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Antimicrobial Properties of Sonochemically Treated Graphene Oxides Sheets

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Abstract

Graphene oxide (GO) has been used as a promising material for antimicrobial surface due to its contact-based antimicrobial activity. The antimicrobial activity of GO was thought to be mediated by physical and chemical interactions when sheets come in direct contact with bacterial cells. Antimicrobial surfaces have important applications in the biomedical field for preventing microbial contamination of medical devices, or in environmental systems where bio-fouling is a major cause of increased operation costs in marine transport, membrane-based water treatment, and heat exchangers. It has been reported that, the antimicrobial activity depends up on the size of GO sheets. Herein, In order to know the accurate assessment of the antimicrobial activity of GO through the suspension assays and surface coatings, we used ultrasonic irradiations to tune the size of GO synthesized through an electrochemical exfoliation method assisted by a surfactant, SDS, in a concentration of 0.01 M to study the antimicrobial activity towards a gram-positive bacteria, *Enterococcusfaecalis* (cocci) and, a gram-negative bacteria, *Escherichia coli* (rod-shaped). It has been found that, the ultrasonication time has an effect on the introduction of edge-plane sites on the GO sheets, and their effect on the antimicrobial activity.

Selection and/or Peer-review under responsibility of International Conference on Advanced Materials(SCICON ‘16).

*Keywords:*Graphene Oxide; Antimicrobial Activity; Suspension Assay; Surface Coating.

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1. Introduction

Graphene is a 2D carbon nanomaterial made up of a single layer of sp^2-bonded carbon atoms arranged in a honey comb like structure, isolated from graphite in 2004[1]. It has attracted researcher's interest because of its wonderful electronic, mechanicaland thermalproperties[2]. Common approaches used to synthesize graphene-based materials aredependenton their further applications.Among these materials, graphene oxide (GO) are usually water–dispersible, havinglarge density of oxygen functional groups in the planes and edges of the sheets. Due to its scalability,cost effectiveness,and stability, ithas been used as a promising precursor for chemically reduced graphene or as building blocks for graphene-based composite materials[3,4].

There are several studies revealing the strong antimicrobial properties of GO against various microorganisms, including gram positive and negative bacterial pathogens, plant pathogens, and also for biofilm forming microorganisms. The antimicrobial activity shown by GO may be due to the physical, and chemical interactions occurring when the bacterial cells encounter withsheets. Usually the cell membrane is the primary target of thecytotoxicity of GO. The membrane damage is usually caused by the atomically sharp edges of graphene, which can easily penetrate into the cell membrane, and may physically disrupt its integrity. There are lots of mechanisms studied, whichvery well describe or explain the antimicrobial nature of GO[5,6,7]. The antimicrobial property of GO has been utilized extensively in the development of GO-based antimicrobial surfaces, sinceGO has the contact-mediated mode of action. This antimicrobial mechanism of GOcan be utilized as an alternative to biocide-releasing surfaces using antibiotics or silver, which deplete from the surface over time. Eventhough, there are lot of studies conducted using GO for antimicrobial surfaces, still there is no deeper understandingabout the required GO material properties, which is responsible for effective antimicrobial activity. Most of the studies conducted till date has focused on the antimicrobial properties of GO sheets in suspension assays, where aggregation and cell wrapping mechanisms occur. For example, GO sheet size has been found to influence its antimicrobial activity in solution, because the larger sheets has the capacity to completely wrap around the cells and hence to isolate them from their natural environment[8]. However, in the case of GO-coated surfaces, where sheets are immobilized on the surface, the interactions between GO sheets and bacterial cells will be different than in suspension, and therefore changes in the physicochemical properties of GO sheets, such as sheet size, may have a different effect when applied on a surface. In the presentwork, we investigated how GO sheet size alters the antimicrobial activity of GObased surface coatings using gram-negative rods and gram-positive cocci, i.e., *Escherichia coli*(*E. coli*)and *Enterococcusfaecalis*(*E. faecalis*). GO used in this study was synthesized in an electrochemical exfoliation method, which was assisted by a surfactant, SDS, used in a concentration of 0.01 M[9], and usedto study the size dependency in the antimicrobial activity against both rods and cocci, both in suspension assay and as surface coatings. The differencesbetween the suspension assays and surface coatings highlighted the importance of selecting antimicrobial nanomaterials. These findings may be applied for the fabrication of graphene-based antimicrobial surfaces.

2. Experimental Procedure

2.1 Sonochemical Treatment of GO.

The GO used in this experiment was synthesized as mentioned previously[9].At firstthe synthesized GO (2 mg mL^{-1}) was diluted to 200 μg mL^{-1} and sonicated for different interval of time (10 min, 20 min, 30 min)at 20 WL^{-1}, which breaks the GO sheets into smaller fragments, and generate GO sheets of decreased average size. GO sample without sonication (0 min) also was used, and its effect on the same bacteria was also studied. Positive cultures for both the bacteria were maintained to compare the antibacterial effect. The experiments were executed by employing aProbe Sonicatordepicted in Fig. 1. This sonochemically treated graphene oxide was further used for the antibacterial study for two bacterial species *E. coli* and *E. faecalis*.

Fig. 1 Probe Sonicator

2.2 Antimicrobial Activity of GO in Suspension.

E.coli and *E.faecalis* cultures were grown overnight in LuriaBertani broth at 37°C. The cultures were then diluted in fresh medium and grown until log phase (2 hr), which was verified by measuring the optical density(OD) at 600 nm. All four different (0 min, 10 min, 20 min and 30 min sonicated) GO samples (2 mg mL^{-1} stock) wereadded to the medium for a final concentration of 200 µg mL^{-1} maintained separately.Cellswere exposed to suspended GO for 3hr at room temperatureunder constant agitation(Fig. 2). At the end of the exposure period,the change in OD was recorded.

2.3 Antimicrobial Activity of GO-Coated Surfaces.

*E. coli*and *E.faecalis*cultures were grown as described in the previous section. For a homogeneous GO surface coating was obtained by simpledrop coating,and subsequentair drying. A 500µLof four different (0 min, 10 min, 20 min, 30 min)GO suspensions (200 µg mL^{-1}) was drop coated onWhatman filter paper 1 separately,and air-dried. Then, 500µLof diluted bacterial suspension were slowly added on top of the GO surface (Fig. 2). Bacterial cells were kept in contact with the GO-coated surface for 3 hr. After the 3 hr incubation, the bacteria suspension was removed, and the GO-coated paperswere washed with sterile 0.9% NaCl suspension to remove unattached cells, andadded to broth, subsequently OD changes were recorded.

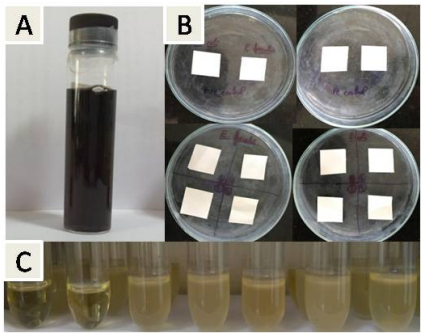

Fig. 2(A) GO (B) Surface Coating Assay and (C) Suspension Assay

3. Result and Discussions

3.1 Characterization of the synthesized GO.

The synthesized GO were characterized by employing different spectroscopic studies. A typical absorption peak at 230nm was obtained in the UV–vis spectrum (Fig. 3). The FT-IR spectra for the GO revealed the oxygen functionalities, such as the C=O,C-O and hydroxyl functionalities (Fig. 4). AFM and TEM imaging revealed that the GO sheets (Fig. 5) were arranged in layers. The nature of the oxygen containing functional groups in GO was

identified as C=O, C-O, and O-C=O bonds by XPS. In addition, XPS survey scansalso revealed that GO was free of any metal residues (Fig. 6).

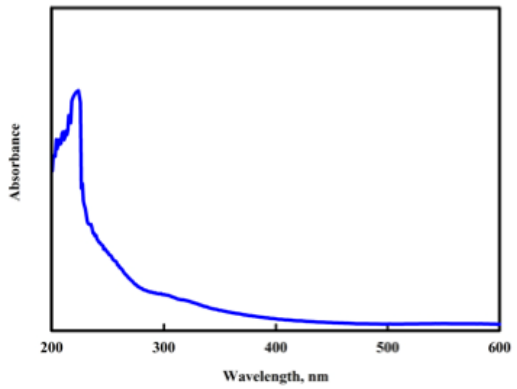

Fig. 3 UV spectra of GO

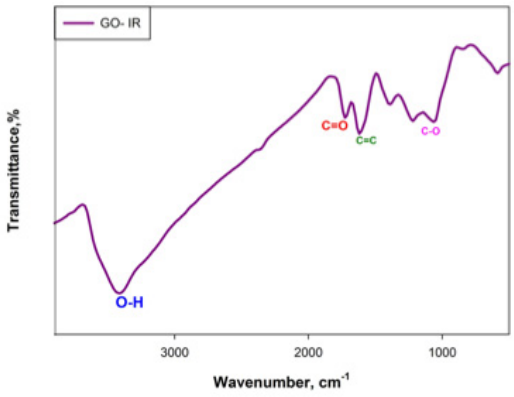

Fig. 4 IR spectra of GO

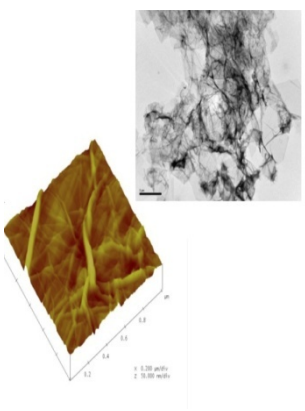

Fig. 5 TEM and AFM image of GO

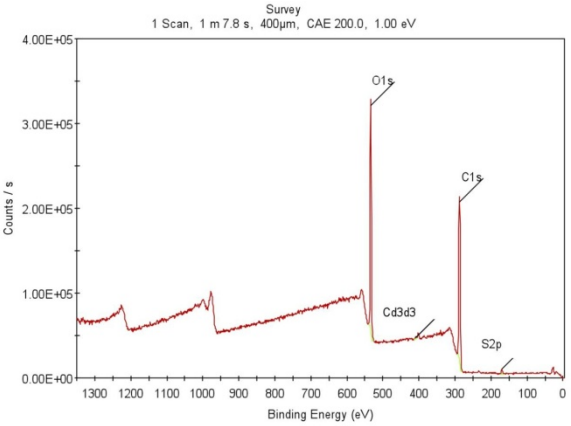

Fig. 6 XPS survey scan of GO

3.2 Bacterial Culture

Fig. 7 represents the microscopic image of cultured *E. coli*(gram negative, rod shaped), and *E. faecalis*(gram positive cocci) for the GO suspension and surface antimicrobial activity.

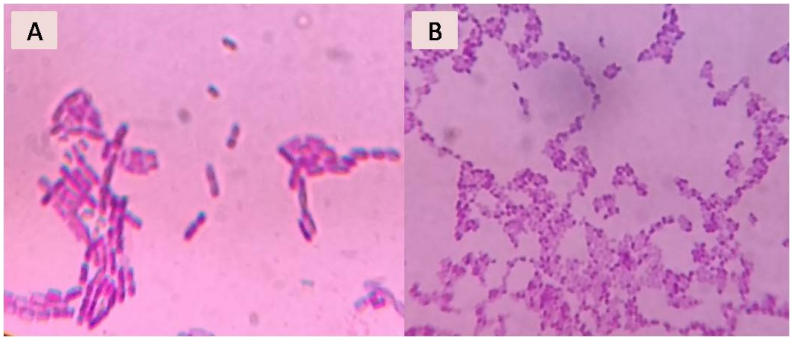

Fig. 7 Gram staining (A) *E. coli*; (B) *E. faecalis.*

3.3 Antimicrobial Activity of GO in Suspension.

The sonochemically treated GOexhibited antibacterial activity, and showed different effects on the two bacterialspecies investigated, i.e., *E. coli* and *E. faecalis*. In suspension assay, for the *E.coli*, as the sonication time increased, the antibacterial activity was found to be reduced, indicating that the size of the GO sheet matters (Fig. 8a). The GO without sonication (0 min) showed greater antibacterial activity when compared with other sonicated GO sample (10 min, 20 min, 30 min). However,*E. faecalis*showed good antibacterial activity as the sonication time increased (Fig. 8b).It is believed that, since*E. coli* are rod shaped, the large sheets effectively wraps around the rods, and hence the larger sheet of GO shows greater antimicrobial activity than the smaller sheets. In *E. faecalis,* the antimicrobial activity increased as the sonication time was increased, indicating that the introduction of more edges (defects) on the smaller sheetsduring the longer sonication timeplayed a major role in the antimicrobial property of GO.Interestingly, the observed phenomenon is comparable with the previously published data [8].

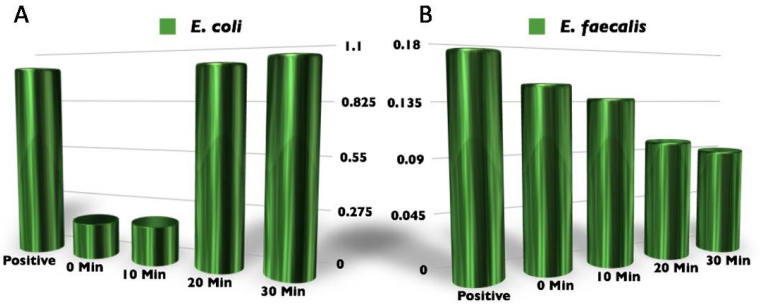

Fig. 8 Antibacterial activity in suspension assay (A) *E. coli*; (B) *E. faecalis*

3.4 Antimicrobial Activity of GO-Coated Surfaces.

In the surface assay method, for the *E. coli* cells, the sonication time (10 min, 20 min, and 30 min) did not showedmuch effect on antibacterial property when compared with *E. faecalis,* but the GO sample without sonication (0 min) showed higher antibacterial activity for *E. coli*, when compared with the other sonicated samples and the positive culture.However, when compared to *E. coli*suspension assay it had an increased antibacterial activity (Fig. 9a).On the other hand, The *E. faecalis* cells exhibited increased antibacterial property as the sonication time increased when compared with the positive culture and the non-sonicated sample (0 min) in the surface assay method (Fig. 9b). In general, when compared with the suspension assay, surface-coating assay showed good antibacterial activity, since the bacterial cells were in direct contact with the GO (Fig. 9). These results indicate that GO can be used as surface coatings for delivering the antibacterial property for any surface.

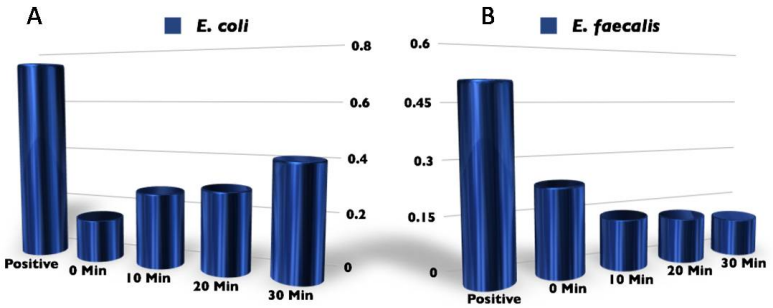

Fig. 9Antibacterial activity in surface coatings (A) *E.coli*; (B) *E. faecalis*.

4. Conclusion

The effects of sonochemically treated GOas antimicrobial agent towards two bacteria, *E. faecalis* and *E.coli* was studied. The results obtained revealed that the surface coating of GO on any material(herein paper substrate)would be more effective antibacterial agent. Hence, could be applied in the fabrication of GO coated surfaces for various applications, especially in the biomedical field, wherethe challenges of bacterial infection are increasingalarmingly.It is envisaged that coating of medical equipments or implants with GO could reduce bacterial infections, and the use of antibiotics.However, the exact mechanism of antibacterial activity towards different cell wall types(rods, cocci, etc) by GO, and herein the specific interaction of sonochemically treated GOtowards a higher antimicrobial activity oncocci than rods need to be studied.

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