Online Journal of Biological Sciences 14 (3): 230-239, 2014
ISSN: 1608-4217

doi:10.3844/ojbsci.2014.230.239 Published Online 14 (3) 2014 (http://www.thescipub.com/ojbs.toc)

GRAPHENE OXIDE AS ANTIMICROBIAL AGAINST TWO GRAM-POSITIVE AND TWO GRAM-NEGATIVE BACTERIA IN ADDITION TO ONE FUNGUS

[1]Roda F. Al-Thani, [2]Noorunnisa Khanam Patan and [2]Mariam A. Al-Maadeed

[1]Department of Biological and Environmental Sciences,
College of Arts and Sciences, Qatar University, P.O. Box 2713 Doha, Qatar
[2]Center for Advanced Materials, Qatar University, Qatar

Received 2014-07-20; Revised 2014-08-29; Accepted 2014-09-08

ABSTRACT

Graphene based materials have wide potential applications in biology, biomedical, agriculture environmental and biotechnology. Graphene Oxide (GO) is one of those materials and has a promising substance as antimicrobial agents. GO in this study was prepared by a modified Hummers method and was characterized by different techniques for confirmation of formation of GO. To study the antimicrobial activities of GO, it was tested against these microorganisms, one eukaryotic fungus (*Candida albicans*, *C. albicans*) two Gram negative bacteria (*Escherichia coli* (*E. coli*) ATCC 41570 and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 25619) and two Gram positive bacteria (*Streptococcus faecalis* (*S. faecalis*) ATCC 19433 and *Staphylococcus aureus* (*S. aureus*) ATCC 11632). Anti-microbial activity of GO was detected by spectrophotometer as indirect method to measure the growth and viable cell count as direct method. Readings were taken at successive incubated times. Results revealed that GO has antibacterial and anti-fungal activity against microorganisms used in this study. In conculosion the developed GO exhibit excellent antimicrobial property and GO affects more on Gram positive bacteria than Gram negative bacteria and fungi.

Keywords: Antimicrobial, Graphene Oxide, Gram Negative Bacteria, Gram Positive Bacteria, Eukaryotic Cells

1. INTRODUCTION

Graphene Based Materials (GBMs) include few-layer graphene, graphene nanosheets, graphene oxide and reduced graphene oxide. Graphene comprising of single-atom-thick sheets of sp2- bonded carbon. It is a typical two-dimensional material made of carbon atoms packed densely in a honeycomb crystal lattice (Geim and Novoselov, 2007; Sanchez *et al*., 2011). Also they have unique electronic and mechanical properties and demonstrate great potential for applications in many areas such as field effect transistors, solar cells, sensors and adsorbent for heavy metal removal (Zhang *et al*., 2010). Graphene Oxide (GO) is chemically modified graphene, containing hydroxyl, carbonyl and epoxy functional groups, which is obtained by synthesis of graphite with strong oxidizing agents (Alves *et al*., 2014). Also it has been used as a promising material for preparing new composites (Tang *et al*., 2013). It is well known that GO and its composites possess anti-microbial properties and have been used as anti-bacterial and anti-fungal agents (Santos *et al*., 2012; De Faria *et al*., 2014).

The effect and interaction of GBMs on microbial cells structure, metabolism and viability has been shown to depend on the materials' concentration, time of exposure and physical-chemical properties, as well as on the characteristics of microorganisms used in the tests (Akhavan and Ghaderi 2010; Hu *et al*., 2010; Liu *et al*.,

Corresponding Author: Roda F. Al-Thani, Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, P.O. Box 2713 Doha, Qatar Tel: +974 4403 4539 Fax: +974 44034531

2011; De Faria *et al*., 2014). There are different mode of action of GBM into microbial cells some studies suggests disruption cell wall and membranes because of sharp edges of GO or because of generation of Reactive Oxygen Species (ROS) which may be fatal factor for microbial cells (Chen *et al*., 2013). GBMs have been tested as antibacterial against food borne pathogens bacteria e.g., *E. coli* and *S. aureus* (Santos *et al*., 2012; Liu *et al*., 2012; Tang *et al*., 2013); opportunistic pathogens bacteria e.g., *P. aerogenosa* and *Klebseilla* sp. (Lim *et al*., 2012; Bykkam *et al*., 2013); plant pathogen e.g., *Xanthomonas oryzae* (Chen *et al*., 2013) and against fungi e.g., *C. albicans* and *C. tropical* (Li *et al*., 2013). Also several studies demonstrated and developed an environment friendly, cost effective, simple method and green approaches for the reduction of GO using microbial cells such as *E. coli*, *Shewanella* and Yeast (Gurunathan *et al*., 2013; Wang *et al*., 2011; Khanra *et al*., 2012).

In this research study, the GO prepared by modified Hammers method and its antimicrobial activity against one fungus (*C. albicans*) two prokaryotic bacteria Gram-negative bacilli (*E. coli* ATCC 41570 and *P. aeruginosa* ATCC 25619) and two prokaryotic bacteria Gram-positive cocci (*S. feacalis* ATCC 19433 and *S. aureus* ATCC 11632) was tested. Also the effect of incubated time was studied, start after 24 h incubation for 4 days.

2. MATERIALS AND METHODS

2.1. Preparation of Graphene Oxide (GO)

GO was prepared from natural graphite flakes by a modified Hummers method (Akhavan and Ghaderi 2012; Shahriary and Athawale 2014; Zheng *et al*., 2013). Briefly, graphite (3.0 g) was added to concentrated H_2SO_4 (70 mL) under stirring at room temperature. Then NaNO3 (1.5 g) was added and the mixture was cooled to 0ºC. Under vigorous agitation, KMnO4 (9.0 g) was added slowly to keep the temperature of the suspension lower than 20ºC. The mixture was stirred at 35ºC for 2 h. Then distilled water (150 mL) was added and the solution was stirred at 90ºC for 15 min. Additional 500 mL of distilled water was added and followed by a slow addition of 15 mL of H_2O_2 (3%), turning the color of the solution from dark brown to yellow. The mixture was filtered and washed with 1:10 HCl aqueous solution (250 mL) to remove metal ions followed by washing with 200 mL of distilled water to remove the acid. The resulting solid was dried in air and diluted to get a GO aqueous dispersion (0.5 wt%).

2.2. Structure and Characterization of the Prepared GO

Structure and characterization of GO was confirmed by four methods include, Powder X-ray diffraction which was carried out by X-ray diffract meter, model Rigaku MiniFlex2. XRD was used to measure the size of the particle; the thermo gram of the graphite and graphene oxide was recorded by Perkin Elmer thermo gravimetric analyzer. Thermo Gravimetric Analysis (TGA) was carried out under nitrogen atmosphere by using TA instrument. The samples were heated from room temperature to 600°C at 10°C/min. Fourier Transform Infrared spectroscopy (FT-IR) spectral analysis of all samples were cryogenically cooled and powdered. The powders were diluted to 1% using potassium Bromide (KBr) and pellets were prepared. The FT-IR spectra of the samples were recorded in the 4000-500 cm^{-1} region on a FT-IR spectrophotometer model 670 (NEXUS) Nicolet in transmittance model with resolution of 4 cm^{-1} with 34 scans. FT-IR was performed to identify types of chemical bonds, i.e., functional groups in a molecule. The surface morphological analysis of samples was carried out by using a Scanning Electron Microscope (SEM-EDX Philips). Samples were used without any coating for SEM analysis.

2.3. Antimicrobial Activity of GO

2.3.1. Preparations of Microorganism Cells

The 5 microorganisms used were as follows one eukaryotic fungus (*C. albicans*) two prokaryotic bacteria Gram negative bacilli (*E. coli* ATCC 41570 and *P. aeruginosa* ATCC 25619) and two prokaryotic bacteria Gram positive cocci (*S. faecalis* ATCC 19433 and *S. aureus* ATCC 11632). Microorganisms were cultured on Nutrient Agar (NA) for 24 h. Four to five well isolated colonies from overnight culture were transferred using sterile loop to the tube of sterilized 0.8% saline solutions (10 mL). The inoculums was emulsified inside the saline tube to avoid clumping of the cells and incubated at 37°C for 10 min to adjust the inoculums standard to a 0.5 McFarland which equals approximately 108 CFU/mL.

2.3.2. Agar Diffusion

Within 10 min of preparing the adjusted inoculums, a sterile cotton swab was dipped into the inoculums (separately for the 5 selected microorganisms) and streaked over the entire surface of the 5 nutrient agar plates. Subsequently, GO disk (5 mm in diameter) was placed on surface of each inoculated plate using sterile

forceps. After application, insure that the disk has made complete contact with the agar surface by touching the top of the disk with forceps. Also a piece of filter paper is immersed into GO solution and placed over the agar previously inoculated with the microorganisms. The plates are then incubated at 37°C for 24 h.

2.3.3. Spectrophotometer and Viable Count

Each microorganism were grown in 10 mL of nutrient broth with GO disk (10 mg) at 30°C for 24 h under 100 rpm shaking speed. After 24 h. incubation the reading of turbidity for each cultures with GO for each microorganisms was record using the spectrophotometer at 600 nm and compared with control to monitoring the microorganisms growth and multiply of cells in liquid media. By spectrophotometer analysis the increased culture turbidity of the reading reflects the index of microbial growth and cell numbers (biomass) and the amount of transmitted light decreases as the cell population increases. The absorbance, or Optical Density (OD), was read at wavelength 600nm and it gives an indirect measurement of the number of microbial population. To this purpose, the absorbance of 1 mL of each broth culture was measured by the spectrophotometer (JENWAY 6305 UV-VIS Spectrophotometer). The spectrophotometer measurement was made by standardizing the machine on the sterile nutrient broth with its concentration equal to zero. The spectrophotometer measurements were performed at 1, 2, 3 and 4 days of incubation with GO disk. This spectrophotometer analysis was paralleled by measurement of the loss of microorganism viability which was counted by transferred and spread of 100 µL of the cultures to sterilized NA plate and incubated at 37°C for 24 h. Media with GO and without bacteria was used as control.

2.4. Scanning Electron Microscope (SEM)

SEM was used to examine the interactions between GO disk and microorganisms cells. From each 5 different cultures GO disk (5mm in diameter) were examined using a scanning electron microscope (SEM-EDX Philips) without any coating.

3. RESULTS AND DISCUSSION

3.1. Preparation and Characterization of GO

3.1.1. X-ray Diffraction Analysis (XRD) of Graphite and GO

The XRD pattern of graphite and GO are shown in **Fig. 1A and B** respectively. As shown in XRD of graphite powder, a strong sharp reflection peak appeared at 26.52º indicating a higher ordered structure, that corresponding to an interlayer spacing of about 3.35 A° (0.335 nm). The XRD patterns of GO samples (**Fig. 1B**) shows that with oxidation, a formation of new broad peak at 2θ = 10.92º with interlayer spacing of about 8.09 A° (0.809 nm). This peak has lower intensity compared to the graphite peak. This change comes from the heterogeneous nature of the oxidized graphite. The peak of graphite (26.52º) starts decreases in GO, due to the oxidation and this peak decreases and the appearance of a peak at 24.2º is observed with interlayer spacing of 3.7018 A° (0.37018 nm). The observed interlayer spacing of S-3 was 3.7018 A° (0.37 nm), due to the presence of oxygenated functional groups and intercalated water molecules which corresponds to the GO. The XRD results of GO samples are in good agreement with the literature (Kaniyoor *et al*., 2010; Du *et al*., 2010; Gurunathan *et al*., 2012).

3.1.2. Thermogravimetric Analysis (TGA)

The TGA for graphite and GO are shown in **Fig. 2A and B** respectively. It is clear from the **Fig. 2A** that, graphite was thermally stable up to 600°C. After oxidation, for GO (**Fig. 2B**) slight mass decrease at 177°C and significant decrease to 209°C are noticed. It shows two degradation step and maximum weight loss takes place at 231.29°C. This was caused by loss of water molecules, loss of oxygen-containing groups and above 500°C relates to an unstable carbon remaining in the structure and the pyrolysis of oxygen functional groups in the main structure to yield CO and CO_2. (Bagri *et al*., 2010; Fan *et al*., 2010; Loryuenyong *et al*., 2013).

Figure 3 shows the FT-IR spectrum of GO. The stretching vibrations of hydroxyl groups, O-H stretching was observed at 3489 cm^{-1}. The peak at 1730 cm^{-1} shows C = O (carbonyl/carboxy) stretching and peak at 1618 cm^{-1} can be assigned to the skeletal vibrations of un oxidized graphitic domains. The peak at 1300 cm^{-1} show for C-O (carboxy) and peak at 1238 cm^{-1} for C-O-H deformation peak. The C-O stretching vibration peak shows at 1027 cm^{-1}. All these bands related with the oxygen containing functional groups in GO and the presence of these oxygen-containing groups reveals that the graphite has been oxidized. The polar groups, especially the surface hydroxyl groups, result in the formation of hydrogen bonds between graphite and water molecules; this further explains the hydrophilic nature of GO (Guo *et al*., 2009; Shahriary and Athawale, 2014).

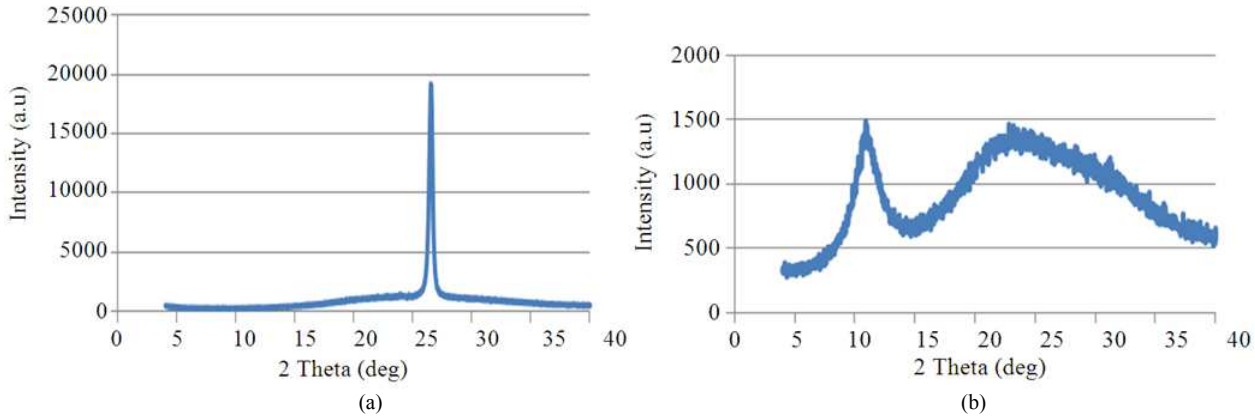


Fig. 1. XRD Patterns of graphite (A) and GO (B)

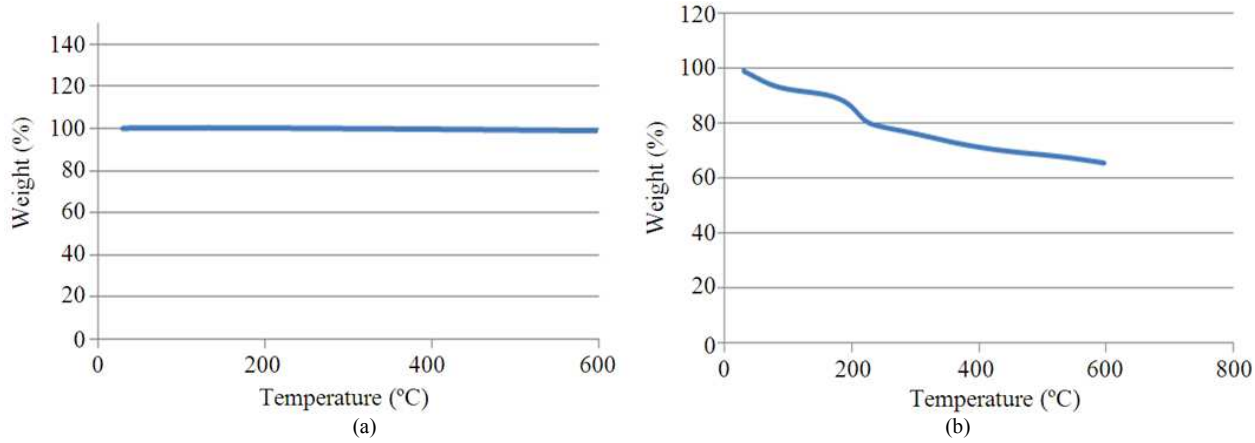


Fig. 2. TGA patterns of graphite powder (A) and GO (B)

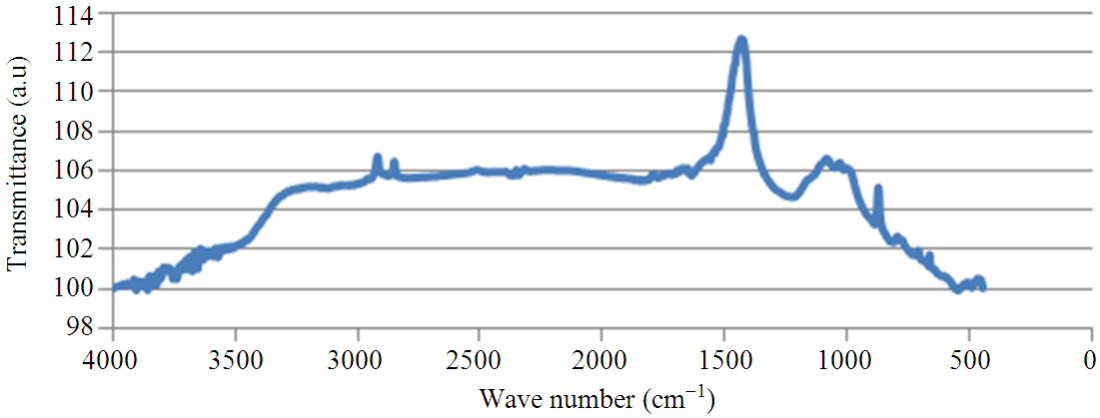
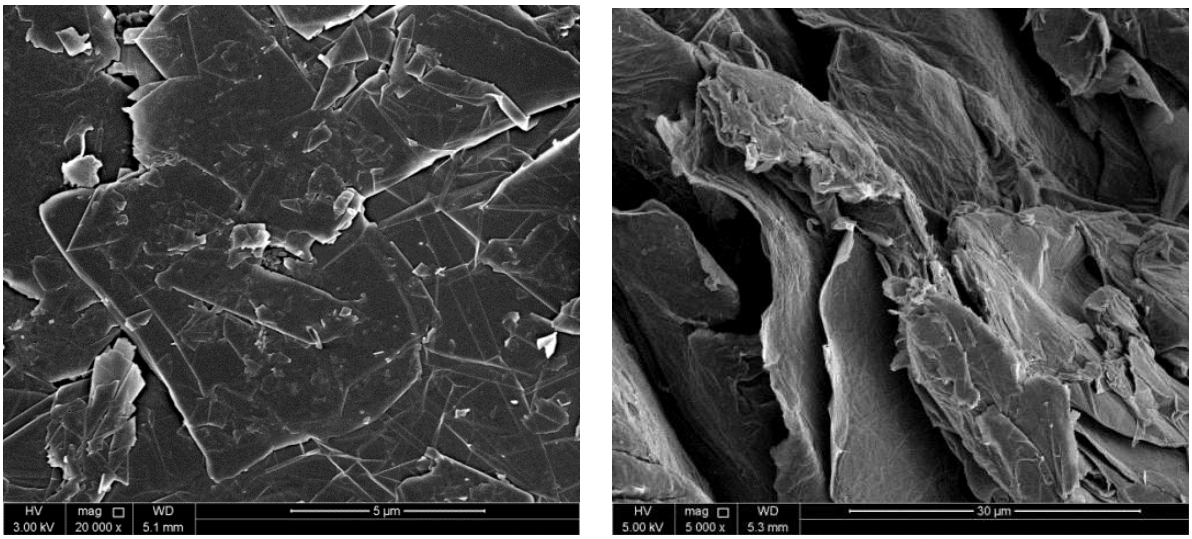


Fig. 3. FT-IR spectrum of GO



(a) (b)

Fig. 4. SEM images of graphite powder (A) and GO (B) before incubation with the microorganisms

3.1.4. Scanning Electron Microscope

Figure 4A and B show the SEM images of graphite and GO respectively before incubation with the microorganisms. SEM photos of graphite shows flaky shape and irregular, thicker platelets are also present in the powder. SEM photos of GO (**Fig. 4B**) shows that, the exfoliated graphite particles were smaller than graphite and the sheets were smooth with small wrinkles at the edges.

3.2. The Antimicrobial Activity of GO

3.2.1. Agar Diffusion

The agar diffusion assay is a fast and simple to estimate the susceptibility of microorganisms toward an antimicrobial agent such as GO. This test is based on the diffusion of the nanomaterial from high concentrations (disk or filter paper) to the agar surface. Das *et al*. (2011) however, it allows only a qualitative result about the susceptibility of the microbial strain. For fast grower microorganisms such as what we used in this study, the results interpreted after 24 h. incubation at 37°C, if the GO has activity, clear zones (no growth of microorganism) will be observed around the disk or filter paper. The presence or absence of growth inhibition zone was interpreted as sensitive or resistant of microorganisms to the GO agent.

E. coli and *P. aerogenosa* are Gram negative bacteria, facultative anaerobic, motile, non-sporulation and cells are typically rod-shaped while *S. aureus* and S. *faecalis* are Gram positive bacteria, facultative anaerobic, non-motile, non-sporulation and cells are typically spherical-shaped. The cell wall is different between both types; Gram negative bacteria possess a thin peptidoglycan layer with another layer structured called the outer Lipopolysaccharide membrane (LPS) whereas Gram positive bacteria possess a thick peptidoglycan layer and no outer lipopolysaccharide membrane (Tortora *et al*., 2013). The cell wall is very important because it can serve as a resistant barrier to some particles and other cells or it can be serve as a target for many antibiotics. A cell wall lets a bacterial cell have its defining shape. The results shows that GO (both if it was apply as disk or filter paper immersed into GO solution) affected more on Gram positive bacteria, which not have the outer lipopolysaccharide membrane in their cell wall, than Gram negative bacteria and fungi (**Fig. 5A and B** respectively).

3.2.2. Spectrophotometer and Viable Count

Figure 6 indicated the OD values of the growth of the 5 microorganisms incubated with GO at different time (24, 48, 72 and 96 h), the OD value of a control sample (broth with GO film). This behavior confirmed the good antibacterial activity of such material. For all types of microorganisms, the growth was totally inhibited when cultured on NB with GO film. The OD 600 of microbial growth through 4 days indicated that, first and second days of incubation the growth of bacterial species was increased and not affected by GO

but after that time the growth was stop and no increase of cells biomass which indicated antimicrobial activities of GO against those bacteria. These results compared and confirmed by the viable cell culture which give the same results with no growth after 4 days. Similar results were obtained with other researchers which also suggested that antibacterial activities of GO are time, concentration and size dependent in addition to the negatively charged membranes of bacterial cells (Liu *et al*., 2012; Li *et al*., 2014). All microbial cells used in this study have the same generation time, the time required for a cell to divide or a population to double, about 60min except the *E. coli* which is about 30min that why this species is very fast grow bacteria than the other (**Fig. 6**).

Toxic by-products known as Reactive Oxygen Species (ROS) are produced by GO, which would affect microorganisms viability. These ROS include hydrogen peroxide, superoxide anion radicals, singlet oxygen, hydroxyl radicals and nitric oxide. To help protect against the destructive effects of ROS, aerobic organisms and facultative anaerobic microorganisms produce protective antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase. Catalases are proteins that catalyse the conversion of Hydrogen Peroxide (H_2O_2) to water and molecular oxygen, thereby protecting cells from the toxic effects of hydrogen peroxide. Catalases are produced by all microorganisms used in this study except *S. faecales* which is microaerophilic. Li *et al* (2014) suggested the antibacterial activity of graphene does not stem from ROS mediated damage, but through electron transfer interaction from microbial membrane to graphene.

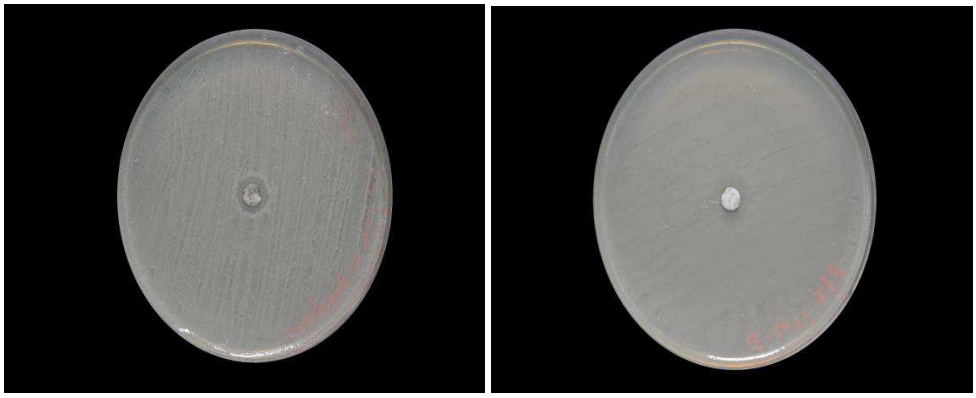


Fig. 5. Antibacterial activity of filter paper immersed into GO solution, positive result (growth inhibition zone) against *S. aureus* (A) and negative result (no growth inhibition zone) against *E. coli* (B)

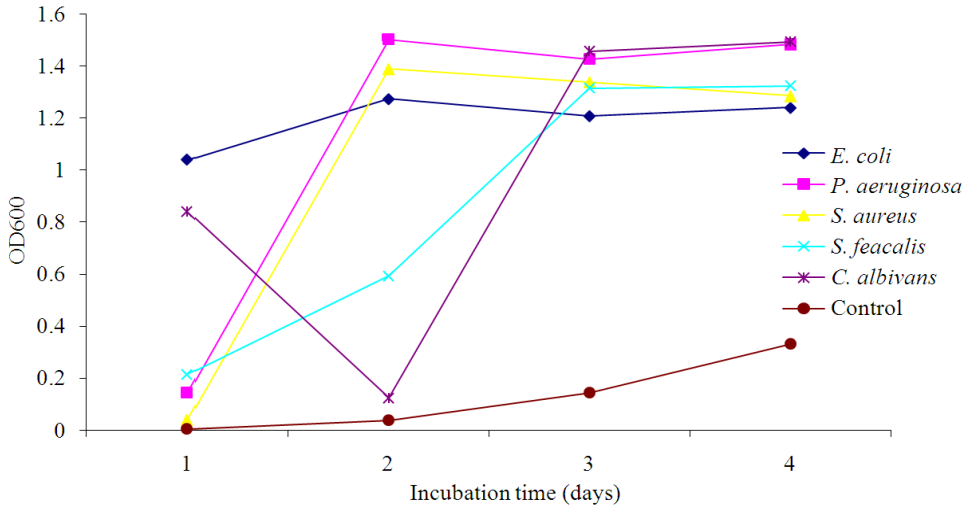


Fig. 6. The OD values of the 5 microorganisms growth incubated with GO at different time

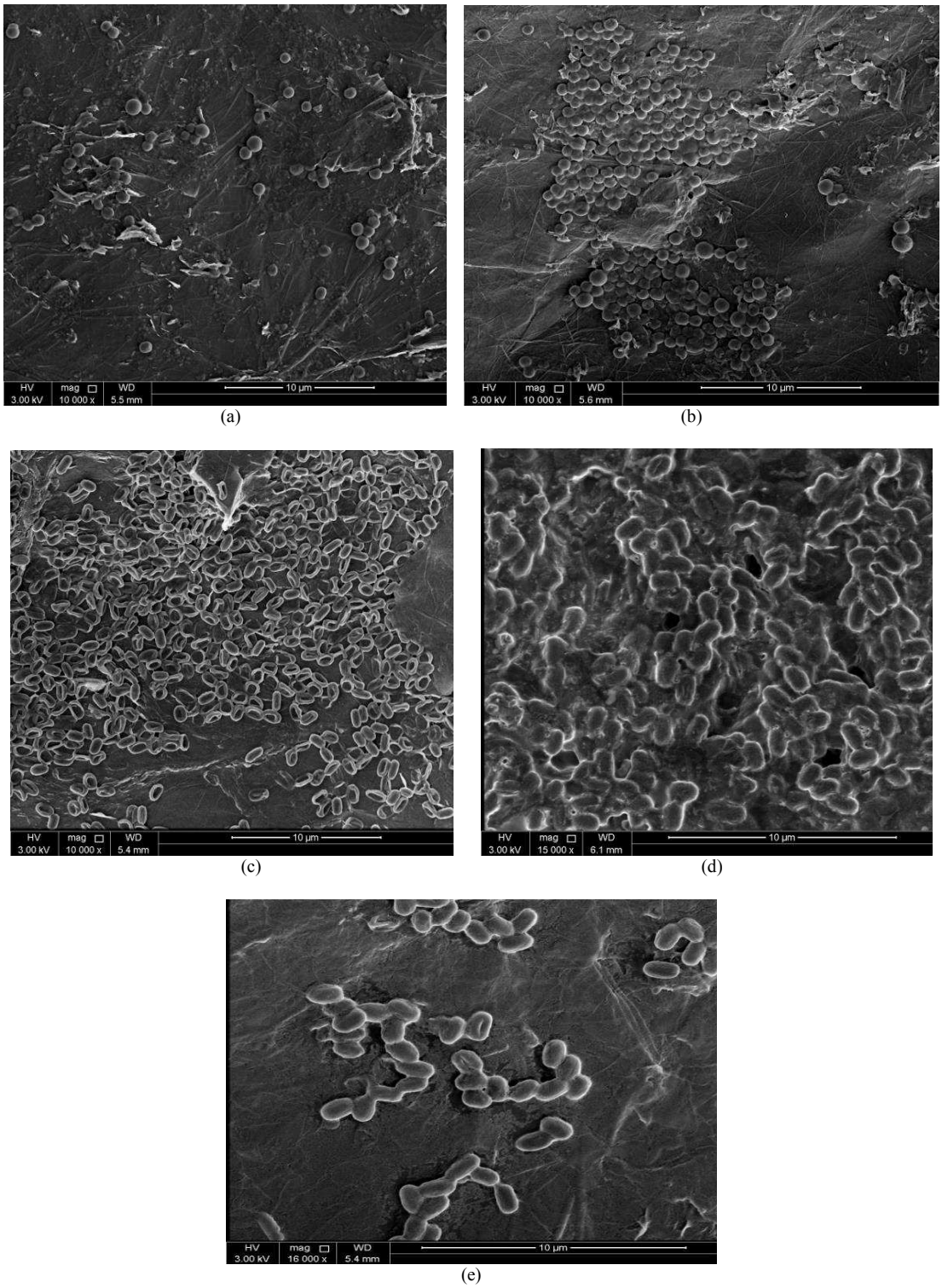


Fig. 7. SEM images of *S. aureus* (A), *S. feacalis* (B), *E. coli* (C), *P. aeruginosa* (D) and *C. albicans* (E)

*C. albicans*is eukaryotic fungus and cell structure and metabolism are more complex than bacterial cells that why not any antimicrobial can affect or stop their growth. Li *et al*., (2013) found promise anti-fungal activity of GO against *C. albicans*. **Figure 6** shows the loss of viability of *C. albicans*incubated with GO at different incubator time. The loss of viability was increased with incubation time.

3.2.3. Scanning Electron Microscope

The action mechanisms of antimicrobial drugs into living cell could be inhibition of cell wall synthesis or inhibition of cell membrane function or inhibition of protein synthesis or inhibition of nucleic acid synthesis (Tortora *et al*., 2013). To know more about the interaction between microbial cells and GO disks, SEM was used to demonstrate these interactions.

Figure 7A and B show Gram positive cells (*S. aureus* and *S. faecales* respectively) the morphologies of most of the survived cells remained unchanged with round shape and smooth surface, no cell divided within demonstrating at SEM images which indicted the inhibition of cell division of those cells by the GO. **Figure 7C and D** show that most of Gram negative cells (*E. coli* and *P. aerogenosa)* become flattened wrinkled and damaged and lose their integrity after exposure to GO. The destruction of cells in SEM images is consistent with previous images obtained by scanning electron microscope (Tang *et al*., 2013). **Figure 7E** demonstrate the *C. albicans* cells with less effective, the reason why the GO was less effective to inhibit the fungi cells, is probably due to the cell walls type of fungi. The structure and metabolism of fungal cells are more complex and resistant to antimicrobial and to high osmotic pressure.

In addition to the cell wall bacteria secrete a variety of Extracellular Polymeric Substances (EPS), including polysaccharides, proteins and nucleic acids that vary in molecular mass and structural properties (Notley *et al*., 2013). **Figure 7B and D** indicated EPSs as attached capsular polysaccharides and as free polysaccharides released into the growth medium. This layer acts as affording the cell proytection from major bacterial pathogens, play a major role in the bacterial colonization of surfaces, biotic and abiotic, by enabling cell adhesion and co-aggregation via dipole interactions, covalent or ionic bonding, steric interactions and hydrophobic association, making the target surface more attractive for bacterial attachment.

4. CONCLUSION

This study demonstrates the preparation of GO by using modified Hummers method and characterization results confirm the GO formation. We studied the antimicrobial activity of prepared GO toward four prokaryotic bacterial species and one eukaryotic fungal species. All microorganisms used in this study are common, can cause disease in animals, including humans and can be found in soil, water, skin flora and colonized many natural and artificial environments. All microbial tests were carried out with different timings for every 24 h. The developed GO exhibit excellent antimicrobial property and GO affects more on Gram positive bacteria than Gram negative bacteria. The cell growth was decrease with the increase of the incubation time. The antimicrobial activates may be attributed to membranes disruption or stop cell division or oxidative stress, which leads the cell death. GO inhibits the microbial growth which prove that GO is useful as anti-microbial agent for different microorganisms. The cell death was lower in *C. albican* when compare with bacteria because these types of cells are more complex in there structures and metabolism than prokaryotic bacterial cells. The emergence of antibiotic-resistant strains of pathogenic bacteria is a universal problem in clinical medicine and can cause life-threatening infections in humans, especially in the nosocomial environment So it is very important to look for more materials that can be used as antimicrobial agents in addition to focus in and studies the mechanisms of interactions between GBMs and different living prokaryotic and eukaryotic cells to concern the potential impact of graphene and its derivatives on humans and environmental health. Also further investigations necessary to understand the molecular basis of GO action and genetic materials of microbial cells (mutation, expression and resistant genes).

5. ACKNOWLEDGMENT

This study is supported by QF/NPRP fund for project titled “Tailored Structuring and Properties of Polymer-Graphene and Polymer-Graphite Nanoplateletes Composites”. The authors would like to thank the Department of Health Sciences at Qatar University for microorganisms cultures.

6. REFERENCES

Akhavan, O. and E. Ghaderi, 2010. Toxicity of graphene and graphene oxide nanowalls against bacteria. ACS Nano., 4: 5731-5736. DOI: 10.1021/nn101390x

Akhavan, O. and E. Ghaderi, 2012. *Escherichia coli* bacteria reduce graphene oxide to bactericidal graphene in a self-limiting manner. Carbon, 50: 1853-1860. DOI:10.1016/j.carbon.2011.12.035

Alves, O.L., C.M. Ana de Moraes, M.B. Simoes, L.C. Fonseca and R.O. do Nascimento *et al*., 2014. Nanomaterials. In: Nanomedicine and Nanotoxicology, Duran, N., S.S. Guterres and O.L. Alves (Eds.)., Springer, New York, pp: 1-29.

Bagri, A., C. Mattevi, M. Acik, Y.J. Chaba and M. Chhowalla *et al*., 2010. Structural evolution during the reduction of chemically derived graphene oxide. Nat. Chem.. 2: 581-587. DOI: 10.1038/NCHEM.686

Bykkam, S., V.K. Rao, S.C.H. Chakra and T. Thunugunta, 2013. Synthesis and characterization of graphene oxide and its antimicrobial activity against *Klebseilla* and *Staphylococcus*. Int. J. Adv. Biotech. Res., 4: 142-146.

Chen, J., X. Wang and H. Han, 2013. A new function of graphene oxide emerges: Inactivating phytopathogenic bacterium *Xanthomonas oryzae* pv. *Oryzae*. J. Nanopart. Res., 15: 1658-1672. DOI: 10.1007/s11051-013-1658-6

Das, M.R., R.K. Sarma, R. Saikia, V.S. Kale and P.S. Shelke *et al*., 2011. Synthesis of silver nanoparticles in an aqueous suspension of graphene oxide sheets and its antimicrobial activity. Colloids Surfaces B, 83: 16-22. DOI:10.1016/j.colsurfb.2010.10.033

De Faria A.F., A. C. M. de Moraes and O. L. Alves, 2014. Toxicity of Nanomaterials to Microorganisms: Mechanisms, Methods, and New Perspectives. In: Nanomedicine and Nanotoxicology, Duran, N., S.S. Guterres and O.L. Alves (Eds.)., Springer, New York, pp: 363-405.

Du, Q., M. Zheng, L. Zhang, Y. Wang and J. Chen *et al*., 2010. Preparation of functionalized graphene sheets by a low-temperature thermal exfoliation approach and their electrochemical supercapacitive behaviors. Electrochim. Acta, 55: 3897-3903. DOI: 10.1016/j.electacta.2010.01.089

Fan, Z., K. Wang, T. Wei, J. Yan and L. Song *et al*., 2010. An environmentally friendly and efficient route for the reduction of graphene oxide by aluminum powder. Carbon, 48: 16861689. DOI: 10.1016/j.carbon.2009.12.063

Geim, A.K. and K.S. Novoselov, 2007. The rise of graphene. Nat. Mater., 6:183-191. DOI: 10.1038/nmat1849

Gurunathan, S., J.W. Han, A.A. Dayem, V. Eppakayala and J.H. Kim, 2012. Oxidative stress-mediated antibacterial activity of graphene oxide and reduced graphene oxide in *Pseudomonas aeruginosa*. Int. J. Nanomed., 7: 5901-5914. DOI: 10.2147/IJN.S37397

Gurunathan, S., J.W. Han, V. Eppakayala and J. Kim, 2013. Microbial reduction of graphene oxide by *Escherichia coli*: A green chemistry approach. Colloids Surfaces B, 102: 772-777. DOI: 10.1016/j.colsurfb.2012.09.011

Guo, H.L., X.F. Wang, Q.Y. Qian, F.B. Wang and X.Y. Xia, 2009. A green approach to the synthesis of graphene nanosheets. ACS Nano, 3: 2653-2659. DOI: 10.1021/nn900227d

Hu, W.B., C. Peng, W.J. Luo, M. Lv and X.M. Li *et al*., 2010. Graphene-Based Antibacterial Paper. ACS Nano. 4: 4317-4323. DOI: 10.1021/nn101097v

Kaniyoor, A., T.T. Baby and S. Ramaprabhu, 2010. Graphene synthesis via hydrogen induced low temperature exfoliation of graphite oxide. J. Materials Chem., 20: 8467-8469. DOI: 10.1039/C0JM01876G

Khanra, P., T. Kuila, N.H. Kim, S.H. Bae and D. Yu *et al*., 2012. Simultaneous bio-functionalization and reduction of graphene oxide by baker's yeast. Chem. Eng. J., 183: 526-533. DOI: 10.1016/j.cej.2011.12.075

Li, C., X. Wang, F. Chen, C. Zhang and X. Zhi *et al*., 2013. The antifungal activity of graphene oxide-silver nanocomposites. Biomaterials., 34: 3882-3890. DOI: 10.1016/j.biomaterials.2013.02.001

Li, J., G. Wang, H. Zhu, M. Zhang and X. Zheng *et al*., 2014. Antibacterial activity of large-area monolayer graphene film manipulated by charge transfer. Scientific Rep., 4: 4359-4367. DOI: 10.1038/srep04359

Lim, H.N., N.M. Huang and C.H. Loo, 2012. Facile preparation of graphene-based chitosan films: Enhanced thermal, mechanical and antibacterial properties. J. Non-Crystalline Solids, 358: 525-530. doi:10.1016/j.jnoncrysol.2011.11.007

Liu, S., T.H. Zeng, M. Hofmann, E. Burcombe, J. Wie, R. Jiang, J. Kong and Y. Chen, 2011. Antibacterial activity of graphite, graphite oxide, graphene oxide and reduced graphene oxide: Membrane and oxidative stress. ACS Nano, 5: 6971-6980. DOI: 10.1021/nn202451x

Liu, S., M. Hu, T.H. Zeng, R. Wu and R. Jiang *et al*., 2012. Lateral dimension-dependent antibacterial activity of graphene oxide sheets. Langmuir, 28: 12364-12372. DOI: 10.1021/la3023908

Loryuenyong, V., K. Totepvimarn, P. Eimburanapravat, W. Boonchompoo and A. Buasri, 2013. Preparation and characterization of reduced graphene oxide sheets via water-based exfoliation and reduction methods. Adv. Materials Sci. Eng.. 2013: 923403-923407 . DOI: 10.1155/2013/923403

Notley, S.M., Russell J.C. and E.P. Ivanova, 2013. Bacterial Interaction with Graphene Particles and Surfaces, Nanotechnology and Nanomaterials, In: Advances in Graphene Sciences. Aliofkhazraei, M. (Ed.)., ISBN 978-953-51-1182-5.

Sanchez, V.C., A. Jackhak, R.H. Hurt and A.B. Kane, 2011. Biological interactions of graphene-family nanomaterials: an interdisciplinary review. Chem. Res. Toxicol., 25: 15-34. DOI: 10.1021/tx200339h

Santos, C.M., J. Mangadlao, F. Ahmed, A. Leon and R.C. Advincula *et al*., 2012. Graphene nanocomposite for biomedical applications: fabrication, antimicrobial and cytotoxic investigations. Nanotechnology, 23: 395101-395101. DOI: 10.1088/0957-4484/23/39/395101

Shahriary, L. and A. Athawale, 2014. Graphene oxide synthesized by using modified hummers approach. IJREEE., 2: 58-63.

Tang, J., Q. Chen, L. Xu, S. Zhang and L. Feng *et al*., 2013. Graphene oxide-silver nanocompositeas a highly effective antibacterial agent with species-specific mechanisms. ACS Appl. Mater. Interfaces., 5: 3867-3874. DOI: 10.1021/am4005495

Tortora, G.J., B.R. Funke and C.L. Case, 2013. Microbiology: An Introduction. 11th Edn., Pearson Benjamin Cummings, San Francisco.

Wang, G.M., F. Qian, C.W. Saltikov, Y.Q. Jiao and Y. Li, 2011. Microbial reduction of graphene oxide by shewanella. Nano. Res., 4: 563-570. DOI: 10.1007/s12274-011-0112-2

Zhang, Y.B., S.F. Ali, E. Dervishi, Y. Xu, and Z.R. Li *et al*., 2010. Cytotoxicity effects of graphene and single- wall carbon nanotubes in neural phaeochromocytoma-derived PC12 cells. ACS Nano, 4: 3181-3186. DOI: 10.1021/nn1007176

Zheng, L., D. Ye, L. Xiong, J. Xu and K. Tao *et al*., 2013. Preparation of cobalt-tetraphenylporphyrin/reduced graphene oxide nanocomposite and its application on hydrogen peroxide biosensor. Analyticachimicaacta, 768: 69-75. DOI: 10.1016/j.aca.2013.01.019