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THE INFLUENCE OF THE BIOCHEMICAL COMPOSITION OF THE BACTERIAL CELL WALL ON THE ANTIBACTERIAL PROPERTY OF MONOLAYER GRAPHENE

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Abstract

Graphene is an allotrope of carbon consisting of a single tightly packed layer of sp^2 carbon atoms arranged in a hexagonal lattice. Graphene has unique properties that could revolutionize different areas, including biology, biomedical science, environmental agriculture and biotechnology. Many studies are aimed mainly at antibacterial effect of graphene. Antibacterial properties of graphene are manifested by directly interacting with the cell membrane. In this study we aim to investigate the aspects of the role of the biochemical composition of the bacterial cell wall in the manifestation of the antibacterial activity of the monolayer graphene on metallic and non-metallic substrates. Antibacterial activity was tested on Gram-positive and Gram-negative bacteria via cell-viability test. The differences in structure and composition of the Gram-positive and Gram-negative cell wall induce different interactions with monolayer graphene. At the same time, the different substrate for the graphene film significantly influences its antibacterial properties. Our observations from this study provide new insights for future studies.

***Key words**: antimicrobial activity, bacterial cell wall, graphene.*

INTRODUCTION

A relatively recent area of research focuses on the study of the interactions between nanomaterials and cells. Over the past decade research into the properties and uses of graphene has rapidly expanded. Graphene is a two-dimensional ultra-thin nanomaterial composed only of hybridized-sp^2 carbon atoms, arranged in a hexagonal structure, similar to that of a honeycomb. Graphene displays specific and unique physico-chemical properties which vary significantly to the material graphite, particularly in terms of electron mobility. These particular properties determine complex interactions with cells (Wolf, 2014). Many researches are aimed mainly at antibacterial effect of graphene, its ability to inhibit bacterial proliferation on its surface, even when the bacteria have all the conditions for optimal growth (Li Jinhua *et al.,* 2014; Kurantowicz *et al.,* 2015; Hegab *et al*., 2016). The factors influencing antibacterial effect of graphene are still not fully understood. The biochemical composition of bacterial cell wall, surface charge and hydrophobicity of non-polar molecules are factors that lead the occurrence specific and non-specific interaction between the bacterial cell and graphene (Y. Luan, 2018).

The bacterial cell wall contains numerous complex biological molecules. The distinction between Gram-positive and Gram-negative bacteria is given by their cell walls chemical composition. In both Gram-negative and Gram-positive bacteria the major components of the cell wall are peptidoglycans, macromolecules composed of sugars and amino acids that provide structural strength and confer the characteristic cell shape (Hogan *et al.,* 2010). The cell wall peptidoglycans of Gram-positive bacteria contain polysaccharide chains highly cross-linked with peptides, while the cell wall peptidoglycans of Gram-negative bacteria have polysaccharide chains partially cross-linked with peptides.

The peptidoglycan layer is thicker in Gram-positive bacteria (20 to 80 nm) and help to support the cell membrane. The cell walls of Gram positive bacteria also contain chains of teichoic and lipoteichoic acids, polysaccharides peptidoglycolipids, covalently attached to the peptidoglycan (Brown *et al.,* 2013). Wall teichoic acids are anionic glycopolymers that can account for as much as 60 % of the total cell wall mass in Gram-positive bacteria. They assist in cellular integrity and play a role in cell division and Gram-positive bacterial physiology (Swoboda *et al.,* 2009). The peptidoglycan layer of Gram-negative cells is thinner (1 to 10 nm). But unlike in Gram-positive bacteria, Gram-negative bacteria have an outer membrane layer that is external to the peptidoglycan cell wall. The outer membrane contains a lipopolysaccharide component, a large glycolipid complex that protects bacteria. The lipopolysaccharide molecules are characteristic of Gram-negative bacteria and are not found in Gram-positive bacteria (Coleman *et al.,* 2014).

In this context, we have been following the link between the biochemical composition of the bacterial cell wall and the antibacterial activity of monolayer graphene. The antibacterial activity of graphene against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacterial strains was compared.

MATERIALS AND METHODS

Two types of monolayer graphene films on different substrates were synthesized by chemical vapor deposition (CVD): graphene on copper (G-Cu) and graphene on silicon (G-Si), foils with a surface area of 2 cm^2 and thickness of 35 μm. We used these monolayer graphene films to evaluate their antibacterial activity against both Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria.

The antibacterial activity was done by cell-viability test. In brief, the pure cultures of organisms were subcultured in nutrient agar substrate, incubated for 24 hours at 35°C ± 2°C and brought up by dilution according to the 0.5 McFarland standards to approximately 10^8 colony-forming units (CFU)/ml.

Each bacterial suspension was placed upon graphene sheets. After overnight incubation at 35°C ± 2°C each bacterial suspension previously overlaid with graphene is removed from the graphene surface, recultivated in solid growth media and evaluation of bacterial survivability immediately. Viable bacterial colonies were counted and recorded by the naked eye by cell-viability test (CFU plate counting). The experiments were carried out in triplicate, and averages were reported.

RESULTS AND DISCUSSIONS

The observed antibacterial activity was found to be bacterial cell wall dependent (his biochemical composition) and types of monolayer graphene. Colony forming units (CFU) counting was used to assess the bactericidal effect of both types of monolayer graphene. It was demonstrated that both graphene matrices effectively inhibit *S. aureus* and *E. coli* cell growth with viability loss. Antibacterial effects of the graphene sheets on copper (G-Cu) and silicon (G-Si) are significantly different for the Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria. To explain the antibacterial mode of action of graphene on different substrates we suggest production of reactive oxygen species, oxidative stress and damage to bacterial cell wall integrity. The cell membranes are severely damaged and even are missing their cytoplasm entirely. The *Staphylococcus aureus* strain exhibited significant reduction ratio of bacterial CFU, compared to viability of *Escherichia coli* strain (Figure 1).

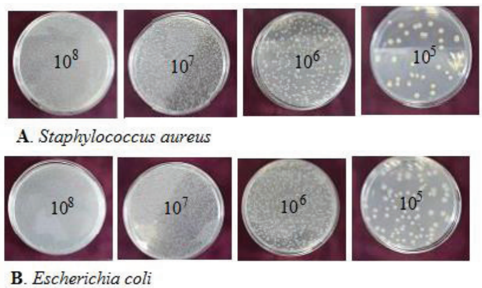

Figure 1 Viable bacterial cell of *Staphylococcus aureus* (A) and *Escherichia coli* (B) on graphene (G-Si) for various concentrations of bacterial suspension

The outer membrane of Gram-negative bacteria acts as a permeability barrier and is partly responsible for this differential antibacterial activity. The destructive effect of graphene on the bacterial cell wall is induced by a lower surface phospholipid density which results in a loss of cell membrane integrity. Furthermore, the destructive effect of graphene coated surfaces is related and to the electronic properties of the substrate. The monolayer graphene on copper substrate has been found to be a much more efficient as inhibitor of the bacterial multiplication. (Table 1).

Table 1. Bacterial response (CFU) for *Staphylococcus aureus* and *Escherichia coli* strains after being incubated on the different monolayer graphene

Monolayer graphene	*Staphylococcus aureus*			*Escherichia coli*		
	10^7 CFU/mL	10^6 CFU/mL	10^5 CFU/mL	10^7 CFU/mL	10^6 CFU/mL	10^5 CFU/mL
G-Cu	56 ± 3 CFU	21 ± 4 CFU	2 ± 1 CFU	82 ± 2 CFU	46 ± 2 CFU	12 ± 2 CFU
G-Si	110 ± 4 CFU	47 ± 2 CFU	18 ± 3 CFU	202 ± 2 CFU	61 ± 3 CFU	22 ± 2 CFU

Graphene-on-substrate junctions can act as electrons pump that by interfering with membrane receptors and with bacterial electron transport away from the microbial membrane, thus producing oxidative stress in the membrane. For *Staphylococcus aureus* strain the teichoic acids in the bacterial cell wall composition form a dense network of negative charges on Gram-positive cell surfaces, a gradient of ions which amplifies and accelerates the osmotic pressure change between the inside and outside of the cell, oxidative stress induced by graphene.

For *Escherichia coli* strain (Gram-negative bacteria) the outer membrane and the thin layer of peptidoglycan stabilizes the inner membrane such that withstand the high osmotic pressures within the cell and oxidative stress induced by graphene.

CONCLUSIONS

The graphene sheet on copper substrate may have antibacterial effect by reducing or inhibiting the viability of certain bacteria that are in direct contact with it, depending on the specific biochemical composition of the bacterial cell wall. Losing viability may be an effect of an oxidative stress generated by conductive character of graphene. The antibacterial properties of monolayer graphene have been shown to be lower for *Escherichia coli* strain (Gram-negative), due to cell wall biochemical composition and structure, more complex than that of the *Staphylococcus aureus* strain (Gram-positive).

REFERENCES

Brown, S., Santa Maria, J., Walker, S. (2013). Wall Teichoic Acids of Gram-Positive Bacteria. *Annual Review of Microbiology,* 67:313-336

Coleman, J.P., Smith, C. J. (2014). Structure and Composition of Microbes. *Reference Module in Biomedical Sciences*

Hegab, H.M., El-Mekawy, A., Mulcahy, D., Saint, C.P. (2016). The controversial antibacterial activity of graphene-based materials. *Carbon,* 105: 362-376.

Hogan, M.C. (2010). Bacteria. *Encyclopedia of Earth*. Eds. Sidney Draggan and C.J.Cleveland, National Council for Science and the Environment, Washington

Kurantowicz N, Sawosz E, Jaworski S, Kutwin M, Strojny B. (2015). Interaction of graphene family materials with Listeria monocytogenes and Salmonella enterica. *Nanoscale Res Lett*, 10: 23.

Li J, Wang G, Zhu H, Zhang M, Zheng X. (2014). Antibacterial activity of large-area monolayer film manipulated by charge transfer. *Scientific Reports*, 4: 4359.

Luan, Y., Liu, S., Busscher, H.J. (2018). Bacterial interactions with nanostructured surfaces. *Current Opinion in Colloid & Interface Science.* 38:170-189

Swoboda, J. G., Campbell, J, Meredith, T.C., Walker, S. (2009). Wall Teichoic Acid Function, Biosynthesis, and Inhibition. *ChemBioChem,* 11(1): 35–45

Wolf, E. L. (2014). Applications of Graphene: An Overview. *Springer Briefs in Materials*