Activity of Engineered Nano-Semiconductor Oxides against Gram Positive and Gram Negative Bacteria

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Abstract: Antibacterial activities of semiconductor oxides such as ZnO and TiO_2 have induced research on the design of engineered nanoparticles (ENPs) for the last few decades. We had synthesized modified ZnO and TiO_2 using rare earth salts and analyzed their antibacterial activities against Bacillus brevis. (Gram positive) and E. coli (Gram negative) by modified well diffusion method. Among the seventeen nanoparticles studied, Ag-TiO_2 showed highest antibacterial activity against Gram positive and Gram negative bacteria. Antibacterial activities of Ni-Ag-TiO_2, ZnO, Eu_2O_3-ZnO, $DyMoO_4$-ZnO, $GdMoO_4$-ZnO, $DyVO_4$-ZnO, $GdVO_4$-ZnO and $HoVO_4$-ZnO were found to be significant. From the microbial plating experiments it was shown that the synthesized nano materials show a wide spectrum of antibacterial activities performed against Bacillus brevis, and E. coli. In this study we help to understand the influence of the engineered nanoparticles (ENPs) against microbes, as these ENPs finds wide applications in medicine, water treatment, in food industry as anti-coating agents.

Keywords : : Engineered nano particles (ENPs), Bacillus brevis, E. coli, Antibacterial activity.

I. INTRODUCTION

Application of nano materials ranging from environmental care to human health are highly practiced (Sahoo et al., 2007; Koo et al., 2005; Lopez et al., 2012) Nanoparticles are being viewed as fundamental building blocks of nanotechnology. Engineered nanoparticles (ENPs) have great potential in achieving specific processes and selectivity of conventional materials. It has enormous applications in the field of biological and pharmaceutical engineering (Brigger et al., 2002; Wu et al., 2003). The influence of engineered nano materials on microbes can lead to two different avenue of studies (i) anti-coating agents for applications in medicinal instruments, water treatment reactors, food packaging etc., (ii) the survival and selection of natural microbes on disposal of these nano material after their usage on to the environment (Pattan and Kaul 2014; Swaminathan and Naresh 2017). TiO_2 and ZnO are well known metal oxide semiconductors and are studied extensively for their chemical stability and efficient photocatalytic characteristics. The special electronic, chemical and physical properties of metal oxide nanoparticles
(especially TiO_2 and ZnO) have attracted considerable attention (Wahab et al., 2010).

Specially formulated metal oxide nanoparticles have high antibacterial activity (Stoimenov et al., 2002; Sarah et al., 2013; Rajiv et al., 2013; Xiaoyan et al., 2012; Yanjing et al., 2013). The synthesized TiO_2/ZnO composite powders were highly effective against *E. coli* (Stoyanova et al., 2013). Jaskova et al. (2013) reported that *Escherichia coli* and *Staphylococcus aureus* were effectively static even at the low concentration of nano-ZnO particles. Nanoparticles are proven to be a very good option for antimicrobial additives because of their size which is quite smaller than the size of the cells such that it can easily pass through the membrane (Travan et al., 2011) The nanoparticles interact with bacterial surface or with the bacterial core where it enters inside the cell and subsequently exhibits distinct bacterial mechanisms which leads to either the inactivation or bacterial death (Seil and Webster, 2012).There is an ever increasing demand in developing novel antibacterial agents against bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, , Enterococcus faecalis* and *Campylobacter, Clostridumper fringe* which are well-known pathogens. Synthesis of nano Ag-TiO_2 was reported first time from our group in 2004 for the degradation of direct azo dyes (Sobana et al., 2006). In continuation we prepared modified TiO_2 and ZnO for enhancing the efficiency of these oxides. Recently we reported the synthesis of rare earth oxides loaded ZnO for multiple applications (Thirumalai et al., 2017). In the present work, we investigated the antibacterial effects of seventeen nanoparticles such as ZnO (Zinc oxide) TiO_2 (Titanium dioxide), AGT (Ag/TiO_2), CAT (Cd/Ag/TiO_2), NAT (Ni/Ag/TiO_2), TUB (Bare Eu_2O_3), TGB (Bare Gd_2O_3), TUZ (Eu_2O_3/ZnO- Europium oxide doped Zinc oxide), TDM (Dy MoO_4- Dysprosium molybdate doped Zinc oxide), TGM ($GdMoO_4$/ZnO- Gadolinium molybdate doped Zinc oxide), TDV ($DyVO_4$- Dysprosium vanadate doped Zinc oxide), TGV ($GdVO_4$/ZnO- Gadolinium vanadate doped Zinc oxide), THV ($HOVO_4$/ZnO- Holmium vanadate doped Zinc oxide), TGT ($GdWO_4$/ZnO - Gadolinium tungstate doped Zinc oxide), TDT ($DyWO_6$/ZnO - Dysprosium tungstate doped Zinc oxide), TZN (Commercial ZnO), THT

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(HO_2WO_6- Holmium tungstate doped Zinc oxide) against *Bacillus brevis*, (Gram positive) and *E. coli*, (Gram negative). One of the important application nanoparticles is to control the microbial contaminants in food, water (as a potential safety measure in food preservation) and in medicine and to understand the influence of the ENPs once discharged to 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-coating agents.

II. EXPERIMENTAL SETUP

A. Preparation of engineered nano materials

The characterization and procedure of engineered nanoparticles preparation are described in our earlier papers (Thirumalai et al., 2017). Distilled water was used as solvent to dissolve 100 mg/ml of prepared nanoparticles. This sample was then mixed gently before inoculating into well for proper dispersion of nanoparticles in the distilled water. The naming and category of different nanoparticles are given in Table 1.

B. Antibacterial activity by well diffusion method

Overnight culture of *Bacillus brevis* and *E. coli* (100 µL) were swabbed on the surface of Luria-Bertani agar (Becton Dickinson) prepared using nutrient agar medium. For 10 min the plates were the kept to dry before the well punch. The antimicrobial activities were determined by modified agar well diffusion method (Heatley, 1994). Approximately 5 mm well was made by using a cork borer and 40µL of the test sample was poured into the wells. The plates were then incubated at 37°C for 24 hours. A standard millimeter scale or by a vernier caliper scale was used to measure the diameter of the zone of inhibition as indicated by the clear area which was devoid of growth of microbes (Magaldi et al., 2004; Valgas et al., 2007). All the antibacterial tests were carried out in triplicate and the average is reported.

C. Statistical Analysis

All the experiments were performed under controlled conditions and with appropriate controls. Each experiment was carried out in triplicates and the data are expressed as mean ± standard deviation. To define statistically significant differences, the data are analyzed with one-way analysis of variance ANOVA assuming equal variances at $p<0.01$.

III. RESULT AND DISCUSSION

To our knowledge this is the first study showing the effect of novel engineered tri metallic nanoparticles with significant antibacterial properties. Using well diffusion method, the antibacterial activity of the prepared 17 engineered nanoparticles against *Bacillus brevis* (gram positive) and *E. coli* (gram negative) bacteria was tested. Fig. 1 shows the Clear zones of inhibition of respective engineered nanoparticle (in various plate cultures) against gram positive and gram negative bacteria as seen in Fig. 1. All the values mentioned for zones of inhibition are in mm scale. Fig. 2 shows the comparison of antibacterial activity of the 17 nanoparticles against *Bacillus brevis* and *E. coli*.

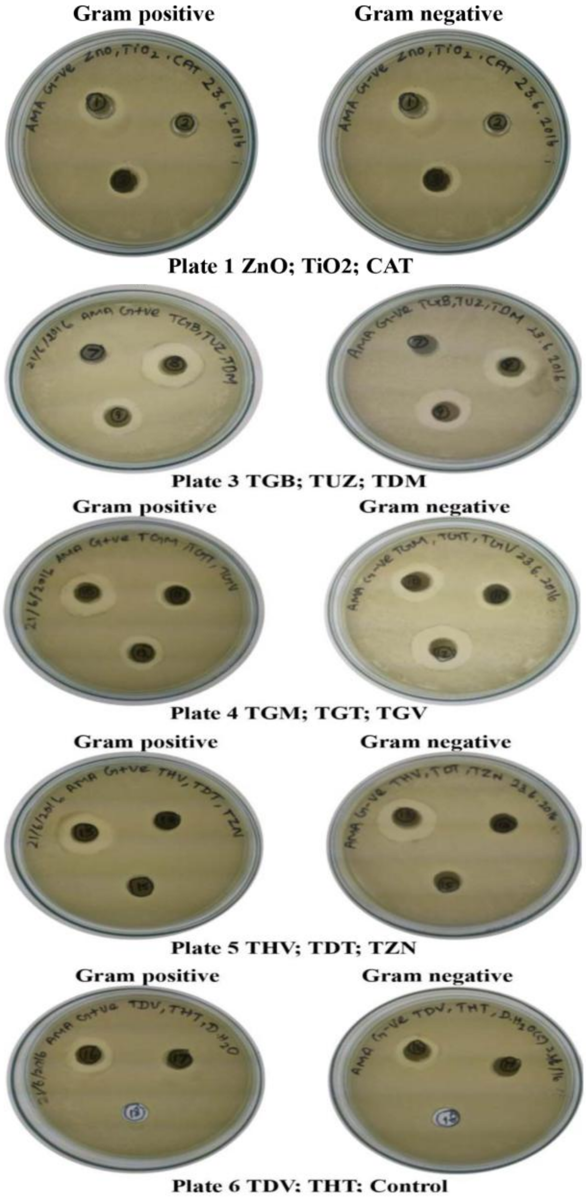

Figure: 1 Inhibition zone comparison of *Bacillus brevis* (Gram +ve) and *E. coli* (Gram -ve)

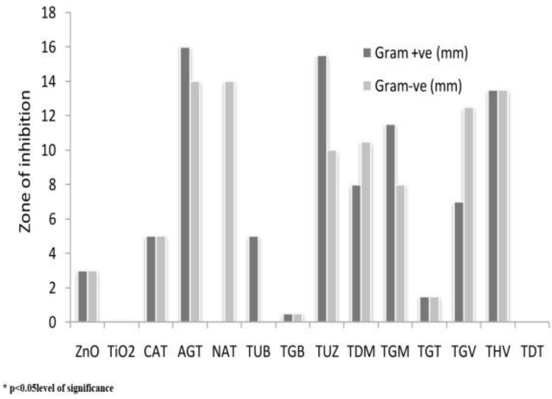

Figure: 2 Comparison of antibacterial activity of synthesized nanoparticle

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 Silver Titanium 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 nano particle 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 nano particle 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 no inhibition.

Table 2 Zone of inhibition (mm) by the nanoparticle in gram positive and gram negative bacteria.

Nano particle	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 and CAT

The effect of bactericidal activity of the prepared nano particles was recorded. Plate 1 demonstrated that ZnO and CAT nano particles inhibited both the *E. coli* and *Bacillus brevis* growth effectively. The zone of inhibition in ZnO was found to be 3.0± 0.05 (Gram +ve); 3.0± 0.05 (Gram -ve). In TiO_2 it was found to be 0.1± 0.05 (Gram +ve) 0.1± 0.05 (Gram -ve). Whereas in CAT it was found to be 5.0± 0.05 (Gram +ve); 5.0± 0.05 (Gram -ve) respectively in Table 2. These results are correlated by the findings of Amna et al., (2015). Alhadrami & Al-Hazmi (2017); Salehi et al., (2014) using zinc oxide, titanium oxide, cadmium. ZnO and TiO_2 shows good antibacterial activity which has been previously reported by Amna, (2015); Yanping, (2011); Alhadrami & Al-Hazmi, (2017). Jayaseelan et al (2012) have showed excellent anti-microbial effect of ZnO nano particles due to its important properties such as hydrophilicity, uniform water dispersion and stability (Jayaseelan et al., 2012). The bactericidal properties of TiO_2 nanoparticles can be explained by its stability and oxidative attack on outer/inner cell wall membrane (Sarah et al., 2013; Fidel et al., 2010). In our study also ZnO and TiO_2 has been proved to be an effective microbial inhibitor. The combination of cadmium silver titanium oxide, however shows good antibacterial effect. The antibacterial effect of cadmium was very well studied by Salehi et al. (2015); Salehi et al. (2014) Hence it is very well understood that the addition of cadmium along with silver and titanium oxide shows good antibacterial activity.

B. Effect of AGT, NAT and TUB

AGT and NAT nano particles inhibited both the *E. coli* and *Bacillus brevis* growth effectively (Plate 2). The zone of inhibition in AGT was found to be 16.0± 0.05 (Gram +ve); 14.0± 0.05 (Gram -ve), whereas in NAT it was found to be 14.0± 0.05 (Gram -ve) and no effect in Gram positive. And for TUB it was found to be 5.0± 0.05 (Gram +ve) and no effect in Gram negative (Table 2). Joanna et al., (2014) also reported in their study that europium ions do not show any bactericidal effect on *E. coli*.

The antibacterial effect of Ag and TiO_2 have been reported separately by Ming et al., (2015) Besinis et al. (2014); Fidel et al. (2010). Further Ag and TiO2 combinations by Lopez et al. (2012). In this study it was found that TiO_2 itself showed less significant microbial inhibition but when combined with Ag in AGT, the antimicrobial property was found to increase by several folds (Lopez et al., 2012). This is due to the release of Ag ions from AGT which binds with DNA decreasing its replication activity; also certain cellular proteins become deactivated on binding with Ag ions (Guzman et al., 2012). According to Morteza et al. (2017); Ashtari et al. (2014) the nickel nanoparticles have best antimicrobial properties. In this study, the Ni nanoparticles were combined with Ag and TiO_2 Therefore a significant bactericidal effect was found on *E. coli* (inhibition zone 10.0 mm) while *Bacillus* sp., was not influenced in presence of NAT. Similarly, TUB showed no activity on gram negative, Joanna et al. (2014) study also says that europium doesn't show bactericidal activity. But TUB shows some antibacterial effect in gram positive bacteria.

C. Effect of TGB, TUZ and TDM

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 high antibacterial effect. Also Dysprosium (Moradi et al., 2017), Molybdate (Meng and Xiong 2008) Zinc oxide (Pawan et al., 2017) itself has their antimicrobial properties. Hence by the combination of all the three TDM (Dysprosium Molybdate doped Zinc oxide) shows good antibacterial properties. TGB Bare Gadolinium itself shows minor antibacterial effect on 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 brings about the antibacterial activity.

D. Effect of TGM, TGT and TGV

TGM, TGT and TGV nanoparticles inhibited both the E. coli and *Bacillus brevis* growth (Plate 4). The zone of inhibition in TGM was found to 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 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. There are several reference for combination of metal oxide in anti-microbial properties. The antibacterial activity of combination of Gadolinium with cerium were reported by Syed et al. (2017). As well as Gadolinium with samarium oxide and erbium oxide were already reported by Dedkova et al. (2017).

E. Effect of THV, TDT and TZN

THV nanoparticle inhibited both the E. coli and Bacillus growth (Plate 5). The zone of inhibition in THV was found to 13.5± 0.05 (Gram +ve); 13.5± 0.05 (Gram -ve). Whereas the zone of inhibition in TZN was found to 0.1± 0.05 (Gram +ve); 0.1± 0.05 (Gram -ve). TDT showed no activity (Table 2).

The combination of vanadate with holmium THV (Tungstate holmium vanadate) shows good activity. But with Dysprosium (TDT-Dysprosium tungstate doped zinc oxide) it doesn't show any bactericidal effect. Tungstate (Moodi et al., 2012) and dysprosium metal (Raffi et al., 2008) individually were able to produce antibacterial properties. But when came into mixture it fails to produce bactericidal effect. TZN (zinc oxide) shows minor antibacterial properties (Jayaseelan et al., 2012).

F. Effect of TDV, THT and Control

TDV nanoparticle inhibited the microbial growth effectively (Plate 6). The zone of inhibition in TDV was found to 10.5± 0.05 (Gram +ve); 10.5± 0.05 (Gram -ve). The antibacterial effect for combination of vanadate with compounds like Dysprosium TDV were already studied by Syed et al. (2010) and Moodi et al. (2012). The combination of Holmium with tungstate (THT-Holmium tungstate doped zinc oxide) shows no activity.

By comparing all the different nanoparticles, AGT (Ag/TiO_2- Silver Titanium Oxide) shows high antibacterial activity against gram positive and gram negative bacteria. The inhibitory action of silver nanoparticles is higher amongst all nano semiconductor oxides tested. This is because the silver ions degrade the bacteria by interacting with the nucleic acid and thereby stop the DNA repletion (Raffi 2008). According to Lee et al. (2007) silver ions bind with the protein molecule and inhibit the cellular metabolism and causes death to the microorganism.

One-way ANOVA results show that the zone of inhibition formed between gram positive and gram negative are significantly different. The level of significance is obtained as * $p<0.05$.

IV. CONCLUSION

The antibacterial effect of Nano particle has been studied to know its applications in biomedicine, pharmaceuticals, food industry etc., The Nanoparticle possess unique properties and excellent stability for acting as a novel antibacterial agent. The achieved data revealed that the synthesized nano particles have potential antibacterial property. Comparing all the 17 nano particles AGT (Ag/TiO_2) showed high antibacterial activity just by incorporating Ag antibacterial agent. The interaction of silver ions with the cellular component is the basic mode of action of the antibacterial properties of the nanoparticles. Those silver ions adhere the cell wall and penetrate through the cell membrane and inhibit the bacterial growth. Hence Ag/TiO_2 develop multidrug resistant against microorganism and act as a substitute to antibiotics.

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