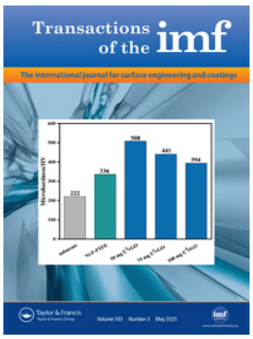

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Antibiofilm potential of graphene-dispersed alkoxysilane coatings: a materials science perspective

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ABSTRACT

This study investigates the antibiofilm properties of alkoxysilane-based coatings incorporating dispersed multilayer graphene powder. Graphene, synthesised *via* a proprietary method, was incorporated into a resin matrix at various concentrations (0.1%, 0.5%, and 1.0% by weight) and applied to glass substrates. Raman spectroscopy confirmed the presence of defect-rich, multilayer graphene, which is known to enhance antimicrobial surface properties. Biofilm formation by *Staphylococcus epidermidis* was evaluated using ISO 4768-standardised crystal violet staining. Results indicated that biofilm development was effectively suppressed at concentrations up to 0.5%, whereas an unexpected increase was observed at 1.0%, possibly due to graphene aggregation and reduced surface exposure. The findings suggest that both graphene concentration and dispersion quality critically influence antibiofilm efficacy. The study highlights the dual role of graphene, both as a physical and chemical antibacterial agent, and its potential application in medical, industrial, and hygienic materials. Further investigation is warranted to optimise dispersion and explore microbial-material interactions in real-world conditions.

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Graphene-dispersed coatings; alkosysilane resin; biofilm suppression; crystal violet assay; Raman spectroscopy

1. Introduction

Biofilms are structured communities of microorganisms that adhere to surfaces and are enclosed in a self-produced polymeric matrix, primarily composed of extracellular polymeric substances (EPS), bacterial cells, and water (Figure 1)[1]. These biofilms are responsible for persistent problems across various fields, as illustrated in Figure 2. In industrial settings, they contribute to corrosion and clogging in piping systems.[2] In the medical field,[3,4] they cause chronic infections on devices such as catheters and implants.[5] In the food industry and domestic environments, they are associated with hygiene issues and material deterioration. Despite some beneficial applications of biofilms in environmental and energy-related technologies, their resistance to conventional cleaning and disinfection methods poses a significant challenge. Hence, developing new surface treatments that prevent biofilm formation is a critical objective in materials science.

When designing surface treatments to combat biofilm formation, two complementary structural strategies must be considered. The first is the incorporation of antimicrobial agents into the surface matrix.[4] Although antimicrobial and antibiofilm properties often overlap, the relationship between them is not symmetric. While antimicrobial activity may contribute to biofilm suppression, a material exhibiting antibiofilm properties does not necessarily possess antimicrobial effects. This distinction arises from the multistep nature of biofilm formation, where the initial bacterial adhesion to surfaces is a critical stage. Inhibiting this attachment and subsequent proliferation is an effective strategy to suppress the production of extracellular polymeric substances (EPS), which form the structural basis of biofilms. Therefore, antimicrobial action plays a vital role in the early defence against biofilm development.[6,7]

On the other hand, a separate and equally important concept involves the interfacial adhesion between the formed biofilm and the underlying material. If the biofilm does not adhere strongly to the surface, it may detach naturally during the growth process. This weak adhesion can result from various factors. One observed by the authors is the hydrophobicity of the substrate: in such cases, moisture within the EPS matrix or at the interface may repel the biofilm, promoting spontaneous detachment. Silane-based resins, for instance, often exhibit this behaviour.

These fundamentally different antibiofilm mechanisms emphasise that biofilm formation is a dynamic, time-dependent process, rather than a static event, and therefore cannot be fully addressed by antimicrobial action alone.

This article focuses on the first approach – dispersing antibacterial agents on the surface – and introduces an example of a material developed using this concept. In particular, it explores the use of graphene, a nanomaterial with unique physicochemical properties, and discusses its potential for biofilm suppression along with relevant evaluation methods.

2. Biofilm and graphene

Biofilms are inhomogeneous, film-like structures formed on material surfaces through the aggregation of microorganisms. These structures are embedded in a self-produced matrix composed primarily of water, bacterial cells, and extracellular polymeric substances (EPS), including polysaccharides, proteins, lipids, and nucleic acids. The majority of

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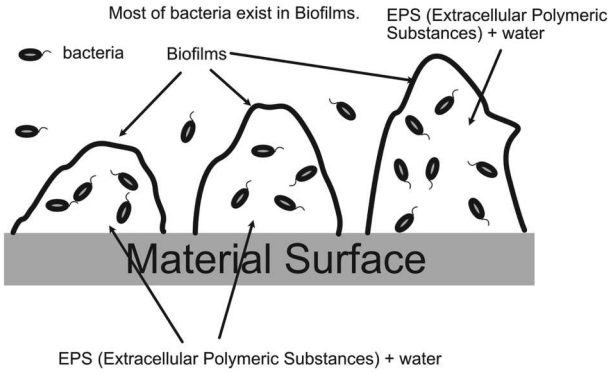

Figure 1. What are biofilms? Schematic illustration.

bacterial populations in natural and artificial environments are found within these biofilms, rather than existing as free-floating cells. This structural organisation allows microorganisms to survive in hostile environments and resist antimicrobial treatments, making biofilms particularly troublesome in medical, industrial, and environmental contexts.

The development of a biofilm occurs in several sequential stages, as shown in Figure 3. Initially, surfaces are rapidly conditioned by the adsorption of organic molecules and metal ions from the surrounding environment, forming a so-called 'conditioning film' (Step 1 in Figure 3). This modified surface facilitates the adhesion of planktonic (free-swimming) bacterial cells (Step 2). Once adhered, the bacteria proliferate and secrete EPS (extracellular polymeric substances), which form a protective and structural matrix (Step 3). As the EPS layer thickens, it encapsulates the growing bacterial colony, leading to the formation of a mature biofilm (Step 4). Over time, biofilms can expand and release cells back into the environment, thereby contributing to the colonisation of new surfaces.

One of the most effective strategies for inhibiting biofilm formation is to prevent the initial bacterial adhesion and subsequent proliferation. If the material surface can repel planktonic bacterial cells or inactivate them upon contact, the progression to a mature biofilm can be significantly suppressed. Surface design plays a crucial role in this context, and one promising approach is to incorporate antimicrobial agents directly into the surface layer. Materials such as silver, copper, and various organic compounds are well-known for their antimicrobial properties. When these agents are embedded into a bulk material or dispersed within a coating matrix, the result is often termed an antimicrobial composite. Among the various candidates for such composites, graphene has emerged as a particularly interesting option due to its unique physicochemical characteristics, which may offer both antimicrobial activity and compatibility with diverse substrates.

Graphene is a two-dimensional nanomaterial composed of a single layer of sp^2-hybridised carbon atoms arranged in a hexagonal lattice. Since its isolation in 2004, graphene has garnered tremendous interest across scientific disciplines due to its remarkable physical properties, including high mechanical strength, exceptional thermal conductivity, and outstanding electron mobility. These features have made graphene a promising candidate in electronics, energy storage, sensors, and biomedical applications.[8,9]

In the context of microbiology and surface engineering, graphene has shown considerable potential for antimicrobial and antibiofilm applications.[10–14] The mechanism by which graphene exerts antimicrobial effects is still under investigation but is believed to include multiple pathways: (1) physical disruption of cell membranes due to sharp edges of graphene sheets, (2) oxidative stress induced by electron transfer or reactive oxygen species (ROS), and (3) cell entrapment and isolation from nutrients. However, these effects are highly dependent on the form, concentration, and functionalisation of the graphene material.

For example, pristine single-layer graphene fabricated *via* chemical vapour deposition (CVD) offers excellent uniformity and can be engineered into highly ordered films. Yet, the high cost and complex production processes limit its applicability in large-scale coatings or industrial surfaces. In contrast, graphene oxide (GO) and reduced graphene oxide (rGO), which contain various oxygen-containing functional groups, are more chemically versatile and can be dispersed in polymers more easily. Nevertheless, their antimicrobial behaviour is more variable and sometimes controversial, depending on their chemical state and processing history.

To address the trade-off between performance and scalability, a practical approach involves incorporating multilayer graphene powder, typically produced *via* graphite exfoliation, into resin-based coating matrices. This method enables cost-effective application on various substrates while retaining the beneficial properties of graphene. Yet, one of the unresolved questions in this field is whether such dispersed graphene materials consistently suppress

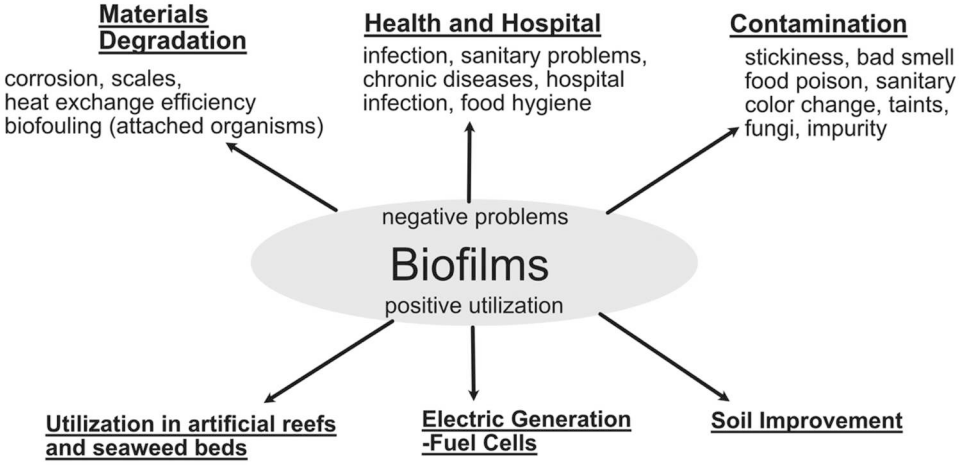

Figure 2. Biofilms affecting industries and medical fields.

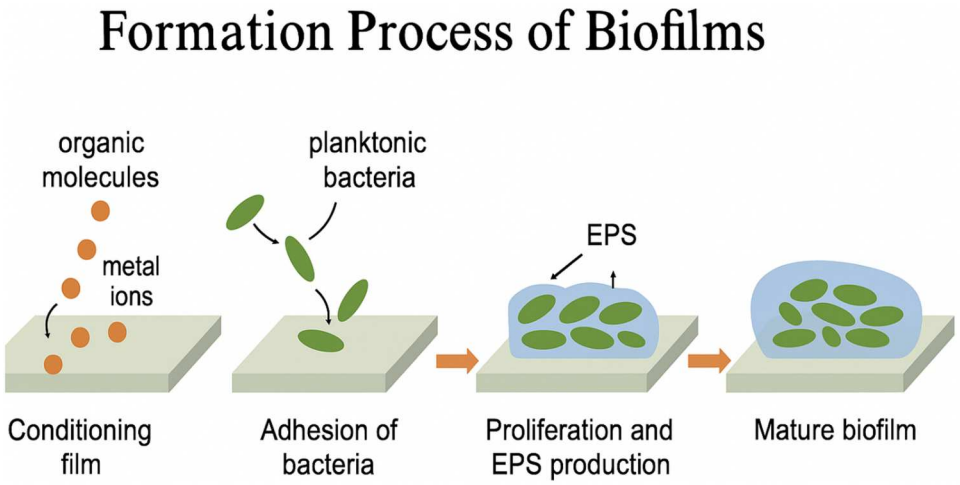

Figure 3. Biofilm formation process.

biofilm formation or, under certain conditions, inadvertently promote microbial colonisation by providing additional surface roughness or adsorption sites.

In this study, the authors have investigated the antibiofilm potential of alkoxysilane-based coatings containing dispersed multilayer graphene powder, as schematically shown in Figure 4. These coatings represent a realistic formulation for industrial or medical surfaces, and the goal is to clarify their influence on biofilm formation by combining surface characterisation with microbiological evaluation techniques.

The central theme of this study is the incorporation of graphene into an alkoxysilane-based resin. Alkoxysilane resins are known to possess intrinsic free volume within their polymer networks. Although nanoparticles may potentially enter and stabilise within these nano-scale voids, in the present case, the graphene appears to be physically mixed and dispersed as a distinct second phase. Considering the reactivity at the material–environment interface, it is reasonable to assume that graphene dispersed near or at the outermost surface layer – where interaction with the surrounding medium occurs – is particularly effective in suppressing biofilm formation.

3. Experimental

3.1. Evaluation methods and international standards

Accurate assessment of biofilm formation is critical for evaluating the anti-biofilm properties of coated surfaces. However, no single method can fully capture both the qualitative and quantitative aspects of biofilm behaviour. Therefore, a combination of complementary techniques is currently considered the most effective approach.

At the qualitative level, initial screening often begins with optical microscopy. Using reflected light, one can observe water-like droplets adhered to the surface – presumed to be biofilms due to their hydrated, slimy nature. While experienced operators may develop an intuition for identifying biofilms this way, the scientific reliability of such identification is limited. Morphological observation alone cannot confirm whether the observed substance is indeed a biofilm or condensed water.

To enhance reliability, various advanced microscopy and spectroscopic techniques have been developed. Scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM), and vibrational spectroscopy methods, such as Fourier Transform Infrared (FTIR) and Raman microscopy, offer greater specificity. These tools can detect molecular features typical of biofilms, such as proteins, polysaccharides, which are key components of extracellular polymeric substances (EPS), enabling more definitive identification. Nonetheless, such methods require significant expertise, are costly, and often lack consistent quantifiability, which limits their industrial practicality.

The evaluation methods for biofilms can be broadly categorised into biological and materials science-based approaches. In the latter category, conventional techniques from materials science – particularly surface analysis – include various forms of microscopy, as noted above, such as optical microscopy and SEM-EDX. Representative and widely adopted techniques are illustrated in Figure 5.

On the other hand, biological approaches typically involve bacterial culture, genetic analysis, and various staining methods. These methods can also be classified along a different axis: those that focus on topographical observation and those aimed at analysing the components of the biofilm, namely the bacteria and extracellular polymeric substances (EPS). Notably, unlike general antimicrobial testing, the presence of EPS is a defining feature of biofilms. Thus, analytical techniques that target EPS are particularly useful for identifying biofilms. Raman spectroscopy and FTIR are prime examples of such methods.

Among these, confocal laser scanning microscopy stands out as a highly valuable tool, as it bridges both categories – providing topographical imaging as well as limited compositional insights. However, from a practical standpoint, the most promising evaluation method is crystal violet staining. This technique allows for simultaneous staining of both EPS and bacterial cells. Given that biofilm formation cannot be accurately assessed based solely on bacterial count, crystal violet staining offers a more holistic and practically advantageous means of quantifying biofilms by capturing all structural components. In fact, as will be discussed later, this method forms the basis of the standardised biofilm evaluation protocol defined in ISO 4768, established by the Society of International Sustaining Growth for Antimicrobial Articles (SIAA).

From an industrial standpoint, quantitative analysis is essential. In this regard, colourimetric staining methods such as crystal violet (CV) staining provide a robust and

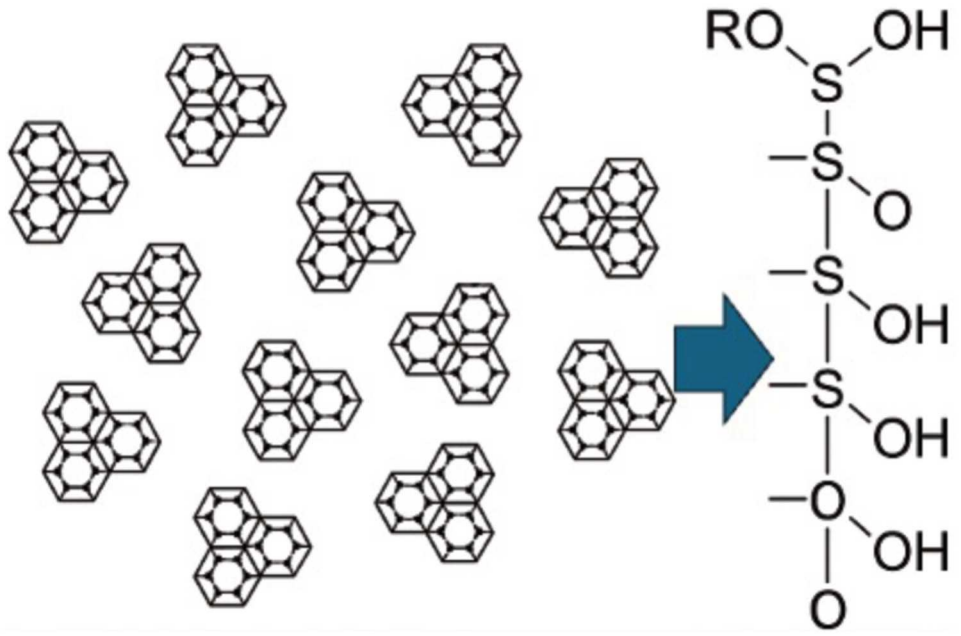

Figure 4. Schematic illustration of Graphene-dispersed Alkoxysilane coating.

cost-effective solution. Crystal violet is an organic salt that ionises. The organic cations electrostatically adsorb to the negatively polarised regions of polymers, resulting in colouration. This property can be utilised to detect EPS (extracellular polymeric substances) in biofilms. Figure 6 shows the actual appearance of biofilms formed on glass specimens stained with CV.

This technique is well-suited for measuring total biofilm biomass by staining surface-attached biofilm with 0.1% CV and then quantifying the dye retained by absorbance at 590 nm. Notably, this method has been standardised internationally through ISO 4768:2023, which defines evaluation procedures for anti-biofilm activity on non-porous surfaces. Although it offers limited structural or compositional information, its reproducibility and scalability make it a practical tool for comparative studies. Figure 7 shows the schematic illustration of ISO 4768.

In this study's evaluation framework, the authors employed both CV staining and Raman spectroscopy to capture complementary information. CV staining enabled quantitative benchmarking, while Raman analysis provided molecular-level insights without requiring destructive sample preparation. Together, these methods provided a multidimensional perspective on biofilm behaviour, striking a balance between reliability, cost efficiency, and scientific rigour.

3.2. Materials and experimental procedure

In this study, multilayer graphene powder, synthesised by a proprietary method developed by one of the authors (Prof. Shochiku Kure).[15,16] was dispersed into an alkoxysilane-based resin known as HS-200. The specific preparation method of graphene powder cannot be disclosed due to proprietary considerations, as the process is still under development. The graphene concentrations investigated were 0.1%, 0.5%, and 1.0% by weight. Coatings were applied to glass substrates using a sponge technique and subsequently cured. Initial Raman analysis of the coatings confirmed the presence of defect-rich, multilayer graphene through prominent D-band peaks. The coated samples were then immersed in bacterial suspensions of *Staphylococcus epidermidis* (Gram-positive, ATCC35984) for 48 h.

To prepare the coating samples, graphene powder was dispersed into an alkoxysilane-based resin (HS-200) using a paint shaker. Specifically, 3.00 g of the mixture, comprising 0.030 g of graphene (1 wt%) and 2.970 g of resin, was

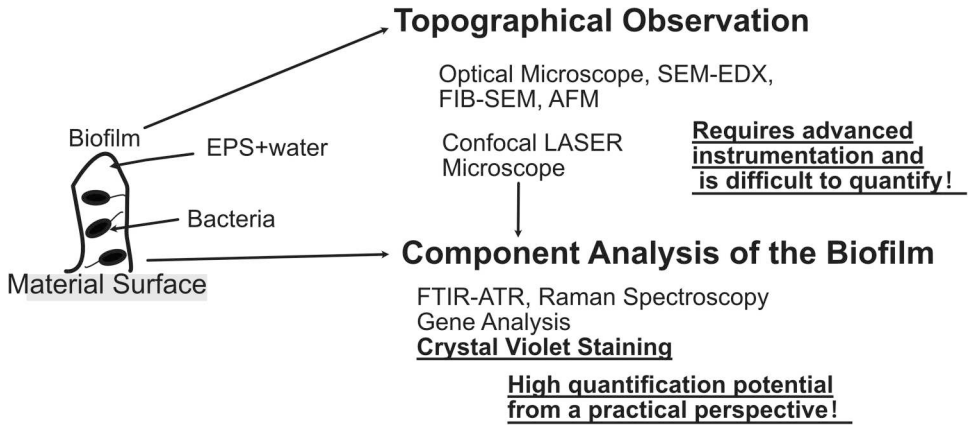

Figure 5. Various evaluation methods available theoretically and practically.

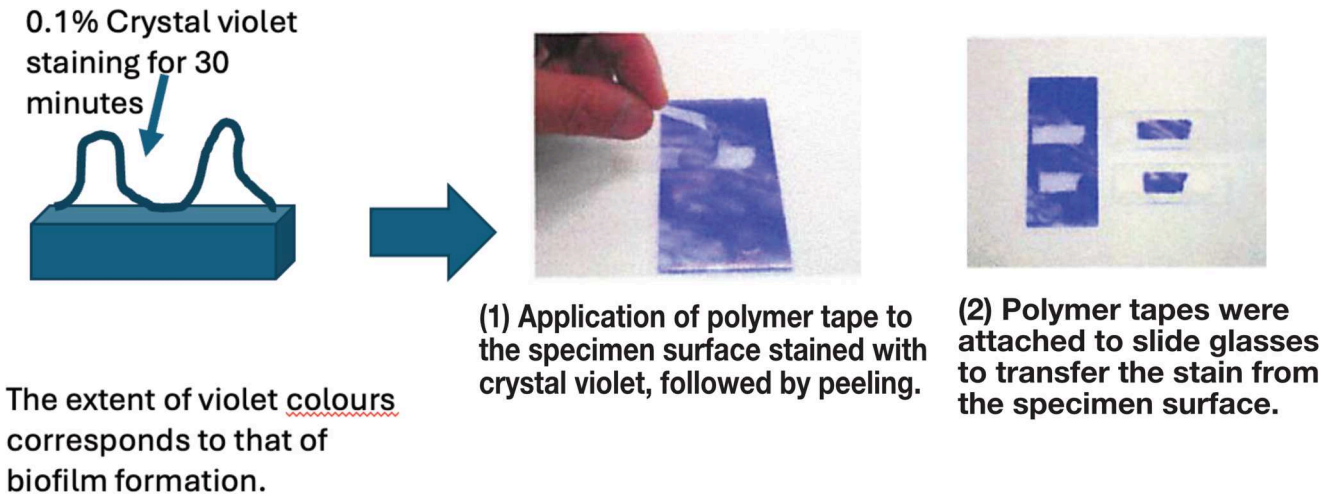

Figure 6. The appearance of stained biofilms formed on glass specimens.

placed in a 9 mL capped glass vial along with 5 g of zirconia beads (dia. 0.8 mm). The vial was shaken for 1 h to ensure uniform dispersion. The resulting 1 wt% graphene dispersion was further diluted with the same resin to obtain 0.5 and 0.1 wt% dispersions. Coatings were applied onto pre-cleaned glass substrates (wiped with acetone and paper) using a sponge applicator ($5 \times 2 \times 20$ mm) soaked with each dispersion. The coatings were uniformly spread to achieve an approximate application rate of 20 g m^{-2} (about 0.018 g per specimen). After curing, the dry film thickness was approximately 10 µm. In this study, it was empirically determined that the coating agent used is a silane-based resin, and that the coating amount (g m^{-2}) corresponds to half the film thickness (µm). Accordingly, the cured film thickness was estimated by measuring the weight of the coating applied to the substrate.

The concept is illustrated schematically in Figure 8.

4. Experimental results

Figure 9 shows the Raman spectroscopic result of the graphene powder used in this experiment.

The Raman spectrum of the graphene powder used in this study, as shown in Figure 9, exhibits two prominent peaks centred around 1350 and 1580 cm^{-1}. These peaks correspond to the D-band and G-band, respectively. The D-band is associated with breathing modes of sp^2 carbon rings that become Raman-active in the presence of structural defects, such as vacancies, edges, or grain boundaries. The G-band, on the other hand, arises from the in-plane stretching of sp^2 carbon atoms and is characteristic of graphitic domains.[17] The relatively high intensity of the D-band compared to the G-band indicates that the graphene powder synthesised by Prof. Shochiku Kure contains a significant number of defects. This is consistent with the expected features of defect-rich, multilayer graphene. In contrast to monolayer graphene, which typically displays a sharp 2D-band near 2700 cm^{-1}, the absence or minimal presence of this band further supports the conclusion that the sample is composed of several stacked graphene layers.

Such a structure, with high defect density and multiple layers, is known to enhance chemical reactivity and surface interactions. Although detailed results are discussed in a later section, the antimicrobial behaviour observed in this

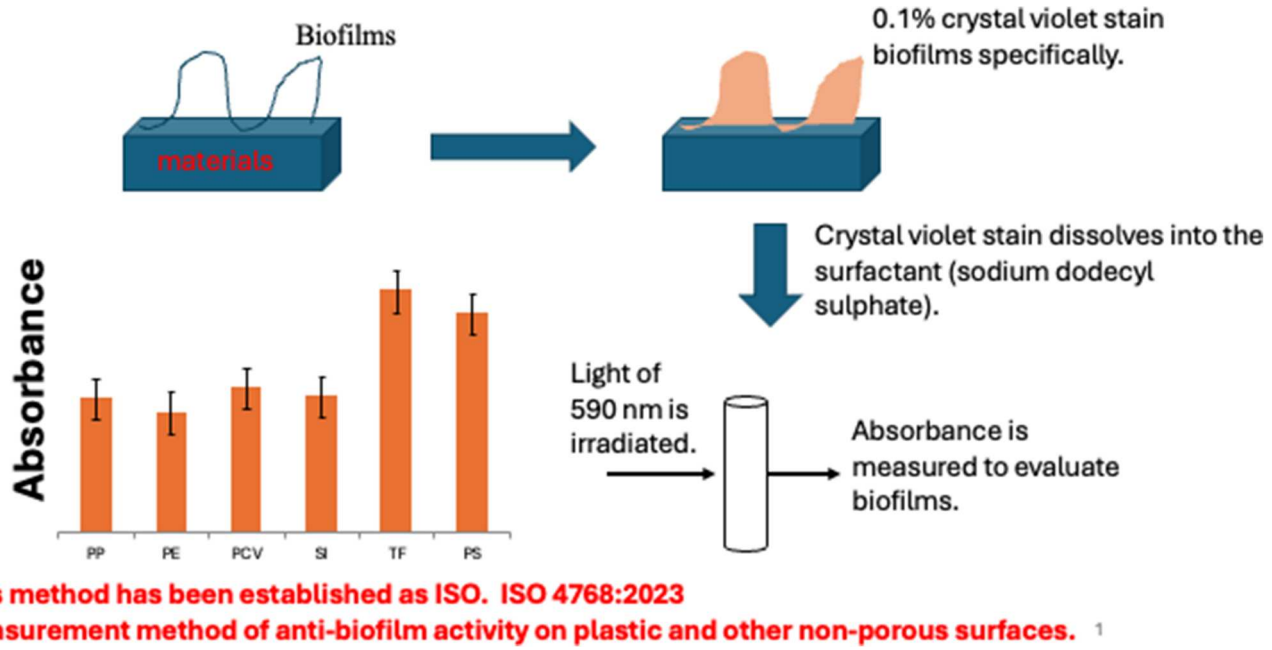

Figure 7. The typical procedure of ISO 4768.

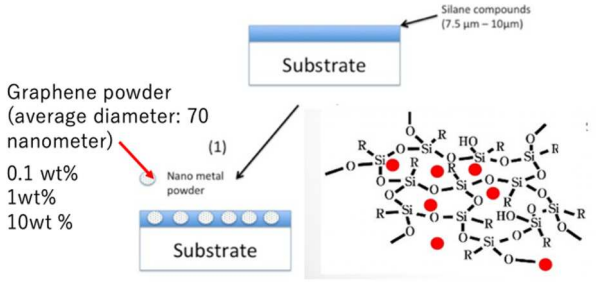

Figure 8. Composition and preparation of composite coatings in this experiment.

study may be partially attributed to the structural characteristics of the graphene employed.[18–21]

Figure 10 presents the absorbance results obtained using a plate reader, in accordance with ISO 4768 protocols, after forming *Staphylococcus epidermidis* biofilms on alkoxysilane-based coatings containing various concentrations of graphene powder. The tested samples include a control (0.0% graphene) and coatings with 0.1%, 0.5%, and 1.0% graphene by weight. Each experiment was conducted in triplicate (N = 3), and error bars represent twice the standard deviation.

As shown in the figure, a general decreasing trend in absorbance is observed as the graphene concentration increases from 0.0% to 0.5%, indicating enhanced resistance to biofilm formation. This suppression may be attributed to the nature of the graphene used in this study, which was identified as defect-rich multilayer graphene. Such a structure exhibits high surface activity and is presumed to interact strongly with microbial cells, thereby exerting antimicrobial effects. These effects likely inhibit bacterial adhesion – specifically that of *S. epidermidis* – onto the coated surface, resulting in reduced biofilm development.

Notably, the suppressive trend was reversed at 1.0% graphene, where an increase in absorbance was observed, almost to the level of the control sample. This reversal may be related to the dispersion state of graphene in the coating. At higher concentrations, graphene particles tend to aggregate, leading to sedimentation during the coating process. As a result, less graphene may be exposed at the coating surface. Since the antimicrobial action of dispersed

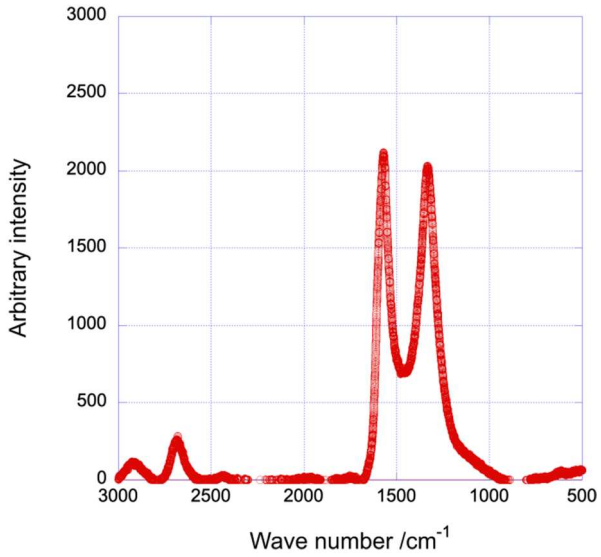

Figure 9. Raman spectroscopy of the powder used in this experiment.

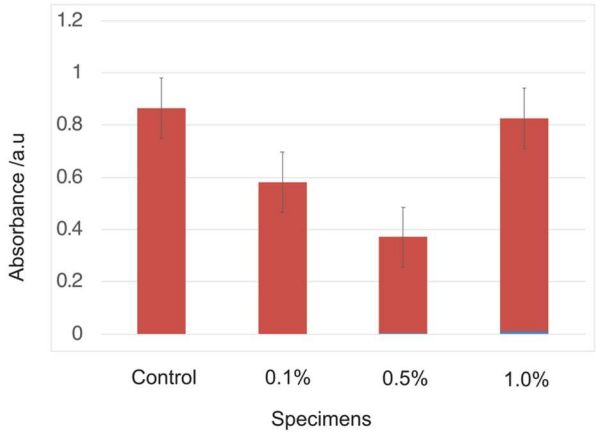

Figure 10. Absorbance data after crystal violet staining for graphene-dispersed alkoxysilane-based resin coatings.'Control' refers to the alkoxysilane-based resin coating without graphene dispersion. The labels 0.1%, 0.5%, and 1.0% indicate the weight concentrations of graphene incorporated into the resin.

graphene is surface-dependent, such a change in surface composition could diminish its effectiveness.

Additional contributing factors may include changes in surface roughness or microstructure that favour bacterial colonisation, or an increase in surface hydrophobicity at high graphene loadings. These factors could unintentionally promote biofilm formation under certain conditions.

Further investigation involving a larger number of replicates and more direct or indirect assessments of graphene dispersion behaviour is required to fully elucidate the observed effects.

5. Discussion: mechanisms of biofilm suppression by graphene

As demonstrated in the experimental results, the coatings containing graphene exhibited a significant suppression of biofilm formation. This effect is believed to be largely due to the intrinsic antibacterial properties of the graphene powder used in this study. Given its defect-rich and multilayered nature, as confirmed by Raman spectroscopy, the graphene dispersed in the alkoxysilane-based resin likely enhanced surface activity and microbial interaction, leading to antimicrobial behaviour and reduced bacterial adhesion. These structural characteristics may have played a key role in the observed inhibition of *Staphylococcus epidermidis* biofilm formation.

More broadly, several mechanisms have been proposed to explain the antibiofilm and antibacterial effects of graphene-based materials.[22–27] These include:

- **Physical damage**: The sharp edges of graphene nanosheets can mechanically disrupt bacterial cell membranes.
- **Oxidative stress**: Graphene may facilitate the production of reactive oxygen species (ROS), which can damage bacterial components.
- **Membrane destabilisation**: The hydrophobic nature of graphene can interact with and compromise bacterial lipid bilayers.
- **Intracellular interference**: Graphene may penetrate bacterial cells and interfere with vital processes such as DNA replication and protein synthesis.
- **Metabolic disruption**: Its high electrical conductivity may impair electron transport chains within bacterial metabolism.

However, under certain conditions, factors such as increased surface roughness or aggregation of graphene sheets may conversely promote bacterial adhesion. Therefore, the net effect on biofilm formation likely depends on a delicate balance between graphene concentration, dispersion quality, and the physicochemical characteristics of the coating surface.

6. Conclusion

This study demonstrates the potential of graphene-dispersed alkoxysilane coatings as an effective strategy for suppressing biofilm formation. The incorporation of defect-rich, multilayer graphene, characterised by pronounced D-band peaks in Raman spectroscopy, into the resin matrix contributed to enhanced antimicrobial surface properties. Biofilm formation by *Staphylococcus epidermidis* was inhibited in a concentration-dependent manner up to 0.5 wt%, as revealed by crystal violet staining.

While Raman spectral changes at higher concentrations may suggest molecular-level interactions with biofilms, the quantitative absorbance data point to a more complex relationship between graphene loading and antibiofilm efficacy. In particular, the diminished effect at 1.0 wt% indicates that excessive graphene may compromise surface uniformity due to aggregation and sedimentation, reducing the availability of active sites on the coating surface.

These findings underscore the importance of optimising not only the graphene concentration but also its dispersion quality to achieve consistent and reproducible antibiofilm performance. With further refinement, such coatings could be applied in a variety of contexts, including medical devices, industrial surfaces, and hygiene-critical environments. Future work should focus on clarifying the physicochemical interactions at the microbe-material interface and validating the long-term performance of these materials under real-world conditions.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

1. R. M. Donlan: Biofilms: microbial life on surfaces. *Emerg. Infect. Dis.*, 2002, **8**(9), 881.
2. H. C. Flemming, J. Wingender, U. Szewzyk, P. Steinberg, S. A. Rice and S. Kjelleberg: Biofilms: an emergent form of bacterial life. *Nature Rev. Microbiol.*, 2016, **14**(9), 563–575.
3. J. W. Costerton, K. J. Cheng, G. G. Geesey, T. I. Ladd, J. C. Nickel, M. Dasgupta and T. J. Marrie: Bacterial biofilms in nature and disease. *Ann. Rev. Microbiol.*, 1987, **41**, 435–464.
4. E. M. Hetrick and M. H. Schoenfisch: Reducing implant-related infections: active release strategies. *Chem. Soc. Rev.*, 2006, **35**(9), 780–789.
5. L. Hall-Stoodley, J. W. Costerton and P. Stoodley: Bacterial biofilms: from the natural environment to infectious diseases. *Nat. Rev. Microbiol.*, 2004, **2**, 95–108.
6. H. Kanematsu and D. M. Barry. (Eds.) Biofilm and materials science. New York, NY, USA: Springer. (2015).
7. H. Kanematsu and D. M. Barry. Formation and control of biofilm in various environments. (2020). Springer Nature Singapore Pte Ltd.
8. K. S. Novoselov, A. K. Geim, S. V. Morozov, D. E. Jiang, Y. Zhang, S. V. Dubonos, I. V. Grigorieva and A. A. Firsov: Electric field effect in atomically thin carbon films. *Science*, 2004, **306**(5696), 666–669.
9. A. K. Geim and K. S. Novoselov: The rise of graphene. *Nat. Mater.*, 2007, **6**(3), 183–191.
10. V. Palmieri and M. J. N. T. Papi: Can graphene take part in the fight against COVID-19? *Nano. Today.*, 2020, **33**, 100883.
11. S. Liu, T. H. Zeng, M. Hofmann, E. Burcombe, J. Wei, R. Jiang, J. Kong and Y. Chen: Antibacterial activity of graphite, graphite oxide, graphene oxide, and reduced graphene oxide: membrane and oxidative stress. *ACS Nano*, 2011, **5**(9), 6971–6980.
12. W. Hu, C. Peng, W. Luo, M. Lv, X. Li, D. Li, Q. Huang and C. Fan: Graphene-based antibacterial paper. *ACS Nano*, 2010, **4**(7), 4317–4323.
13. K. Krishnamoorthy, N. Umasuthan, R. Mohan, J. Lee and S. J. Kim: Antibacterial activity of graphene oxide nanosheets. *Sci. Adv. Mater.*, 2012, **4**(11), 1111–1117.
14. D. Bitounis, H. Ali-Boucetta, B. H. Hong, D. H. Min and K. Kostarelos: Prospects and challenges of graphene in biomedical applications. *Adv. Mater.*, 2013, **25**(16), 2258–2268.
15. S. Sakaida, S. Z. S. Kure-Chu, T. Matsubara, J. Liu, T. Hihara and H. Yashiro: Hybrid electrodeposition of Ag-graphene composite films on Cu alloys for high-conductive and wear-resistive automotive connectors in Electrochemical Society meeting abstracts prime 2020. The Electrochemical Society, Inc; 2020. No. 16, p. 1461–1461.
16. S. Sakaida, S. Z. S. Kure-Chu, T. Hihara and H. Yashiro: Hybrid electrodeposition and characteristics of Ag-graphene composite films on Cu sheets toward high-performance electric connectors in Electrochemical Society meeting abstracts 235. The Electrochemical Society, Inc.; 2019. No. 18, p. 1064–1064.
17. A. C. Ferrari and J. Robertson: Interpretation of Raman spectra of disordered and amorphous carbon. *Phys. Rev. B*, 2000, **61**(20), 14095.
18. A. C. Ferrari: Raman spectroscopy of graphene and graphite: Disorder, electron–phonon coupling, doping and nonadiabatic effects. *Solid State Commun.*, 2007, **143**(1-2), 47–57.
19. L. G. Cançado, A. Jorio, E. M. Ferreira, F. Stavale, C. A. Achete, R. B. Capaz, M. V. O. Moutinho, A. Lombardo, T. S. Kulmala and A. C. Ferrari: Quantifying defects in graphene *via* Raman spectroscopy at different excitation energies. *Nano Lett.*, 2011, **11**(8), 3190–3196.
20. I. Childres, L. A. Jauregui, W. Park, H. Cao and Y. P. Chen: Raman spectroscopy of graphene and related materials. *New Develop. Photon Mater. Res.*, 2013, **1**, 1–20.
21. R. Saito, M. Hofmann, G. Dresselhaus, A. Jorio and M. S. Dresselhaus: Raman spectroscopy of graphene and carbon nanotubes. *Adv. Phys.*, 2011, **60**(3), 413–550.
22. X. Zou, L. Zhang, Z. Wang and Y. Luo: Mechanisms of the antimicrobial activities of graphene materials. *J. Amer. Chem. Soc.*, 2016, **138**(7), 2064–2077.
23. F. Perreault, A. F. De Faria, S. Nejati and M. Elimelech: Antimicrobial properties of graphene oxide nanosheets: why size matters. *ACS Nano*, 2015, **9**(7), 7226–7236.
24. J. Hu, J. Liu, L. Gan and M. Long: Surface-modified graphene oxide-based cotton fabric by ion implantation for enhancing antibacterial activity. *ACS Sustainable Chem. Eng.*, 2019, **7**(8), 7686–7692.
25. N. Mitik-Dineva, J. Wang, V. K. Truong, P. R. Stoddart, M. R. Alexander, D. J. Albutt, C. Fluke, R. J. Crawford and E. P. Ivanova:: Bacterial attachment on optical fibre surfaces. *Biofouling*, 2010, **26**(4), 461–470.
26. O. Akhavan, E. Ghaderi, H. Emamy and F. Akhavan: Genotoxicity of graphene nanoribbons in human mesenchymal stem cells. *Carbon. N. Y.*, 2013, **54**, 419–431.
27. S. Gurunathan, J. W. Han, A. A. Dayem, V. Eppakayala and J. H. Kim: Oxidative stress-mediated antibacterial activity of graphene oxide and reduced graphene oxide in Pseudomonas aeruginosa. *Int. J. Nanomedicine*, 2012, **7**, 5901–5914.