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TiO_2: ZnO nanocomposites in treatment of dental plaques

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Abstract: Dental caries is a major concern effecting most of the individuals today. Statistics show that 69% of adults aged 35 to 44 years have lost permanent tooth due to accident, gum disease failed root canal or tooth decay. Dental plaque is a general term for the diverse microbial community found on tooth surface, embedded in matrix of polymers of bacteria. Nanoparticles considered being of a size not greater than 100 nm have unique properties to combat infection. Nanocomposite is a matrix to which nanoparticles have been added to improve a particular property of the material. The present study deals with the antimicrobial studies of nanocomposite preparations of Titanium dioxide and Zinc oxide. Titanium dioxide and Zinc oxide nanocomposites are found to be effective in inhibiting the growth of bacteria. The present work is to prepare different proportions of nanocomposites of these oxides and find out if the effectivity is more when compared to using the nanoparticle alone. Some nanocomposite materials have been shown to be 1000 times tougher than the bulk component materials. The study carried out shows that the antimicrobial activity of nanocomposites is more effective than the nanoparticle alone providing best alternative to dental implant surgery.

Key words: Nanocomposites; antimicrobial activity; implant surgery.

Introduction

Dental caries is one of the most common chronic infectious diseases in the world [1,2]. Bacterial plaque accumulated on dental surface 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-cell communication [4], and exchange of genetic material [5]. These mechanisms benefit bacterial survival and can make dental biofilms which lead to dental diseases. Dental caries cause destruction of enamel, dentin or cementum of teeth due to bacterial activities. The burden of dental caries is still a major health problem in most industrialized countries as it affects 60% - 90% of school-aged children and the vast majority of adult. This is largely due to the increasing consumption of sugar and inadequate exposure to fluorides [6].

The cells divide and generate a biofilm. Initially, the biofilm is soft enough to come off by using the fingernail. Later, it starts to harden within 48 h, 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 suitable solution for the present problem was the application of nanotechnology. Nanotechnology deals with nanometer sized objects [8]. Nanoparticles have a very high surface to volume ratio. They provides tremendous force in diffusion, especially at high temperatures. Nanocomposites differ from conventional composite materials due to the exceptionally high surface to volume ratio of the reinforcing phase and its exceptionally high aspect ratio. [9] [10]

Sample collection and isolation of organisms:
The samples were collected from patients having signs and symptoms of dental caries from Savitha Dental clinic, JNTU. The swabs were inserted at the site of caries lesion and kept for few min and taken out and placed in a tube containing sterile nutrient broth. The swabs were collected in sterile nutrient broth solution and transported to laboratory for processing. Dental samples were isolated from patients and streaked onto nutrient agar media. The inoculated plates were incubated at 37^0C for 24 h.

Synthesis of Titanium dioxide nanoparticles:
20 ml Titanium chloride (S.D Fine Chemicals) is mixed with 60 ml of 0.1N Ammonium hydroxide and this mixture is stirred for 48h at RT. Titaniumdioxide nanoparticles formation is

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indicated by change in color 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 characterized by XRD, UV visible spectroscopy and checked for antimicrobial activity.

Synthesis of Zinc oxide nanoparticles:

5.68 gms of zinc acetate was added to 120 ml of DMSO and stirred for 30 min. 4.038 gms of KOH in 60 ml of chilled ethanol is added to the mixture drop wise and stirred for 5 min then 0.36 ml of thioglycerol was added to the above mixture and stirred it for 1h. When the solution turns milky it is washed with methanol and air dried.

Preparation of Titanium and Zinc oxide Nanocomposites:

Diffferent ratios of Titanium dioxide and Zinc oxide nanoparticles, were taken mixed and checked for antimicrobial activity. 1:1, 1:2 and 1:3 ratios of Titanium dioxide and Zinc oxide nanocomposite preparations were prepared. Nutrient agar plates were prepared and 0.1 ml of the isolates was inoculated onto nutrient agar media. Wells were bored into the agar and 100µl of the above nanocomposite was incorporated into the wells. Similarly, 1:1, 1:2 and 1:3 ratio of Zinc oxide and Titanium dioxide nanocomposite were made and the procedure was repeated and checked for the antimicrobial activity.

Characteristaion of TiO2: ZnO nanoparticles:

The structural properties of TiO2: ZnO nanocomposite (3:1) were recorded on ADV D8 Bruker X-ray powder diffractometer with Cu radiation, (λ=0.154nm in the range of 20-80^o).

Results and Discussion

Isolation of the Microorganisms:

Dental isolates were collected from Savitha Dental Clinic (JNTU) from patients reported to have dental caries. The isolates were labeled as D2-D6, D10-D15, D17, D19, D23, D24, D29, D30. On gram staining the isolates D2, D14 were identified as gram positive cocci and D3, D4, D5, D9, D10, D20, D24, D29, D30 were identified as gram positive bacilli whereas D5, D11, D12, D19 were identified as gram negative bacilli.

Antimicrobial Activity of Nanocomposites

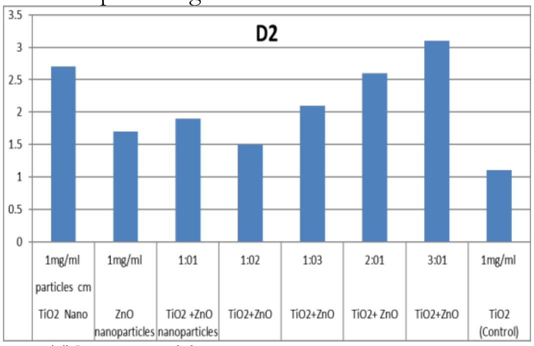
Nanocomposites represent a novel class of nanostructures with profound technological significance. They combine the advantages of the inorganic materials such as rigidity, thermal stability and those of the organic polymers like flexibility, process ability etc. The physical, chemical and biological properties of nanocomposites differ from the properties of individual atoms and molecules or bulk matter. The dental isolates which are isolated were identified as strong biofilm producing strains. A biofilm is any group of microorganisms in which cells stick to each other on a surface. Biofilms have been found to be involved in a wide variety of microbial infections in the body, by an estimate 80% of all infections. The biofilm on the surface of teeth is frequently subjected to oxidative stress [11] and acid stress. Dietary carbohydrates can cause a dramatic decrease in pH in oral biofilms to values of 4 and below (acid stress)[11]. The ever increasing resistance of pathogens towards antibiotics has caused serious health problems in recent years. Hence, by combining nanotechnology and antimicrobial activity of the metal oxides, metal oxide nanoparticles can be used as antimicrobial agents. Recent Studies have demonstrated antimicrobial efficacy against bacteria, viruses and eukaryotic microorganisms of various nanoparticle materials, including silver,[12] copper [13] titanium dioxide[14] magnesium, gold [15] alginate, and zinc oxide. Thus by introducing new antibacterial agents we can control the mortality and morbidity rate of organisms causing dental diseases. From the earlier studies carried on, by microtitre plate assay and tube detection method the isolates D2, D3, D4, D10, D19, D23, D24, were found to be good biofilm forming organisms. The antimicrobial activity of the nanocomposites was determined by agar well diffusion method. The antimicrobial activity of TiO2: ZnO nanocomposites were tested against these test organisms.

Table 1: Antimicrobial activity of TiO_2: ZnO against dental plaque causing organisms

Isolates	TiO2 Nano particles cm	ZnO nanoparticles	TiO2 +ZnO nanoparticles	TiO2+ZnO	TiO2+ZnO	TiO2+ ZnO	TiO2+ZnO	TiO2 (Control)
	1mg/ml	1mg/ml	1:1	1:2	1:3	2:1	3:1	1mg/ml
D2	2.7	1.7	1.9	1.5	2.1	2.6	3.1	1.1
D3	1.9	1.2	2	2.3	2.3	2	2	-
D4	2	2	2.3	2.7	2.8	2.5	3	-
D10	1.8	1.6	1.3	1.5	2.2	1.3	2	-
D12	1.7	2	2	-	-	2	2.1	-
D23	2.2	2.3	2.2	2.7	2.7	-	-	-
D24	1.1	1.1	1.6	1	1	1.7	2.4	-
D19	1.2	1.2	1.5	1.5	1.5	1.7	1.8	-

As seen in Fig 1a, b, c, d Titanium dioxide and Zinc oxide nanocomposites are effective in inhibiting the growth of microorganisms. The antimicrobial tests show that TiO_2 nanoparticles are having good antimicrobial activity against all the test strains. On the other hand ZnO nanoparticles also have fairly good activity against the test strains. In case of D2, D4, D19, D24 when the proportion of TiO_2 was more in the composite. (Table 1) there was greater inhibition. On the other hand in isolates like D3, D10, D23when the proportion of ZnO was more in the composite there was greater antimicrobial activity. The results show that TiO_2 nanoparticles were exhibiting more antimicrobial activity compared to ZnO nanoparticles. When 1:1 proportion of TiO_2 and ZnO was used there was no enhanced antimicrobial action, the synergistic activity of both the nanoparticles did not show much activity. The results also show that with the increasing concentrations of TiO_2 nanoparticles, the activity is more, i.e 3:1of TiO_2: ZnO shows more activity when compared to 1:1 and 2:1 of TiO_2 and ZnO. D2 is inhibited even by nano TiO_2. From these studies we can analyze that TiO_2 as well as ZnO nanoparticles are having effective antimicrobial activity against both gram +ve bacilli as well as gram-ve bacilli and nanoTiO_2 is effective against gram positive cocci D2 (graph 1). The effectivity of TiO_2 nanoparticles is due to the generation of reactive oxygen species such as superoxide ions which can oxidize bacteria resulting in death of the microorganisms. [16] The toxicity of nano TiO_2 on *E. coli* is due to electrostatic interactions between bacteria and nanoparticles leading to adsorption of latter onto the cell surface [8]. Similarly, ZnO-NPs also exhibit attractive antibacterial properties due to increased specific surface area and the reduced particle size leading to enhanced particle surface reactivity. Generation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), OH^- (hydroxyl radicals), and O_2^{-2} (peroxide) [17, 18] has been a major factor for several mechanisms including cell wall damage due to ZnO-localized interaction, enhanced membrane permeability, internalization of NPs due to loss of proton motive force and uptake of toxic dissolved zinc ions. [19] These have led to mitochondrial weakness, intracellular outflow, and release in gene expression and oxidative stress which caused eventual cell growth inhibition and cell death.

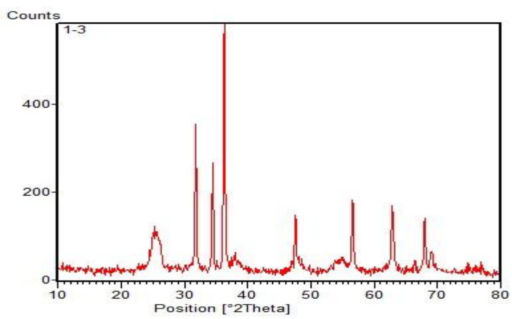
Graph 1: Antimicrobial activity of nanocomposites against D2



1. TiO2 nanoparticle
2. ZnO nanoparticles
3. 1:1 TiO2: ZnO
4. 1:2 TiO2 : ZnO
5. 1: 3 TiO2 :ZnO
6. 2: 1 TiO2 : ZnO
7. 3:1 TiO2: ZnO
8. Titanium dioxide (Bulk)

XRD analysis of Nanocomposites of TiO2: ZnO (3:1 ratio)

The 3:1 proportion of TiO2: ZnO showed greater antimicrobial activity and hence the size of the nanocomposite was determined by XRD studies. TiO2: ZnO nanocomposites were characterized by using XRD which was performed at a scanning range of 2- 80°(2θ) using copper Kα radiation with a wavelength of 0.154nm. Graph 2 shows the X-ray diffraction analysis of TiO_2: ZnO nanocomposites. The major peaks are indexed at 31°, 32°, 35°, 36.5°, 26°,5° respectively. Absence of any other peaks clearly indicates that no impurities are present. The diameter of the nanoparticles is calculated by Debye-Sherrer equation: $D = K\lambda/\beta \cos\theta$ where K is Sherrer constant, λ is the X-ray wavelength, β is the peak width at half maximum, and θ is the Bragg diffraction angle. The average crystallite size D was estimated to be around 14.74 nm using the Debye–Sherrer formula. Thus we can infer that the smaller size of the nanoparticles in nanocomposites could be responsible for greater diffusion into the microbial cells and enhanced antimicrobial activity against microbial cells.



Graph 2: XRD analysis of nanocomposites of TiO2: ZnO (3:1)

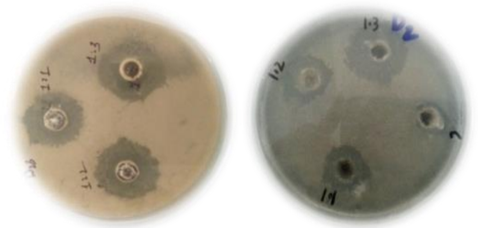


Figure 1 a, b: Anti-microbial activity of different proportions of TiO2: ZnO against (D23, D2).

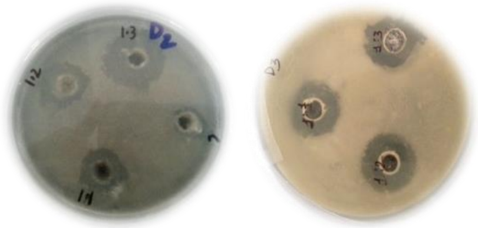


Figure 1 c, d: Anti-microbial activity of different proportions of ZnO: TiO2 (D2, D3)

Conclusion

The present study helped in identification of microorganisms causing dental caries and also gave insight into role of nanocomposites having good antimicrobial activity. The study showed that TiO_2 nanoparticles were having greater activity compared to ZnO nanoparticles against dental plaque causing organisms and 3:1 proportion of TiO2: ZnO has enhanced antimicrobial action in inhibition of dental caries. Dental implants prepared with suitable nanocomposite proportions hold a promising solution to many dental caries related issues.

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