient broth for aerobic bacteria, Brain heart infusion broth for anaerobic bacteria. The culture was inoculated into nutrient broth and brain heart infusion broth and incubated under suitable conditions .For anaerobic growthgas pack system was used for cultivation.

2. Synthesis of Titanium dioxide nanoparticles

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.[14]

2.1 Compartive analysis ofAntimicrobial activity of different TiO_2 nanoparticles

Antimicrobial activity of TiO_2 nanoparticles synthesized by wet chemical method was checked against commercial TiO_2 nanoparticle P23 and bulk TiO2 by agar well diffusion method.

2.2 Comparative analysis of antimicrobial activity of toothpaste and toothpaste with TiO_2 nanoparticles (aerobic & anaerobic isolates)

The antimicrobial activity of commercial toothpaste was checked against dental plaque causing organisms. 1mg of TiO2 was incorporated into toothpaste and the activity of both was compared using bulk TiO2 as control.

3.Synthesis of AstringentMouth Wash

Requirements : Sodium chloride = 2.5gm , zinc chloride, = 0.5gm , menthol = 0.05gm alcohol =9.6gm ,honey =10gm ,water=77.25gm .Dissolve the zinc chloride and the sodium chloride in the water,add honey .Dissolve the rest of the ingredients in alcohol .Mix the two solutions together, and filter it.

3.1Comparative Analysis of The Antimicrobial Activity ofAstringent Mouthwash And Astringent Mouthwash With Nanoparticles

Astringent mouth was synthesized. The antimicrobial activity of astringent mouth wash was checked. Futher TiO2 nanoparticles were also incorporated into astringent mouth to check if there was enhanced antimicrobial action.

4. Gene sequencing of ADC-1 was performed by 16s rRNA

The 16S rRNA gene is used as the standard for classification and identification of microbes, because it is present in most microbes and shows proper changes. Type strains of 16S rRNA gene sequences for most bacteria and archaea are available on public databases. In the present study, the EzTaxon database (a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences) was used.EzTaxon is an open access database that is produced and maintained by ChunLab, Inc. The EzTaxon database contains sequences of type strains of prokaryotic species with validly published names, mainly used for the routine identification of prokaryotic isolates.ADC1 isolate was identified by 16srRNA typing.

III. RESULTS AND DISCUSSION

1. Isolation and identification

8 samples were collected from the dental clinic which was reported to be causative organisms of dental plaques 5 were identified to be aerobic and 3 were found to be anaerobic .These samples were checked for the antimicrobial activity.Out of the 8 samples, TiO_2 showed good activity against 6 samples. These samples were cultured into nutrient broth and brain heart infusion broth. The organisms were further Gram stained to identify their morphology.

The sample ADC-1 is identified to be *Enterobacter cloacae* ECNIH2; CP008823.The sequence analysis also showed similar results (Table 7). After the completion of BLAST analysis the organism was identified to be *Enterobacter cloaceae*with0.995 s_ab score.

Table1 : Different aerobic bacteria of dental plaque

Dental samples	Gram stain	Starch hydrolysis	endospore	Catalase	VP	Glucose fermentation	Oxidase Test	microorganisms
D5	Gram positive rods	+	_	+	_	_	_	*Bacillus circulans*
D4	Gram negative Rods	+	+	_	_	_	+	*Pseudomonas species*
D12	Gram Positive	+	+	_	_	_	_	*Bacillus circulans*
D13	Gram negative rods	_	_	_	_	_	+	*Pseudomonas species*
D15	Gram positive rods	_	_	_	_	_	_	*Lactobacillus fermentii*

Dental samples	Gram stain	Catalase	Indole	Urease	Glucose fermentation	Microorganisms
ADC 1	Gram positive *Cocci*	+	_	_	+	*Enterococcus sps.*
ADC 4	Gram positive *Cocci*	+	+	_	+	*Peptostreptococcus*
ADC 8	Gram positive *Cocci*	_	_	_	+	*Peptostreptococcus*

2.1Compartive analysis of Antimicrobial activity of different TiO_2 nanoparticles

Table3 : Zone of inhibition (cm)

Sr.No.	Sample	TiO_2 bulk	TiO_2 Commercial nanoparticles	TiO_2 synthesized nanoparticles
1	D4	-	1.8	2
2	D13	1.3	1.7	1.8
3	D15	2.3	1.8	2
4	D5	1.3	1.5	2
5	D12	1	1.3	1.7

The nanoparticles synthesized by wet chemical method had greater activity compared to commercial TiO_2 nanoparticles.

Figure 1. Antimicrobial activity of synthesized vs commercial TiO2 nanoparticles.

2.2 a Comparative analysis of antimicrobial activity of toothpaste and toothpastes with TiO_2 nanoparticles (aerobic dental culture)

Table 4: Zone of inhibition in (cm)

SAMPLES	HIMALYA	HIMALYA+ TiO_2 NANO	COLGATE	COLGATE + TiO_2 NANO	DABUR	DABUR+ TiO_2 NANO
D15	1.6	2.5	3.5	3.8	1.4	1.6
D12	1.6	2	3.6	3.7	1.5	1.6

All the three toothpaste Himalaya, Colgate and Dabur showed antimicrobial activity against the aerobic dental isolates D15, D12. When TiO_2 nanoparticles are incorporated into commercial toothpaste there is enhanced antimicrobial action than when the toothpaste was used alone.

Figure 2: Comparative analysis of antimicrobial activity of toothpaste and toothpaste with TiO2 nanoparticles (D15& D12)

2.2 b Comparative analysis of antimicrobial activity of toothpastes and toothpastes with TiO_2 nanoparticles (anaerobic dental culture)

Table 5: zone of inhibition (cm)

Dental samples	Sensodyne (cm)	Sensodyne + TiO_2 nanoparticle	Colgate	Colgate + TiO_2 nanoparticle	Himalya	Himalya + TiO_2 nanoparticle
ADC-1	-	-	2.5	2.5	-	-
ADC-4	1.8	2	2.4	2.7	2.2	2.4
ADC-8	1	2.5	2.4	2.6	2	2.6

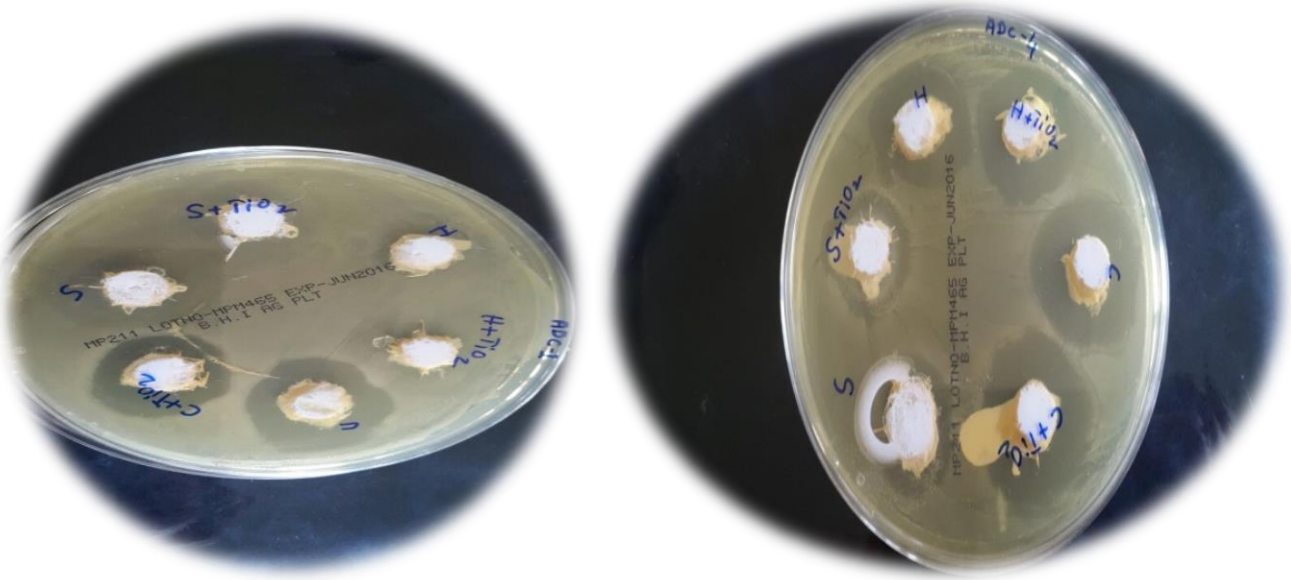

Figure 3. Comparitive analysis of antimicrobial activity of toothpaste and toothpaste with nanoparticle. ADC1, ADC4.

In case of anaerobic isolate ADC,1 Sensodyne and Himalaya toothpaste had no activity whereas ADC4, ADC8 were inhibited against all the three toothpastes. When TiO_2 nanoparticles were incorporated into toothpates there was no activity against ADC1 with Sensodyne and Himalaya. But

there was enhanced antimicrobial activity when the combination of TiO_2 was used with toothpastes incase of ADC4, ADC8.

3.Comparative Analysis ofthe Antimicrobial Activity ofAstringent Mouthwash And Astringent Mouthwash with TiO_2 Nanoparticles

Table 6: zone of inhibition in (cm)

Dental sample	Astringent mouthwash (cm)	Astringent+TiO_2 Nanoparticles (cm)
D15	1.5	2

Astringent mouthwash showed good antimicrobial activity against D15 sample. 1mg of TiO2 npwas incorporated into 1 ml of astringent mouthwash and 100ul of this was incorporated into the well. The result shows that when the combination of $TiO_2$was used with astringent mouth wash there was enhanced antimicrobial activity.

Figure 4: Comparative Analysis Of The Antimicrobial Activity Of Astringent Mouthwash And Astringent Mouthwash With TiO_2 Nanoparticles

4. Gene sequencing of ADC-1 was performed by 16s rRNA

Table 7 Blast report

Short ID	S_ab Score	Unique common oligomers	Sequence full name
S000544285	0.995	1417	Enterobacter cloacae; type strain: EN-314 = DSMZ 16687 = CIP 108490; AJ853889
S000769724	0.995	1396	Enterobacter sp. p2-6+1; EF138627
S002034044	0.992	1295	Enterobacter sp. ARS-3; GU372946
S004069608	0.995	1440	Enterobacter cloacae ECNIH2; CP008823
S004069610	0.985	1439	Enterobacter cloacae ECNIH2; CP008823
S004069611	0.985	1440	Enterobacter cloacae ECNIH2; CP008823
S004445793	0.985	1450	Enterobacter cloacae; 34983; CP010377

The sample ADC-1 is identified to be *Enterobacter cloacae* ECNIH2; CP008823.The sequence analysis also showed similar results (Table 7). After the completion of BLAST analysis, the organism was identified to be *Enterobacter cloaceae*with0.995 s_ab score.

2. Discussion

In the following study plaque forming microorganisms that were isolated were *Bacillus circulans,* which is found in food samples and responsible for wound infection of gums.*Lactobcillusfermentii*is heterofermentative which in addition to lactic acid produces acetate, ethanol and carbon dioxide [8] responsible for tooth decay .*Pseudomonas is* responsible for wound infection. Anaerobic isolate is *Peptostreptococcus* is most frequently found anaerobic microbe produce large amounts of lactic acid during the process fermentation of sugar. It is a commensal organism found in humans, living predominantly in the mouth.

In this study, antibacterial effect of TiO_2 nanoparticles against organisms causing dental plaque was carried out. The study shows that TiO_2 nanoparticles synthesized by wet chemical method showed enhanced antibacterial activity on the dental plaque causing microorganisms than TiO_2 bulk and commercial TiO_2 nanoparticles. The difference in the activity in bulk and nano TiO_2 is due to the smaller size of TiO_2nanoparticles, which leads to better diffusion into the cells. [9]
The antimicrobial activity of three commercially available toothpastes was checked against both aerobic and anaerobic dental isolates. TiO_2 nanoparticles were incorporated into these toothpastes and the comparative analysis was performed. It was observed that all the toothpastes were showing activity against aerobic dental isolates. When the combination of toothpaste was used with TiO_2 nanoparticle there was enhanced antimicrobial activity.(Table 4).

The antimicrobial activity of Sensodyne, Colgate and Himalaya was checked against anaerobic dental isolates ADC1, ADC4, ADC8. It was observed that Sensodyne and Himalaya had no activity against ADC1 isolate where ADC4 and ADC8 were inhibited by all the three toothpastes. When TiO_2 nanoparticles were incorporated into all the three toothpaste, similarly ADC1 was not inhibited with the combination of Sensodyne, Himalaya with TiO2 nanoparticle showing that the strain ADC1 is highly resistant to most of the toothpastes and it is a strong biofilm-forming organism. (table 5). On the other hand, when TiO_2 nanoparticle was incorporated into toothpastes, it showed enhanced activity against ADC4, ADC8 compared to toothpaste alone. (table 5)

Astringent mouthwash synthesized showed good activity against dental plaque sample and when TiO_2 nanoparticle was incorporated into the mouthwash, there was enhanced antimicrobial action.

In molecular studies using 16S rRNA analysis, the predominant microbes in deep caries lesions were *S. mutans*and Lactobacillus. Others included bacteria the genera *Prevotella*, *Selenomonas, Fusobacterium, Bifidobacterium,Pseudoramibacter, Bacillus circulans , Pseudomonas,Lactobacillusfermentii*were isolated from dental plaque .[10,11]

In the present study 16srRNA typing was carried out for ADC1 isolate, which was identified as Enterobacter cloacae, was which is an anaerobicgram positivecocci. *Enterobacter cloaceae*is a member of the normal gut flora of many humans and is not usually a primary pathogen..[16] It is sometimes associated with urinary and respiratory tract infections. Enterobacter cloaceae transplanted into germ fed mice resulted in increased obesity due to presence of Enterobacter gut flora. [17]

The nanomaterials, based on the metal ions, exhibit broad-spectrum biocidal activity towards different bacteria, fungi, and viruses. Nanomaterials are known to deactivate cellular enzymes and DNA by coordinating to electrondonating groups such as thiols, carboxylates, amides, imidazoles, indoles, hydroxyls, and so forth. They cause pits in bacterial cell walls, leading to increased permeability and cell death [12]

IV. CONCLUSION

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, Pseudmonasspp, Peptostreptocci*. Microorganisms are gaining resistance to most of the antimicrobial agents so the present work involved the application of TiO2 nanoparticles, which has good antimicrobial activity gainst broadened spectrum of bacterial strains.

From the above work we could identify that TiO2 nanoparticles have greater activity in inhibiting the

growth of microorganisms and thus can be used in controlling organisms causing dental plaques.

It is effective on both aerobic and anaerobic bacteria. It can be incorporated into the toothpastes and mouthwashes for enhanced antimicrobial activity which is very effective against dental plaque causing organisms.

Furthermore, TiO2 is accepted as a food and pharmaceutical additive. In the United Statesit 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.

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