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Engineered Nano-Semiconductor Oxides' Antibacterial Activity Against Gram-Positive and Gram-Negative Bacteria

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Abstract: *Engineered nanoparticles (ENPs) have been the focus of research on semiconductor oxide antibacterial activity over the last several decades. A modified well diffusion technique was used to test the antibacterial activity of modified ZnO (Gram-positive) and TiO2 (Gram-negative) against Bacillus brevis (Gram-positive) and E. coli (Gram-negative). Nanoparticles made of Ag-TiO2 were shown to be most effective in killing Gram-positive and Negative bacteria. ZnO, Eu2O3, ZnO, DyVO4-ZnO, GdVO4-ZnO, GdVO4-ZnO and HoVO4-ZnO were discovered to have substantial antibacterial properties. Microbial plating studies have shown that the produced nanomaterials have a broad range of antibacterial properties against Bacillus brevis and E. coli. As ENPs are widely used in medicine, water treatment, and the food sector as anti-coating agents, this research aims to provide light on the impact of ENPs on microorganisms.*

Keywords*: *Engineered nanoparticles (ENPs), Bacillus brevis, E. coli, Antibacterial activity.

I. INTRODUCTION

There is a wide range of applications for nanomaterials in the fields of environmental protection, human health, and more (Sahoo et al., 2007; Koo et al., 2005; Lopez et al., 2012) the building blocks of nanotechnology are nanoparticles. Enable particular procedures and selectivity in conventional materials with the use of engineered nanoparticles (ENPs). Biological and pharmaceutical engineering may make extensive use of it (Brigger et al., 2002; Wu et al., 2003). Engineered nano materials' impact on microbes can be studied from two angles: anti-coating agents for medical devices, water treatment reactors, food packaging, and so on; and (ii) the survival and selection of natural microbes on disposal of these nanomaterials after their use in the environment after their use in these nano materials' use (Pattan and Kaul 2014; Swaminathan and Naresh 2017). Metal oxide semiconductors such as TiO2 and ZnO are well-known and widely investigated

Metal oxide nanoparticles that have been specially synthesized have a strong antibacterial activity (Stoimenov et al., 2002; Sarah et al., 2013; Rajiv et al., 2013; Xiaoyan et al., 2012; Yanjing et al., 2013). E. coli was effectively killed by the TiO2/ZnO composite powders that were produced (Stoyanova et al., 2013). Even at low concentrations of nano-ZnO particles, according to Jaskova et al. (2013), Escherichia coli and Staphylococcus aureus were effectively static. Because they are much smaller than cells and hence may readily pass through the membrane, nanoparticles have shown to be an excellent choice for antibacterial compounds (Travan et al., 2011) various bacterial pathways lead to either inactivation or death when nanoparticles engage with the surface or centre of the bacterial cell (Seil and Webster, 2012). To combat infections including Staphylococcus aureus, pseudomonas aeruginosa, enterococci, enterococci, enterococcus faecalis, and campylobacteriosa (which causes Clostridium difficile infection), new antibacterial drugs are needed all the time. In 2004, our group became the first to publish on the synthesis of nano-Ag-TiO2 for the degradation of direct azo dyes (Sobana et al., 2006). In the next step, we changed TiO2 and ZnO to improve their performance. We recently reported synthesising ZnO-loaded rare earth oxides for a variety of purposes (Thirumalai et al., 2017). A total of seventeen nanoparticles were tested for their antibacterial properties in this study: ZnO (Zinc oxide) and TiO2 (Titanium dioxide), AGT (Ag/TiO2), CAT (Cd/Ag/TiO2), NAT (Ni/Ag/TiO2), TUB (Bare Eu2O3) and TGB (Bare Gd2O3). TDM, TGM, TDV, TGV (DyVO4- Dysprosium vanadate doped Zinc oxide) and TGV (GdVO4/ZnO) were also tested for their antibacterial properties.

(HO_2WO_6-HolmiumtungstatedopedZincoxide)against *Bacillus brevis*, (Gram-positive) and *E. coli*, (Gram-negative). One of the important applicationsof nanoparticles is to control the microbial contaminants in food, water (as a potential safety measure in food preservation) and medicine and to understand the influence of the ENPs once discharged into the environment, especially on the natural microbes. This study paves the way for the use of the engineered nanoparticles (ENPs) against the microorganisms in the field of medicine, food packaging, water treatment and anti-coatingagents.

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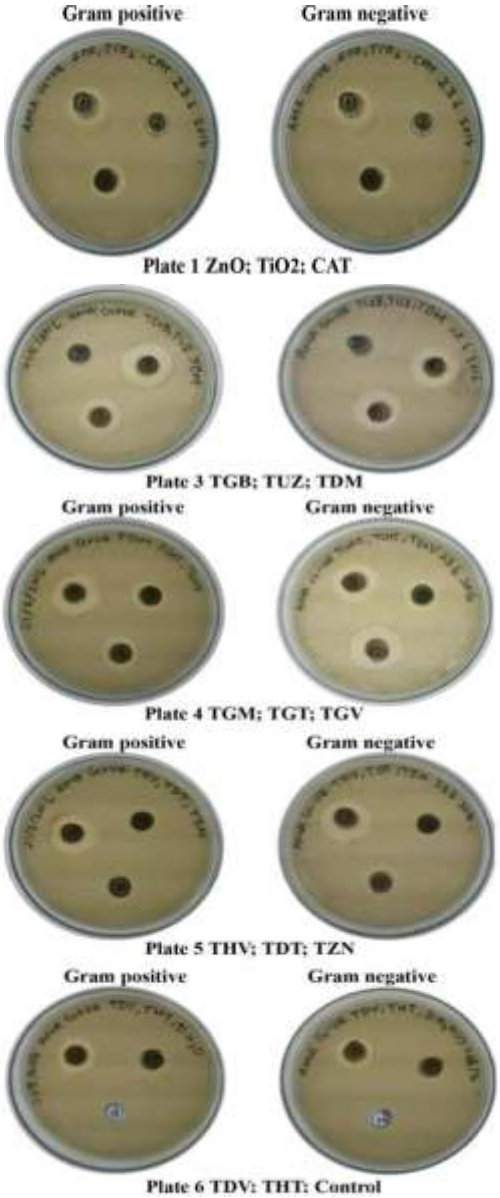

II. EXPERIMENTALSETUP

A. Preparation of engineered nanomaterials

Our previous studies detail the characterisation and preparation process for tailored nanoparticles (Thirumalai et al., 2017). The nanoparticles were dissolved in distilled water at a concentration of 100 mg/ml. For appropriate dispersion of nanoparticles in distilled water, this sample was gently mixed before being inoculated into the well. Listed in Table 1 are the names and categories of several nanoparticles.

B. Antibacterial activity by well diffusionmethod

Swabs were made from overnight cultures of E. coli and Bacillus brevis on the Luria-Bertani agar (Becton Dickinson) supplemented with nutrients. Before the good punch, the plates were let dry for 10 minutes. A modified agar well diffusion technique was used to determine the antibacterial properties (Heatley, 1994). Cork borer holes were drilled into the cork and 40 microliters of test material were put into each well. After that, the plates were incubated for 24 hours at 37°C. This inhibitory zone was identified by the absence of microbe growth in the transparent region, which could be measured using a vernier calliper scale or on a millimetre scale (Magaldi et al., 2004; Valgas et al., 2007). The average of all the antimicrobial tests was calculated.

C. StatisticalAnalysis

All studies were conducted in a laboratory with strict adherence to standard operating procedures and suitable controls. The results are shown as the mean standard deviation of each experiment, which was done in triplicate. The data are evaluated utilising a one-

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way analysis of variance ANOVA, assuming equal variances at p0.01, to establish statistically significant differences.

III. RESULT ANDDISCUSSION

To our knowledge, this is the first research to demonstrate the antibacterial capabilities of unique designed trimetallic nanoparticles. A good diffusion approach was used to assess the antibacterial activity of the generated 17 modified nanoparticles against Bacillus brevis (gramme positive) and E. coli (gramme negative). It is possible to notice in Figure 1 the clear zones of inhibition against gramme positive and gramme negative bacteria of the corresponding designed nanoparticles (in different plate cultures). These numbers are all on millimetres (mm) scale. Antibacterial activity against Bacillus brevis and E. coli is shown in Figure 2.

Figure: 1 Inhibition zone comparison of *Bacillus brevis* (Gram +ve) and *E. coli* (Gram -ve)

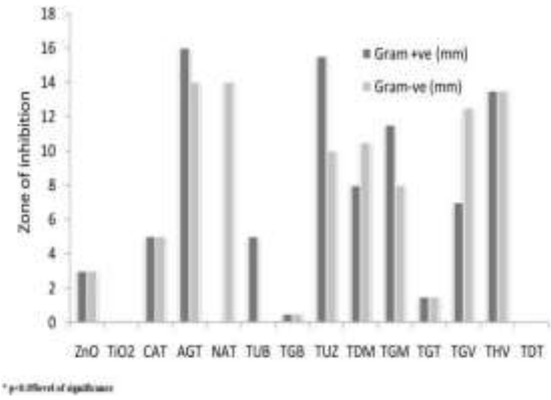

Figure: 2 Comparison of antibacterial activity of synthesized nanoparticle

Table 1 Naming and Category of different nanoparticles investigated.

S. No	Nanoparticle (acronym)	Nanoparticle – Full name	Category
1	ZnO	Zinc Oxide	Metal Oxide – Undoped
2	TiO_2	Titanium Oxide	Metal Oxide – Undoped
3	AGT (Ag/TiO_2)	Silver Titanium Oxide	Dimetalic Oxide – Doped
4	CAT ($Cd/Ag/TiO_2$)	Cadmium SilverTitanium Oxide	Trimetalic Oxide – Doped
5	NAT ($Ni/Ag/TiO_2$)	Nickel Silver Titanium Oxide	Trimetalic Oxide – Doped
6	TUB (Bare Eu_2O_3)	Europium oxide	Metal Oxide – Undoped
7	TGB (Bare Gd_2O_3)	Gadolinium	Metal Oxide – Undoped
8	TUZ (Eu_2O_3/ZnO)	Europium oxide doped Zinc oxide	Dimetalic Oxide – Doped
9	TDM (Dy MoO_4)	Dysprosium molybdate doped Zinc oxide	Trimetalic Oxide – Doped
10	TGM ($GdMoO_4/ZnO$)	Gadolinium molybdate doped Zinc oxide	Trimetalic Oxide – Doped
11	TDV ($DyVO_4$)	Dysprosium vanadate doped Zinc oxide	Trimetalic Oxide – Doped

12	TGV ($GdVO_4/ZnO$)	Gadolinium vanadate doped Zinc oxide	Trimetalic Oxide – Doped
13	THV ($HOVO_4/ZnO$)	Holmium vanadate doped Zinc oxide	Trimetalic Oxide – Doped
14	TGT ($GdWO_4/ZnO$)	Gadolinium tungstate doped Zinc oxide	Trimetalic Oxide – Doped
15	TDT ($DyWO_6/ZnO$)	Dysprosium tungstate doped Zinc oxide	Trimetalic Oxide – Doped
16	TZN	Commercial ZnO	Metal Oxide – Undoped
17	THT (HO_2WO_6)	Holmium tungstate doped Zinc oxide	Trimetalic Oxide – Doped

The zone of inhibition values (mm) are summarized in Table

2. For gram-positive *Bacillus brevis*, the nanoparticle shows high antibacterial activity in the order of AGT>TUZ> THV>TGM>TDV>TDM>TGV>TUB; CAT>ZnO>TGT> TGB>TiO_2; TZN; THT. Further TDT and NAT showed no activity. For gram-negative *E. coli*, the nanoparticle shows high antibacterial activity in the order of AGT; NAT>THV>TGV>TDV; TDM>TUZ> TGM> CAT> ZnO> TGT> TGB>TiO_2; TZN; THT, while TUB and TDT, were found to show noinhibition.
3. Table 2 Zone of inhibition (mm) by the nanoparticle in gram-positive and gram-negative bacteria.

Nanoparticle	**Gram-positive (mm)**	**Gram-negative (mm)**
ZnO	3	3
TiO_2	0.1	0.1
CAT	5	5
AGT	16.0	14
NAT	0	14
TUB	5	0
TGB	0.5	0.5
TUZ	15.5	10.0
TDM	8	10.5
TGM	11.5	8.0
TGT	1.5	1.5
TGV	7	12.5
THV	13.5	13.5
TDT	0	0
TZN	0.1	0.1
TDV	10.5	10.5
THT	0.1	0.1

A. Effect of ZnO, TiO_2 andCAT

There was an evaluation of the nanoparticles' bactericidal activity. ZnO and CAT micro particles successfully reduced the development of both E. coli and Bacillus brevis on Plate 1 of the experiment. An inhibitory zone of 3.0 0.05 (Gram +ve) was discovered in ZnO. (Gram -ve). To be precise, it was observed in TiO2 that it was between 0.1 and 0.05 (Gram +ve) 0.1 and 0.05 (Gram -ve). Table 2 shows that in CAT it was 5.0 0.05 (Gram +ve) and 5.0 0.05 (Gram -ve) The findings of Amna et al., are consistent with these results (2015). Zinc oxide, titanium oxide, and cadmium were used by Alhadrami & Al-Hazmi (2017); Salehi et al., (2014). As previously observed by Yanping and Alhadrami & Al-Hazmi, (2011), ZnO and TiO2 have high antibacterial action (2017). As a result of ZnO nanoparticles' hydrophilicity, homogenous water dispersion, and stability, Jayaseelan et al. (2012) found an outstanding anti-microbial effect (Jayaseelan et al., 2012). Because TiO2 nanoparticles are very stable and oxidatively target both the outside and interior cell wall membranes, their bactericidal capabilities may be explained (Sarah et al., 2013; Fidel et al., 2010). One of the most efficient microbial inhibitors is ZnO and TiO2. Silver titanium oxide in conjunction with cadmium has strong antibacterial activity. Salehi et al. (2015) conducted extensive research on cadmium's antibacterial properties (2014) That's why cadmium and silver/titanium oxide antibacterial action is widely known..

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B. Effect of AGT, NAT andTUB

Both E. coli and Bacillus brevis growth was successfully suppressed by AGT and NAT microparticles (Plate 2). Although the zone of inhibitory activity for AGT was found to be 16.00.05 in Gram-positive, it was only 14.00.05 in Gram-negative for NAT, with no impact on Gram-positive.

Ming et al. (2015), Besinis et al. (2014), and Fidel et al. (2015) have all reported on the antibacterial effects of Ag and TiO2 (2010). By Lopez et al., new combinations of Ag and TiO2 were discovered (2012). It was discovered in this work that TiO2 alone exhibited less substantial microbial suppression, but that the antibacterial property was increased by many times when mixed with Ag in AGT (Lopez et al., 2012). When Ag ions are released from the AGT, DNA replication is inhibited. Certain cellular proteins are also rendered inactive when bound to Ag ions (Guzman et al., 2012). In the opinion of Morteza et al. (2017); Ashtari et al. (2014), nickel nanoparticles have the best antibacterial capabilities. NAT had no impact on Bacillus sp. when mixed with Ni nanoparticles, Ag, and TiO2 in this investigation, resulting in an effective antibacterial effect on E. coli. Similar to TUB, Joanna et al. (2014) found that europium has no bactericidal action on gramme negative bacteria. Gram-positive bacteria, on the other hand, seem to benefit from TUB's antimicrobial properties.

C. Effect of TGB, TUZ andTDM

TUZ and TDM nanoparticles inhibited both the *E. coli* and *Bacillus brevis* growth effectually (Plate 3) (Gram -ve). In TGB the zone of inhibition was found to be 0.5± 0.05 (Gram +ve); 0.5± 0.05 (Gram -ve). In TUZ the zone of inhibition was found to be 15.5± 0.05 (Gram +ve); 10.0±
0.05 (Gram -ve) and the zone of inhibition in TDM was found to be 8.0± 0.05 (Gram +ve); 10.5± 0.05 (Gram -ve) respectively in Table 2.

As europium doesn't have any antibacterial properties (Joanna et al., 2014), the combination of europium with zinc oxide TUZ shows a high antibacterial effect. Also, Dysprosium (Moradi et al., 2017), Molybdate (Meng and Xiong 2008) Zinc oxide (Pawan et al., 2017) itself has its antimicrobial properties. Hence the combination of all the three TDM (Dysprosium Molybdate doped Zinc oxide) shows good antibacterial properties. TGB Bare Gadolinium itself shows minor antibacterial effectson both gram-positive and negative microbes. According to Franchini et al. (2012) and Valappil et al. (2009) exchanges of ions during protein metabolism in the microbial cells in presence of gallium ions bring about antibacterial activity.

D. Effect of TGM, TGT andTGV

TGM, TGT and TGV nanoparticles inhibited both the E. coli and *Bacillus brevis* growth (Plate 4). The zone of inhibitioninTGMwasfoundtobe 11.5±0.05(Gram+ve);8.0±
0.05 (Gram -ve); In TGT the zone of inhibition was found to be 1.5± 0.05 (Gram +ve); 1.5± 0.05 (Gram -ve); In TGV the zone of inhibition was found to be 7.0± 0.05 (Gram +ve); 12.5± 0.05 (Gram -ve) respectively in Table 2.

The combination of Gadoinium along with molybdate and zinc oxide, TGM (Gadolinium molybdate doped zinc oxide) But the combination of tungstate with gadolinium (TGT- Gadolinium tungstate doped zinc oxide) shows minor activity. Thereareseveralreferencesfora combinationofmetal oxide in anti-microbial properties. The antibacterial activity of the combination of Gadolinium with cerium was reported by Syed et al. (2017). As well as Gadolinium with samarium oxide and erbium oxide was already reported by Dedkova etal. (2017).

Effect of THV, TDT andTZN

Both E. coli and Bacillus growth were slowed by the THV nanoparticle. ' (Plate 5). 13.5 0.05 (Gram +ve) was shown to be the zone of inhibition in THV; 13.5 0.05 (Gram -ve). Instead of the TZN's 0.1 0.05 (Gram +ve) zone of inhibition (Gram -ve). TDT was completely inactive (Table 2).

A good activity is shown when vanadate is used in conjunction with holmium THV (Tungstate holmium vanadate). This does not seem to be the case with Dysprosium (TDT-Dysprosium tungstate doped zinc oxide). There were antimicrobial characteristics in both Tungstate (Moodi et al., 2012) and dysprosium metal (Raffi et al., 2008). It does not, however, have a bactericidal action when combined. Zinc oxide, or TZN, has quite weak antimicrobial activity (Jayaseelan et al., 2012).

Effect of TDV, THT andControl

The microbial growth was successfully suppressed by TDV nanoparticles (Plate 6). There was a 10.5 0.05 (Gram +ve) zone of inhibition in TDV (Gram -ve). Studies on the antibacterial properties of vanadate in conjunction with Dysprosium TDV and other chemicals have been done by Syed et al. (2010) and Moodi et al. (2012). (2012). In the THT-Holmium tungstate doped zinc oxide (THT-Holmium tungstate), there is no activity.

AGT (Ag/TiO2-Silver Titanium Oxide) is the most effective nanoparticle in terms of antibacterial activity against both gramme positive and gramme negative bacteria when compared to other nanoparticles. Silver nanoparticles had the strongest inhibitory effect of all nano semiconductor oxides studied. Because the silver ions contact with the nucleic acid of the bacteria, they destroy them and prevent DNA replication (Raffi 2008). A study by Lee et al. (2007) found that silver isotopes may attach to proteins and disrupt cellular metabolism, resulting in cell death.

Results from a one-way ANOVA demonstrate that the inhibitory zone produced between gramme positive and gramme negative is statistically significant. * p0.05 is the level of significance.

IV. CONCLUSION

For biomedical, pharmaceutical, food-processing, and other industries that need antibacterial properties, nanoparticles' antibacterial impact has been thoroughly investigated. As a new antibacterial agent, the Nanoparticle has unique characteristics and good stability. The results showed that the nanoparticles were capable of preventing the growth of bacteria. The antibacterial activity of AGT (Ag/TiO2), when compared to all 17 nanoparticles, was significantly enhanced by the addition of Ag. The antibacterial activities of silver nanoparticles are primarily mediated by silver ion interactions with biological components. They bind to the cell wall and enter the cell membrane, preventing the development of germs. As a result, bacteria that are exposed to Ag/TiO2 become resistant to a wide range of drugs.

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