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Title	Water-resistant antibacterial properties of a graphene oxide/cetylpyridinium chloride complex formed on medical gauze fibers
Author(s)	Kanemoto, Yukimi; Miyaji, Hirofumi; Nishida, Erika et al.
Citation	Journal of oral biosciences, 65(2), 202-205 https://doi.org/10.1016/j.job.2023.02.002
Issue Date	2023-06
Doc URL	https://hdl.handle.net/2115/92702
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Type	journal article
File Information	2023 Kanemoto J Oral Biosci.pdf

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Water-resistant antibacterial properties of a graphene oxide/cetylpyridinium chloride complex formed on medical gauze fibers

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Abstract

Objectives

Graphene oxide (GO) is a nanocarbon material with a high aspect ratio (width:thickness) and abundant anionic functional groups on its surface. In this study, we attached GO to the surface of medical gauze fibers, constructed a complex with a cationic surface active agent (CSAA), and demonstrated that the treated gauze exhibits antibacterial activity even after rinsing with water.

Methods

Medical gauze was immersed in GO dispersion (0.001%, 0.01%, and 0.1%), rinsed with water, dried, and subjected to the Raman spectroscopy analysis. Subsequently, the gauze treated with 0.001% GO dispersion was immersed in 0.1% cetylpyridinium chloride (CPC) solution, immediately rinsed with water, and dried. Untreated, GO-only, and CPC-only gauzes were prepared for comparison. Each gauze was placed in a culture well, seeded with *Escherichia coli* or *Actinomyces naeslundii,* and turbidity was measured after 24 h of incubation.

Results

The Raman spectroscopy analysis of the gauze after immersion and rinsing showed a G-band peak, indicating that GO remained on the surface of the gauze. The turbidity measurements indicated that GO/CPC-treated gauze (GO-treated and rinsed, followed by CPC-treatment and rinsing) significantly decreased turbidity compared to the other gauzes ($P<0.05$), suggesting that the GO/CPC complex remained on the gauze fibers even after water rinsing and showed antibacterial activity.

Conclusions

The GO/CPC complex imparts water-resistant antibacterial properties to gauze and has the

potential to be widely used for the antimicrobial treatment of clothes.

Keywords

Actinomyces naeslundii, Cationic surface active agent, *Escherichia coli*, Graphene oxide, Medical gauze

Abbreviations

CPC, cetylpyridinium chloride; CSAA, cationic surface active agent; GO, graphene oxide.

1 Introduction

The spread of infectious diseases, such as coronavirus 2019, has led to a new phase in the application of antimicrobial materials to improve public health. Although antimicrobial wipes are used to disinfect various areas of the home, textile fibers provide a good environment for microorganisms to proliferate and thus play a critical role in causing secondary infections (human-bacterial mediators) [1]. In the medical field, healthcare-acquired infections caused by medical textiles are a major concern, and periodic cleaning alone cannot prevent these infections [2]. In addition, fabrics used to cover wounds retain material from the body that provide nutrients for bacteria, promoting bacterial growth and delaying wound healing [3]. Attempts to address these issues include the incorporation of heavy metals [4], natural oil microcapsules [5], and antimicrobial synthetic fibers [6] into antimicrobial cloths.

Recently, Miyaji et al. reported the long-term water-resistant antibacterial properties of a complex comprising graphene oxide (GO) and a cationic surface active agent (CSAA) [7]. GO is a nanocarbon material with a large aspect ratio (the ratio of width to thickness) and is approximately one nanometer thick and several micrometers wide [8]. GO has an abundance of oxygen functional groups on its surface, can strongly adhere to a substrate through high surface interactions (e.g., van der Waals forces and ionic interactions), and can thus attract a CSAA through anion-cation interactions to form a complex. A GO/CSAA complex was previously attached to various substrate surfaces, such as glass, polyethylene terephthalate, polystyrene, and human teeth, and was retained even after rinsing with water, showing long-term (at least 2 weeks) antimicrobial activity [7].

In this study, we attempted to develop a cloth treated with GO/CASS that would retain its antimicrobial properties even after being rinsed with water. This process can be performed under mild conditions at room temperature and pressure and thus there is little damage to the fibers of the cloths. The GO monolayer is transparent to the naked eye and does not affect the color of the cloths. Testing the antimicrobial properties of treated medical gauzes showed that the GO/CSAA layer was retained even after rinsing with water and reduced bacterial growth.

2 Materials and methods

2.1 Preparation of the GO dispersion

The GO dispersion was prepared as previously described [7]. Graphite (FUJIFILM Wako Pure Chemicals Co., Ltd., Osaka, Japan) and sulfuric acid were mixed, then potassium permanganate and water were added. Hydrogen peroxide was used to stop the reaction and the mixture was homogenized to obtain the GO dispersion.

2.2 GO treatment of medical gauze

Medical gauze (Hi-cut Gauze 3 × 3, Nichiei Co., Ltd., Sakai, Japan) was used as the fiber substrate. The concentration of the GO dispersion was adjusted to 0.1, 0.01, and 0.001% with water. The gauze was immersed in each GO dispersion solution for 10 s, immediately rinsed, and dried at 50°C. The color of the gauze changed to brown as the GO concentration increased (Figure 1). Each treated gauze was examined by Raman spectroscopy (excitation wavelength 532 nm, NSR-3100, JASCO Corporation, Hachioji, Japan) to detect the presence of GO.

2.3 Preparation of the GO/CPC complex on gauze

An aqueous solution of 0.1% cetylpyridinium chloride (CPC, Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) was used as the CSAA. GO-coated gauze (GO concentration; 0.001%) was immersed in the CPC solution for 10 s, immediately rinsed with water, and dried to provide the GO/CPC complex. For comparison, untreated gauze, gauze treated with GO only, and gauze treated with CPC only were prepared for antibacterial evaluation.

2.4 Antibacterial evaluation

Bacterial suspensions were prepared of two pathogens: gram-negative *Escherichia coli* (ATCC 25922) (2.0×10^6 CFU/mL) and gram-positive *Actinomyces naeslundii* (ATCC 27039) (2.0×10^6 CFU/mL). Brain heart infusion medium (Eiken Chemical Co., Ltd., Tokyo, Japan) was used as the culture medium. Each treated gauze was cut into 10 mm square pieces, placed in 48-well plates, and seeded with 500 μL of bacterial suspension. After 24 h of anaerobic incubation at 37°C, the gauze was removed from the wells and the turbidity of the culture medium was measured at 590 nm using a colorimeter (CO7500 Colourwave, Funakoshi Corporation, Tokyo, Japan). The turbidity was calculated relative to the turbidity of the untreated gauze wells.

2.5 Statistical analysis

Turbidity measurements were analyzed using two-tailed one-way analysis of variance with

Games-Howell post hoc test to determine significant differences. $P<0.05$ was considered a significant difference. Calculations were performed using the SPSS software package (version 11.0; IBM Corporation, Armonk, NY, USA).

3 Results

3.1 Raman spectroscopy analysis

Figure 2 shows the results of Raman spectroscopy analysis. The 0.1% GO-treated gauze clearly showed the G band peak at 1600 cm^{-1} (highlighted in pink), originating from GO, and this peak weakened with decreasing GO concentration. However, the G band was faintly visible even at 0.001% GO. Untreated gauze showed no G-band peak. Instrument noise was observed at about 2800 cm^{-1}.

3.2 Antibacterial test

The results of turbidity measurements using the bacterial strains *E. coli* and *A. naeslundii* are shown in Figure 3. *E. coli* inoculated on gauze treated with GO showed turbidity comparable to that observed using untreated gauze. However, *E. coli* inoculated on gauze treated with CPC only and *A. naeslundii* inoculated on gauze treated with GO only or CPC only showed a tendency to decreasing turbidity, but the difference was not statistically significant compared to untreated gauze ($P > 0.05$). In contrast, GO/CPC gauze (i.e., GO treatment followed by water-rinsing and then CPC treatment followed by water-rinsing) showed a remarkable decrease in turbidity, to approximately 30% of that of untreated gauze for *E. coli* and 20% that of untreated gauze for *A. naeslundii*, and the differences were statistically significant ($P < 0.05$).

4 Discussion

Raman spectroscopy analysis showed a G-band peak, indicating that GO can adhere to the surface of gauze fibers and remain even after rinsing. The G-band is a peak characteristic of graphene and originates from the in-plane stretching motion of carbon atoms [9]. We confirmed the presence of GO on the fibers 10 s after immersion in GO dispersion and subsequent water rinsing, suggesting that a GO coating can be easily obtained by a simple process at room temperature and pressure. In addition, the results of antibacterial evaluation showed that GO/CPC-treated gauze (GO-treated and rinsed, followed by CPC-treatment and rinsing) significantly reduced the turbidity of both gram-positive and gram-negative bacteria, suggesting that bacterial growth was consistently inhibited. Therefore, a water-resistant GO and CSAA complex was likely formed on the gauze fibers and exerted antimicrobial activity.

The mechanism of GO/CPC complex formation in this study is postulated as follows (Figure 4). First, a monolayer of GO adheres to the gauze fiber surface by attractive forces, such as van der Waals forces. Due to its shape and high aspect ratio, GO in direct contact with the fiber substrate does not detach easily, even after water rinsing. Next, the residual oxygen functional group of GO binds to CPC by anion-cation interactions to form a complex [10]. Other attractive forces, such as π-π stacking and CH/π interactions [11], are also involved in binding CPC. Thus, GO may play a role as an adhesive between the gauze fibers and CPC. The adsorbed CPC can detach from the bound GO over time [7, 10], suggesting that CPC is released into the culture medium and attacks bacteria.

Various approaches have been proposed to confer antimicrobial resistance to clothes, but all suffer from limited long-term sustainability [12]. We previously showed that the GO/CSAA complex described here exhibits antimicrobial properties for 2 weeks in water. In addition, the GO monolayer remains on the substrate even after most of the CSAA is released into water. Antimicrobial activity can be re-established by re-immersion in CSAA, regenerating the GO and CSAA complex [7]. However, concerns remain regarding the biotoxicity of antimicrobial clothes. GO has been reported to exhibit dose-dependent cytotoxicity [13], although GO concentrations below 20 μg/mL have little cytotoxic effect [14]. Since 0.001% GO was used in this study (to avoid changing the color of the gauze), and excess GO was removed by rinsing with water, the amount of GO remaining on the gauze is likely to be very small. In addition, GO monolayers exhibit favorable cytocompatibility [7].

5 Conclusion

In this study, we succeeded in forming a water-resistant antibacterial GO/CPC complex on gauze fibers at room temperature and pressure. This approach has the potential to be widely used for the antimicrobial treatment of clothes.

Ethical approval

Ethical approval was not required for this study.

Acknowledgments

This study was supported by Hokkaido University, which provided facilities and resources, and a research grant from Nippon Shokubai Co., Ltd.

CRediT authorship contribution statement

YK: methodology, validation, formal analysis, investigation, writing - original draft, visualization.

HM: conceptualization, methodology, validation, formal analysis, investigation, writing - original draft, visualization, supervision. EN: validation, investigation. AH: investigation. TS: resources. SG: conceptualization, methodology, validation, formal analysis, investigation, writing - review & editing, visualization, supervision. HO: conceptualization, methodology, resources, writing - review & editing. All of the authors have approved the final version of the manuscript.

Conflicts of Interest

SG and HO are full-time employees of Nippon Shokubai Co., Ltd. Parts of the data sets in the current study are patent pending (2021-036701). The other authors have no conflicts of interest to declare.

Data Availability Statement

The datasets are available from the corresponding author upon reasonable request.

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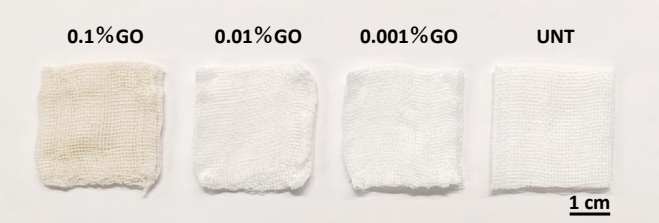


Figure 1.

Digital photograph of GO-treated medical gauze.

Abbreviations: GO, graphene oxide; UNT, untreated.

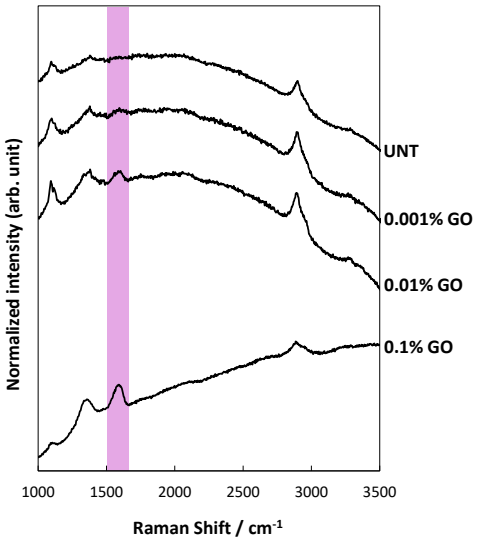


Figure 2.

Raman spectroscopy analyses.

GO (concentration; 0.001, 0.01, and 0.1%) -treated gauze exhibiting the G band (1600 cm^{-1}, highlighted in pink).

Abbreviations: GO, graphene oxide; UNT, untreated.

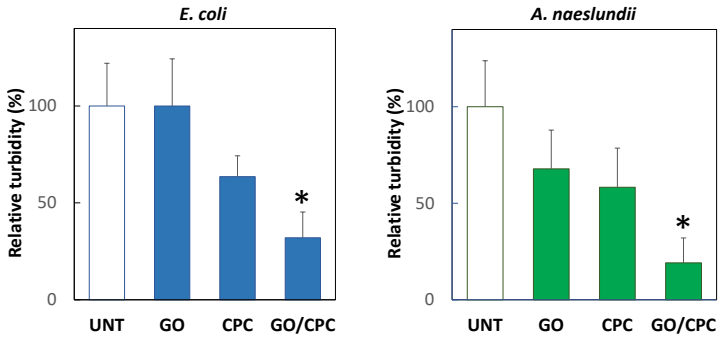


Figure 3.

Antibacterial assessment.

Relative turbidity (n = 5, mean ± standard deviation) after 24 h incubation of *E. coli* (left) and *A. naeslundii* (right) inoculated on each gauze substrate. *P<0.05 versus all other groups. Statistical analysis: two-tailed one-way analysis of variance with Games-Howell post-hoc test.

Abbreviations: CPC, cetylpyridinium chloride; GO, graphene oxide; UNT, untreated.

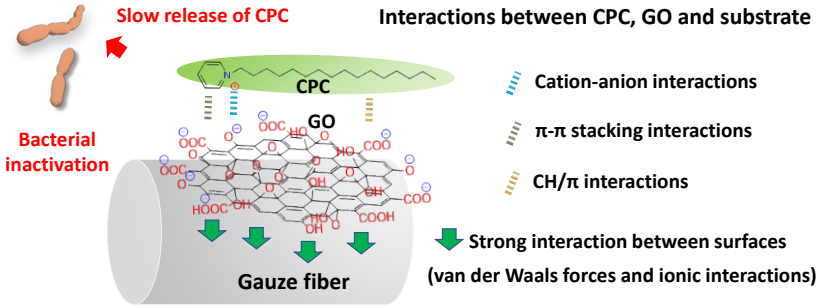


Figure 4.

Schematic representation of GO/CPC complex formation. Modified from Miyaji et al. [7].

Abbreviations: CPC, cetylpyridinium chloride; GO, graphene oxide.