Shape-Dependent Antibacterial Activity against *Escherichia coli* of Zinc Oxide Nanoparticles

Nor Hazliana Harun[1], Rabiatul Basria S.M.N. Mydin[1*], Srimala Sreekantan[2], Khairul Arifah Saharudin[2], Norfatehah Basiron[2], Fakrul Radhi[3], Azman Seeni[1,4]

[1]Oncological and Radiological Sciences Cluster, Advanced Medical & Dental Institute, Universiti Sains Malaysia, Penang, Malaysia

[2]School of Materials & Mineral Resources Engineering, Engineering Campus, Universiti Sains Malaysia, Penang, Malaysia

[3]Faculty of Science, Universiti Malaya 50603 Kuala Lumpur, Malaysia

[4]Malaysian Institute of Pharmaceuticals and Nutraceuticals (IPHARM), National Institute of Biotechnology Malaysia, Penang, Malaysia

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*Corresponding author:
Rabiatul Basria S.M.N. Mydin
E-mail: rabiatulbasria@usm.my

Abstract - Zinc oxide (ZnO) nanoparticles (NPs) has become as promising candidate for antibacterial agents against *Escherichia coli* (*E.coli*), commensal hospital- acquired infections (HAIs). This study investigates the antibacterial action of ZnO NPs in three difference shapes; nanorod, nanoflakes and nanospheres against *E.coli* ATCC 25922. The antibacterial activity of ZnO NPs was determine through two standard protocols known as Clinical Laboratory Standards Institute (CLSI) MO2-A11 under light conditions of 5.70 w/m^2 and American standard test method (ASTM) E-2149. Preliminary screening shows ZnO NPs did not inhibit the growth of *E.coli.* Further analysis using ASTM E-2149 in dynamic conditions revealed antibacterial activity after 3 hours with 100% reduction for ZnO NPs nanoflakes and 6 hours with 94.63% reduction for ZnO nanospheres, respectively. It demonstrated the ZnO NPs in nanoflakes and nanospheres exerted higher antibacterial activity possibly through release of ios, free radicals, ROS generation and electrostatic collision which contribute to bacterial death. Further analysis is needed to investigate biocompatibility of these samples for future biomedical applications.

Keywords **– Zinc oxide nanoparticles, Hospital-acquired infections, Antibacterial activity, *Escherichia coli***

1 INTRODUCTION

Presently scientists have focus on the potential of metal oxide nanoparticles (NPs) in biomedical applications as antibacterial agent due to its photocatalytic properties. An increasing number of patients recurrently exposed to the *Escherichia coli* (*E.coli*) colonization, a gram-negative strains that commonly associated with urinary tract infections (UTI) patients and other hospital-acquired infections (HAIs) leading to increased rates of morbidity and mortality, challenging treatment and healthcare costs. Zinc oxide (ZnO) metal oxide NPs is of the promising candidate as antibacterial agents as it a photocatalytic NPs that's works under light and without light conditions by generating reactive oxygen species (ROS) free radicals and ion release [1-3].

2 METHODOLOGY

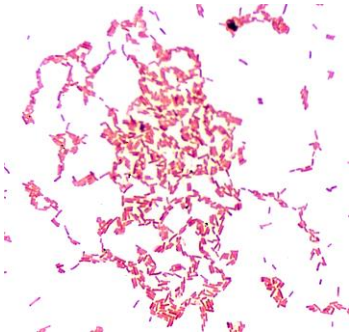
The present study aims to compare the interfacial potential of different shaped ZnO NPs such as nanorod, nanoflakes, and nanospheres against *E.coli* (ATCC 25922). ZnO NPs were produced by solution precipitation method and incorporated with low density polyethylene (ZnO NPs-LDPE) as described by [4]. The bacterial morphology was further studied by using gram staining with crystal violet and safranin method. The preliminary screening was done according to Standard Clinical Laboratory Standards Institute (CLSI) protocol MO2-A11 for test samples with dimension: 6.0 x 6.0 cm^2 under light conditions of 5.70 w/m2 [5] prior incubation for two hours. Further, the antibacterial activity was confirmed by using another standard protocol known as

American standard test method (ASTM) E-2149 on test samples with dimensions 2.7 x 2.7 cm^2 at 1.5-3.0 x 105 cfu/mL *E.coli* bacterial dilution for 1, 3 and 6 hour under light treatment condition

3 RESULTS AND DISCUSSION

Generally, the Gram staining methods is used to study the classification of bacterial either “Gram-positive” and “Gram-negative” and shape morphology either cocci, rods or spiral-shaped. As shown in Figure 1, under the microscope, the *E.coli* appear in pink in color with rod-shaped. The absence of purple color confirming the gram-negative *E.coli*. Noted that difference in cell wall structure and peptidoglycan layer thickness resulting a different color staining results [6], a gram-positive bacteria retain violet-stained due to the thick layer of protein-sugar complexes or known as peptidoglycan and low levels of lipid. However, in case of gram-negative bacteria, it will retain the red-stained due for having a thin layer of peptidoglycan and thick layer of lipids [7]. Crystal violet-iodine complex unable to retain in the gram-negative bacteria cell wall and be washed away together with lipids by decolorizer. Subsequently, it counter-stained by safranin and hence, appears red or pink in color.

a)



b)

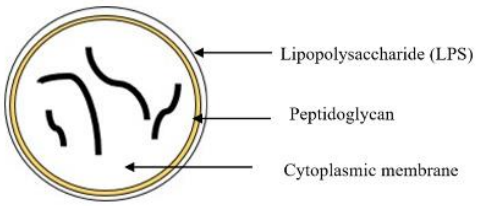


Figure. 1: (a) *E.coli* from the culture stock showed pink-rod shaped morphology which confirm it is a gram-negative at 100X magnification. **(b)** Schematic structure of *E.coli* cell walls. Gram-negative cell walls have an inner and outer membrane, LPS and a thin layer of peptidoglycan compared to gram- positive bacteria.

The preliminary screening data guided by CLSI MO2-A11 shows that all shapes of ZnO NPs did not inhibit the growth of *E.coli*, as shown in Figure 2. The greater complexity of the *E.coli* for having an extra outer membrane as additional shield, increased difficulty for ZnO NPs to penetrate into bacterial cell membrane [8-10]. However, a noteworthy antibacterial activity was observed under ASTM E-2149 assay after 3 hours with 100% reduction for ZnO NPs nanoflakes and 6 hours with 94.63% reduction for ZnO nanospheres, respectively in Table 1. The antibacterial effects that governed by surface area can be explained based on our previous work on scanning electron microscopy (SEM) which showed the morphology characterization for each shapes of ZnO NPs (data not shown here). ZnO NPs inhibit or kill bacteria through various mechanisms such as the released of zinc ions and electrostatic force of attraction. The released ZnO NPs in nanoflakes and nanospheres directly pitting on the bacterial cell wall. The collision between cell wall and ZnO ions due to electrostatic force of interaction between positively charges ZnO ions and negatively charge bacterial cells ruptures the outer membrane of *E.coli* resulting the leakage of cytoplasmic contents [11]. Therefore, this will lead easier penetration for ZnO NPs into bacterial cell membrane and causing the bacterial death. Another study by Maqbool et al. 2016 [12], stated that generation of (ROS) generated by ZnO NPs is responsible as another possible route for antibacterial activity. Upon irradiation under visible light treatment in aqueous suspension of ZnO NPs, two different photochemical reaction pathways may occur; generation of hydroxyl radicals and superoxide ions (electron transfer process,Type I) and formation of singlet oxygen (energy transfer, Type II). Formation of photogenerated ROS can disrupt the bacterial cellular membranes which cause overall damage to bacterial cell.

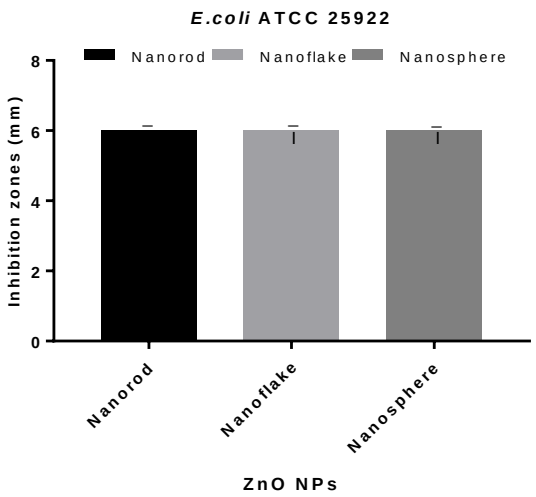


Figure. 2: Effect of different shapes ZnO NPs on the growth of *E.coli* ATCC 25922. Inhibition zones were measured after 72 hours after exposure with light for 2 hours prior incubation treatment. The experiments were done in triplicates.

Table 1: Bacterial reduction percentage results of different shapes ZnO NPs on the growth of *E.coli* ATCC 25922 pathogens. Colony were counted in triplicate and reduction % was measured after 1, 3 and 6 hours after exposure with visible light (Mean ± Standard deviation reduction).

a)

Time (Hour)	ZnO NPs	
	Nanorods	
	Inoculum (CFU/mL)	Bacterial reduction (%)
0	(1.67 ± 0.58) x 10^2	-
1	(0.66 ± 0.58) x 10^2	60.48
3	(1.33 ± 1.15) x 10^2	20.36
6	(0.66 ± 0.58) x 10^2	60.48

b)

Time (Hour)	ZnO NPs	
	Nanoflakes	
	Inoculum (CFU/mL)	Bacterial reduction (%)
0	(3.0 ± 1.73) x 10^2	-
1	(1.0 ± 1.73) x 10^2	66.67
3	0.0	100
6	0.0	100

c)

Time (Hour)	ZnO NPs	
	Nanospheres	
	Inoculum (CFU/mL)	Bacterial reduction (%)
0	(1.2 ± 0.59) x 10^3	-
1	(8.33 ± 1.52) x 10^2	32.28
3	(8.0 ± 2.64) x 10^2	34.96
6	(0.66 ± 1.15) x 10^2	94.63

4 CONCLUSION

This study highlighted that ZnO NPs with nanoflakes and nanospheres exerted higher antibacterial activity possibly through the release of ZnO ions, free radicals and generation of ROS. The electrostatic force of interaction between ZnO ions and bacterial cell walls also enhanced the interaction with the cell membrane, thus leading to bacterial death. Further study is need to explain the interfacial potential of ZnO NPs with nanoflakes and nanospheres shapes as antibacterial agent for biomedical applications.

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REFERENCES

[1]. Jesline, A., John, N. P., Narayanan, P. M., Vani, C., & Murugan, S. (2015). Antimicrobial activity of zinc and titanium dioxide nanoparticles against biofilm-producing methicillin- resistant Staphylococcus aureus. Applied Nanoscience, 5(2), 157-162.

[2]. Aysa, N. H., & Salman, H. D. (2016). Antibacterial activity of modified zinc oxide nanoparticles against Pseudomonas aeruginosa isolates of burn infections. World Scientific News, (33), 1-14.

[3]. McGuffie, M. J., Hong, J., Bahng, J. H., Glynos, E., Green, P. F., Kotov, N. A., & VanEpps, J. S. (2016). Zinc oxide nanoparticle suspensions and layer-by-layer coatings inhibit staphylococcal growth. Nanomedicine: Nanotechnology, Biology and Medicine, 12(1), 33-42.

[4]. Chandraiahgari, C. R., De Bellis, G., Ballirano, P., Balijepalli, S. K., Kaciulis, S., Caneve, L., & Sarto, M. S. (2015). Synthesis and characterization of ZnO nanorods with a narrow size distribution. RSC Advances, 5(62), 49861-49870.

[5]. S. E. Edition, "CLSI document M02-A11", Wayne,

PA: Clinical and Laboratory Standards Institute, 32(1), 76 (2012).

[6]. Thairu, Y., Nasir, I. A., & Usman, Y. (2014). Laboratory perspective of gram staining and its significance in investigations of infectious diseases. Sub-Saharan African Journal of Medicine, 1(4), 168.

[7]. Aryal, S. (2018, June 12). Gram Staining: Principle, Procedure, Interpretation, Examples and Animation. Retrieved from https://microbiologyinfo.com/gram-staining-principle-procedure- interpretation-examples-and-animation/.

[8]. Clifton, L. A., Skoda, M. W., Daulton, E. L., Hughes, A. V., Le Brun, A. P., Lakey, J. H., & Holt, S. A. (2013). Asymmetric phospholipid: lipopolysaccharide bilayers; a Gram-negative bacterial outer membrane mimic. Journal of the Royal Society Interface, 10(89), 20130810.

[9]. Santos, R. S., Figueiredo, C., Azevedo, N. F., Braeckmans, K., & De Smedt, S. C. (2017). Nanomaterials and molecular transporters to overcome the bacterial envelope barrier: towards advanced delivery of antibiotics. Advanced drug delivery reviews.

[10]. Bajaj, H., Acosta Gutierrez, S., Bodrenko, I., Malloci, G., Scorciapino, M. A., Winterhalter, M., & Ceccarelli, M. (2017). Bacterial outer membrane porins as electrostatic nanosieves: exploring transport rules of small polar molecules. ACS nano, 11(6), 5465-5473.

[11]. Ramalingam, B., Parandhaman, T., & Das, S. K. (2016). Antibacterial effects of biosynthesized silver nanoparticles on surface ultrastructure and nanomechanical properties of gram-negative bacteria viz. Escherichia coli and Pseudomonas aeruginosa. ACS applied materials & interfaces, 8(7), 4963-4976.

[12]. Maqbool, Q., Nazar, M., Naz, S., Hussain, T., Jabeen, N., Kausar, R., & Jan, T. (2016). Antimicrobial potential of green synthesized CeO2 nanoparticles from Olea europaea leaf extract. International journal of nanomedicine, 11, 5015