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Zhang, Peng; Guo, Zhiling; Chen, Chunying; Lynch, Iseult

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1 Opinion

² Uncertainties in the antibacterial mechanisms of graphene ³ family materials

4	Peng Zhang ^{1,2} , Zhiling Guo ^{1,} *, Chunying Chen ^{3,4} , Iseult Lynch ¹					
5						
6	¹ School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham					
7	B15 2TT, United Kingdom.					
8 9	² Department of Environmental Science and Engineering, University of Science and Technology of China, Hefei 230026, China					
10	³ CAS Center for Excellence in Nanoscience and CAS Key Laboratory for Biomedical Effects of Nanomaterials					
11	and Nanosafety, National Center for Nanoscience and Technology of China, Beijing 100190, China					
12	⁴ GBA National Institute for Nanotechnology Innovation, Guangzhou 510700, Guangdong, China					
13						
14	*Corresponding author:					
15	Dr Zhiling Guo; E: z.guo@bham.ac.uk					
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31 ABSTRACT

The antibacterial activity of graphene family materials (GFMs) has been explored since 2010, 6 years after the discovery of graphene in 2004. It is proposed that the antibacterial activity is derived from both physical interaction and chemical reaction between GFMs and bacteria. However, whether the two mechanisms work synergistically or whether there are conditions under which one mechanism dominates remains uncertain. This opinion article highlights the uncertainties and controversies in the current understanding of antibacterial mechanisms of GFMs as well as deficiencies in methodologies and provides perspectives on future directions to move this field forward.

39 KEYWORDS

- 40 Graphene family materials, bacteria, physical interaction, oxidative damage, computational modelling
- 41

42 Graphic abstract





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49 Introduction

50 Antimicrobial materials are an attractive approach for improving medical treatments, food packaging, 51 wastewater treatment process, textiles, and dental care. Development of novel and efficient antibacterial 52 agents is urgently required due to increasing bacterial resistance to existing antibiotics. Amongst the 53 numerous nanomaterials that have been demonstrated to be bactericidal, graphene family materials (GFMs), 54 especially graphene, graphene oxide (GO) and reduced graphene oxide (rGO), are undoubtedly attractive. 55 Graphene is a 2D sp²-hybridized carbon nanosheet composed of single-carbon atoms. GO is an oxidized form 56 of graphene, while rGO is a form of GO with less oxygen content obtained by chemical, thermal and other 57 reducing methods. The first report on the antibacterial performance of GFMs appeared in 2010, when Hu et al. produced GO paper with high antibacterial activity using a simple vacuum filtration method [1]. Since then, 58 59 numerous studies have explored the antibacterial mechanism of GFMs [2] and developed GFM-based 60 antibacterial materials including through modification with polymers [3], antibacterial metals [4] and 61 nanomaterials [5].

62 The major reason for GFMs being attractive as antimicrobial materials is the hypothesis that there is much 63 less chance of bacteria developing antimicrobial resistance (AMR) to GFMs because of their physical interaction mechanism [6, 7], compared with antibiotics which operate by interfering with RNA, protein or cell 64 65 wall synthesis or DNA replication [8]. GFMs not only show AMR-independent antibacterial activity but also 66 appear not to trigger long-term secondary resistance [6]. This unique feature allows GFMs to be used for 67 various antimicrobial applications although the potential toxicity to environment and human health needs to 68 be fully understood [9]. The physical mechanisms that have been proposed and most studied include side-on 69 interactions with the sharp edges of graphene sheets that result in cutting [10], penetration and extraction of the 70 lipid membrane [11] causing membrane damage and cell death, and wrapping [12] or trapping [13] of bacteria 71 which starves the cells by reducing the ability to take up nutrients. Bridging effects, whereby GFMs act as a 72 bridge to accelerate the movement of electrons between bacteria and the external environment, is also 73 proposed as a mechanism that causes bacterial death [14]. When a physical mechanism dominates, 74 development of antibacterial resistance is unlikely, as bacteria are not able to deactivate GFMs or evolve to 75 modify the molecular target of the GFMs in bacteria. However, it has also been proposed that chemical 76 reactions between GFMs and bacteria may also play a role in the antibacterial action [15], which increases the 77 likelihood for development of antibacterial resistance. The chemical mechanism includes the self-generation 78 of reactive radicals that kill bacteria or chemical oxidation of cellular components such as antioxidants (e.g. 79 glutathione, GSH) which indirectly cause overload of reactive oxygen species (ROS) in the cell, causing cell 80 death. Radical formation has been suggested to be less likely because the number of radicals generated by

GFMs *per se* is insufficient to produce such pronounced impacts [15, 16]. However, depletion of antioxidants
has been proven to play a critical role in the antibacterial of GFMs. [14, 17-19].

83 While we already know that these mechanisms play certain roles, it is unknown whether it is a simple 84 combination of these or whether each mechanism is controlled by certain factors (e.g. medium, GFMs 85 properties) and thus dominates under specific conditions. This fundamental question has been posed 86 previously [2]; however, isolation of the individual physical and chemical mechanisms and untangling their 87 relative contributions is challenging, so the question remains unanswered. Additionally, there are 88 contradictions in the published literature regarding the effects of GFMs on bacterial growth, i.e. studies 89 showing antibacterial effects, enhancement of growth, or insignificant effects. It is already known that the 90 antibacterial activity are affected by many factors such as the experimental conditions, the bacterial species, 91 and the physicochemical properties of the materials themselves. For example, changing the exposure medium 92 from simple to nutrient-rich can reverse antibacterial effects and induce growth promotion [20]. Reducing the 93 lateral size of the GO also enhances its antibacterial activity [21]. This opinion does not intend to discuss all 94 the controversies associated with the antibacterial performance of GFMs. Rather, it will focus on some of the 95 major uncertainties in the antibacterial mechanisms of GFMs, highlighting the drawbacks in the 96 methodologies that lead to these uncertainties and provide direction on future studies to overcome them.

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1. Physical interaction mode: parallel or perpendicular

99 The most well-accepted physical mechanism of antibacterial activity is the cutting of bacterial cell membranes 100 by the lateral edge of GFMs. This concept was firstly proposed by Akhavan et al., who produced GO and rGO 101 nanowalls with sharp edges that were nearly perpendicular to the stainless steel substrate and demonstrated 102 excellent antibacterial activities [10]. However, later studies provide increasing evidence that parallel arrays 103 of GO also kill bacteria, suggesting that perpendicular orientation is not a necessary condition for antibacterial 104 activity of GFMs [22]. Note that in the aforementioned studies the GFMs are deposited onto substrates where 105 their interaction mode is fixed, i.e. they are arrayed either perpendicular or parallel to the cells. In suspension, 106 different interaction modes may occur simultaneously. However, many studies have reported wrapping or 107 covering bacteria with GFMs, which represents a parallel interaction with the bacterial surface (Fig. 1a). The 108 studies that observed wrapping modes exclusively used GO rather than rGO or pristine graphene. Edgewise 109 contact with bacterial cells by rGO has been observed by scanning electron microscopy (SEM; Fig. 1b); however, 110 whether such contact mode causes cutting or penetration of the bacterial cell membranes needs experimental 111 evidence. The only study that visually identified the penetration of GFMs into cell membranes used pristine 112 graphene and human cell lines (Fig. 1c-1e). Therefore, the dominant physical interaction mode of GFMs in 113 suspension remains uncertain.

114 Fundamentally, the wrapping of GFMs on bacteria is driven by energy minimisation requirements, 115 whereby the more hydrophobic interiors of GFMs are shielded from water by promoting their stacking on the 116 hydrophobic cell wall of bacteria. Similarly, perpendicular penetration of GFMs into a cell membrane 117 maximises the hydrophobic interaction between the fatty acyl tails of lipids and the GFM surface. Since wrapping mode is mainly demonstrated by GO, while rGO or pristine graphene predominantly interact 118 119 perpendicularly (Fig. 1a and 1b) [20], the interaction could be related to the surface oxygen content of GFMs. 120 Furthermore, the surface oxygen content is related to the mechanical properties of GFMs: GO is relatively soft 121 and flexible, readily able to wrap around bacteria [23], while rGO and graphene are rigid and free standing so 122 more likely to interact with cell membranes edge-wise [24]. If the mechanical properties of the material are 123 critical to the mode of interaction, we may deduce that other parameters such as lateral size and number of 124 layers may also play a role by indirectly affecting the mechanical strength. For example, small-sized GO 125 nanosheets may have more chance of contacting the bacterial cell membrane directly than larger sized ones 126 and are more likely to be free standing. Additionally, it is more energetically expensive for a larger graphene 127 sheet to align vertically with a cell membrane than smaller sheets, which are more likely to interact in this way 128 through Brownian motion. Increasing the number of layers increases the thickness of GO, thus making them 129 more rigid and increasing the chance of edge-wise contact while reducing their capacity for wrapping. Lastly, 130 the physical interaction of GFMs with gram-negative bacteria might be different from that with gram-positive 131 bacteria due to their distinct cell wall composition and structure. These hypotheses are still undemonstrated 132 and thus need further studies.



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Fig. 1. Physical interaction mode of GFMs with bacteria and cells. a, b, SEM images of *S. aureus*, a grampositive round-shaped bacterium, after incubation with GO (a) and rGO (b) for 48 h. GO wrapped the bacteria while rGO contact with the bacteria edge-wise. Adapted and printed with permission from [20], Copyright 2017, Elsevier. c, d, SEM images of human lung epithelia cells (A549) exposed to graphene for 24 h at low and high magnification. Graphene penetrated the cell and stood freely. e, SEM image of murine macrophage exposed to graphene for 5 h. Penetration of multiple graphene sheets into a single cell was observed. Scale
bar in d and e indicate 2 µm. Printed with permission from [25]. Copyright, PNAS.

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142 **2.** Computational modelling needs to consider the bacterial structure

143 Computational modelling has been increasingly useful for obtaining mechanistic understanding of the 144 interaction of nanomaterials including GFMs with biological systems at the molecular level. For example, the 145 mechanisms of physical puncture and extraction of lipid membrane components have been proposed based 146 on molecular dynamic (MD) simulations [11]. The usefulness of the computational modelling depends on the 147 construction of the initial model and choice of parameters. Because of their large size, cells are usually 148 represented by a simplified planar lipid membrane. Most studies to date have used a single phospholipid 149 (POPE) or a combination of two lipids (POPE and POPC) to simulate the lipid membrane (Fig. 2a). However, 150 the bacterial cell membrane also contains other components such as proteins, cholesterol and carbohydrates, 151 which have been recently suggested to play important roles in the interaction of nanomaterials with bacterial 152 cell membranes [26]. For example, positively charged gold nanoparticles tend to interact with gram-negative 153 bacteria more than negatively charged gold particles mainly because of the interaction of lipopolysaccharides 154 (LPS) (Fig. 2b-2d) [26]. Another key issue is that current models do not consider bacterial cell structure. The 155 bacterial cell membrane is a complex multi-layered structure that protects them from hostile environments. 156 The plasma membrane of both gram-positive and gram-negative bacteria is surrounded by a thin 157 peptidoglycan cell wall (Fig. 2e). The former has a thicker cell wall, but while the latter has a thinner cell wall 158 it is surrounded by an outer membrane containing LPS. It remains unclear whether GFMs can penetrate the 159 peptidoglycan cell wall and enter the inner plasma membrane. Thus, we suggest that computational modelling 160 might need to consider the cell wall and other membrane components in future studies. This could be 161 combined with experimental studies to understand the role of a specific biomolecule component. Moreover, 162 the biocorona that forms on the GFMs surface immediately after contact with a culture medium will also affect 163 these interactions and should be considered in modelling, although it will increase the cost and time 164 substantially.



166 Fig. 2. MD simulation and role of bacterial cell structure on interactions. a, a representative trajectory of a 167 fully restrained graphene nanosheet docked at the surface of the POPE lipid membrane. The simulation time 168 is 110 ns and the lower snapshot is obtained by rotating the upper snapshot anticlockwise by 180 degrees. 169 Printed with permission from [11], Copyright 2013, Spring Nature Group. b, c, d, Gold nanoparticle (AuNP) 170 association with bacterial cells is directly observable and depends on the cell LPS content. The AuNP was 171 functionalized with either 3-mercaptopropionic acid (MPA) or 3-mercaptopropyl amine (MPNH₂). (b) Cells isolated (sorted) from the total cell population after exposure to MPNH₂-AuNPs. (c) Unsorted cells after 172 173 exposure to MPNH₂-AuNPs. In panels **b** and **c** the arrows point to AuNPs associated with the cells as confirmed 174 by hyperspectral imaging. (d) Association of MPA- or MPNH₂-AuNPs with the marine bacteria Shewanella cells 175 with varying LPS content (indicated by 8-amino-2-keto-3-deoxy-D-manno-octonate (8-aminoKdo) content of 176 lyophilized cells) quantified by flow cytometry. Error bars (representing one standard deviation, n = 3) are 177 smaller than the symbol in some cases. Printed with permission from [26], Copyright 2015, American Chemical 178 Society. e, Schematic illustration of gram-positive and gram-negative bacterial structures. Printed with 179 permission from [27]. Copyright 2015, Spring Nature Group.

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181 **3. Underestimated role of electric conductivity of GFMs**

182 While physical puncturing, nutrient deficiency as a result of wrapping, and oxidative stress are widely accepted 183 mechanisms of GFM- induced antibacterial activity, electric conductivity is a key property of GFMs that has 184 been overlooked and less studied in terms of its role in the antimicrobial activity of GFMs. In eukaryotic cells, 185 electron transport (ET) occurs in the mitochondria but in prokaryotes, which lack mitochondria, it operates in 186 the plasma membrane, i.e. the single membrane in gram-positive bacteria or the outer membrane in gram-187 negative bacteria [28]. The ET is central to the production of the energy needed to support bacterial growth. 188 When the ET in bacteria is disrupted, cell death occurs. In fact, enhancing ET has become increasingly attractive 189 as an effective strategy for electrochemical antibacterial approaches [29]. The typical way to achieve this is 190 by immobilizing electron acceptors (e.g. graphene or other nanomaterials) onto a metal or semiconductive 191 substrate so that electrons are transferred to the nanomaterial-metal system from the bacterial membrane, 192 causing membrane damage. Several studies have shown that immobilization of GFMs on conductive 193 substrates (e.g. Cu, Zn, Ni) can significantly enhance their antibacterial activity [30, 31]. The ET mechanism is 194 further supported by the fact that graphene and GO show little or no bactericidal effects on insulating 195 substrates such as SiO₂ film [31] or glass [30]. This can be explained by the different band structures of 196 conductors, semiconductors and insulators (Fig. 3). The respiratory protein in bacterial cell membranes, which 197 is responsible for oxygen transport, storage and delivery, behaves as a semi-conductor with a bandgap of 2.6 198 eV~ 3.1 eV. Contact between the cell membrane and a (semi) conductor will lead to the alignment of the 199 Schottky barrier and Fermi level, which facilitates electron transfer from the membrane to graphene [31].

200 Note that these studies are all performed on a conductive substrate, which acts to enhance electron 201 transfer so physical puncturing or wrapping of the bacterial cells is unlikely to happen. However, in suspension, 202 e.g. in wastewater, direct evidence for the ET mechanism is lacking and other physical mechanisms may occur 203 simultaneously. A study by Chong et al. reported that sunlight exposure can increase the antibacterial activity 204 of GO which they attributed to light-induced electron-pair holes on GO enhancing the ET from antioxidants 205 (e.g. GSH) [16]. Another study reports that nitrogen doping of GO eliminates their antibacterial activity 206 because nitrogen has one more electron than carbon and thus changes GO from an acceptor to an electron 207 donor, thus preventing energy transfer in the bacteria and reducing the antibacterial activity of GO [32]. While 208 a few studies indicate that the electric conductivity of GFMs may play an important role in their antibacterial 209 activity, in many studies GO shows higher antibacterial performance than graphene or rGO despite having 210 lower electric conductivity. Thus, in suspension, multiple factors might act simultaneously and this needs to 211 be studied and explored under specific conditions.



Fig. 3. Role of electric conductivity of GFMs in their antibacterial activity. a Schematic illustration of an electrochemical antibacterial device utilising GFMs. b-d, Energy band diagrams of graphene-on-substrate junctions on a conductor such as Cu (b), a semiconductor Ge (c) and an insulator SiO₂ (d) substrates. e – h, Schematic illustration for the electrical measurements (e) to obtain the current–voltage (I–V) characteristics of Graphene@Cu (f), Graphene@Ge (g) and Graphene@SiO₂ (h) contacts at room temperature, respectively, indicating three different types of contact of graphene films with the underlying substrates. Printed with permission from [31]. Copyright 2014, Springer Nature Group.

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4. Improved methodologies to study chemical mechanisms of action in bacteria are

222 needed

The primary chemical mechanism for GFMs-induced bacterial death is believed to be mediated by oxidative stress. It can be caused either by reactive oxygen species (ROS) directly generated by GFMs or *via* the depletion of antioxidants which are responsible for capturing excessive ROS in order to maintain a balance in the bacterial cells. Although the mechanisms of ROS generation are still controversial, it is increasingly evident that the number of ROS generated directly by GFMs is minimal, contributing little to the antibacterial activity

[15, 16]. Thus, the oxidation of antioxidants such as GSH seems to be the key chemical mechanism. Indeed, *in vitro* GSH oxidation has been used as a classic method to demonstrate and compare the capacity of GFMs to oxidize the cellular antioxidants and to quantify the subsequent antibacterial effect [14, 33].

The question raised here however, is whether this *in vitro* test represents a realistic scenario in bacteria. Firstly, the bacterial species should be considered as a key factor because GSH is widely found in eukaryotes and gram-negative bacteria but is hardly present in gram-positive bacteria [34, 35]. This means that GSH oxidation assay is not ideal when evaluating antibacterial activity towards gram-positive bacteria.

235 Secondly, in vitro GSH oxidation assays are performed in liquid phase and the reaction is based on 236 direct contact of the GFMs with GSH. In cells, however, GSH is only produced in the cytosol and efflux to 237 extracellular regions is low. Besides, as there is no direct evidence to date showing that GMs can enter 238 bacterial cells, the possibility of direct interaction between GFMs and GSH is very low. Although computational 239 modelling studies suggest that graphene can penetrate cell membranes, experimental evidence of the entry 240 of GFMs into the cytosol has only been reported in mammalian cells through endocytosis [36]. However, 241 endocytosis is not known to occur in bacteria [37]. Unless the bacterial cell wall and membrane are broken, 242 GFMs are unlikely to enter the bacterial cytosol and reach GSH. The cell wall is unlikely to be broken by GFMs 243 under pure physical forces, unless chemical interactions between GFMs and bacterial cells occur to rupture 244 the cell wall and allow entry of GFMs into the cells. Therefore, more experimental evidence is needed to 245 demonstrate the direct oxidation of GSH by GFMs in bacteria. This may need in situ techniques such as labelling 246 to observe GFMs inside bacteria at a subcellular level. Alternatively, GSH oxidation may occur indirectly, e.g. 247 via an electric conductive bridging effect. GFMs may act as a conductive bridge over the insulating cell 248 membrane, accepting electrons transferred from GSH [16].

Lastly, the GSH assay is usually performed in buffer without considering the medium in which the bacteria are cultured. *In vitro* GSH oxidation relies on direct contact with GFMs. Upon contact with the medium, the physiochemical properties of GFMs may change immediately due to the adsorption of ions or biomolecules onto the basal surface (formation of a biocorona) [38]. Thus, to interpret the results thus to determine link with antibacterial activity, pre-incubation of GFMs in relevant culture media might be necessary in future studies.

255

5. Summary and outlook

257 Despite the growing body of literature on the antimicrobial activity of GFMs, there are still several 258 uncertainties in the mechanism of this behaviour / effect. To explore these mechanisms, several fundamental 259 questions need to be answered in the near future, including:

1) What are the main factors driving the interaction mode? It has been demonstrated that the surface oxygen content of GFMs can switch the interaction mode from parallel to perpendicular. Whether other parameters such as sheet size or thickness can affect the interaction mode needs to be understood. The interconnection of these parameters and the combined effects of the GFM physicochemical properties as well as the impact of the medium composition on these, should be explored and understood.

265 2) Can GFMs cut cell membranes and enter bacteria? Computational simulations have provided vital 266 mechanistic information that GFMs may cut the cell membrane and extract the lipid membrane components. 267 However, a limitation is that these simulations are based on a simplified lipid membrane model. More 268 experimental and computational research is required to demonstrate the role of other membrane or cell wall 269 structural components in the interaction of GFMs with bacteria. While it is difficult to incorporate all the cell 270 components into a single simulation system because of the significant computational cost and time, which will 271 remain a challenge in the foreseeable future, this can be studied separately and interpreted together with 272 experimental data. Experimental evidence is also needed as to whether GFMs can cut through cell walls and 273 enter bacteria when in suspension. Sophisticated techniques such as isotope labelling of GFMs and/or imaging 274 of their location in a single bacterial cell may be needed. Besides, machine learning may make it feasible to 275 obtain critical information from data accumulated over the last decade, when combined with molecular 276 dynamics and coarse-grained simulation.

3) *Is there an alternative method for examining the oxidative potential in vivo?* As discussed above, the *in vitro*GSH oxidation assay is not suitable for most gram-positive bacteria. Can other intracellular antioxidants be
used as alternatives for GSH?

280 The complexity of antibacterial activity and its mechanisms are not only the result of the 281 physicochemical properties of GFMs but also different bacterial species and the dynamics of the environment 282 in which they are present. Although antibacterial properties could be driven by multiple factors simultaneously, 283 increasing evidence suggests that they are dominated by certain factors (e.g., size, SOC or culture medium 284 composition) in specific scenarios, which need to be elucidated. Therefore, systematic studies are required in 285 the future. More importantly, these factors are interconnected so an integrated view of their roles is needed 286 (Fig. 4). The biological and chemical activities of GFMs are determined by their physicochemical properties 287 including lateral size/surface area, thickness (number of layers), surface chemistry and electrical conductivity. 288 However, the original identity of GFMs can change when they enter a biological environment, e.g. through 289 agglomeration or formation of a biocorona, which determines the subsequent biological activity of the GFMs. 290 The physical and chemical mechanisms involved in GFM-bacterial interactions, in essence, may become GFM-291 biocorona-bacterial interactions. Thus, bacterial responses to GFM exposure vary in different environments.

- 292 Bacterial species are also critical given their structural differences and that they may excrete different
- 293 extracellular components, which affect their interactions with GFMs.



Fig. 4. Illustration of the interconnection of the properties of GFMs, bacteria and the ambient environment. GFMs can both inhibit or enhance bacterial growth, and which effect they induce is affected or even reversed by changing the growth conditions. Bacterial growth depends on the ambient environment. Bacteria secrete biomolecules and their respiratory activity can alter the properties of GFMs, causing biotransformation, which subsequently affects the antibacterial effect of the materials. Untangling and exploiting these interconnected processes is essential to ensure the safe use of GFMs.

301

302 These critical factors including physical / chemical properties and experimental conditions, however, 303 are not always fully reported. For example, SOC might be a key factor determining whether GFMs interact 304 with bacteria perpendicularly or edge-wise (Fig. 1a and 1b); however, based on our survey of the literature 305 regarding the antibacterial effects of pristine GMs since 2010, only 23 out of the 72 studies (32%) reported 306 SOC. Lack of reporting of SOC renders the results from these studies difficult to compare. To enable a 307 comparison between different studies and address the uncertainties in the mechanisms of action of GFMs, we 308 suggest a checklist of questions for performing antibacterial tests with GFMs. The impact of each aspect of the 309 material, medium, and bacterial species is analyzed. We recommend that this checklist be used as a standard 310 for future studies exploring the antimicrobial properties of GFMs.

Table 1. Checklist for reporting GFMs antibacterial studies to enable comparison between different studies.

Reporting standard for GFMs	Techniques	Impact
antibacterial study		

Are the GFMs fully	Method of synthesis	N/A	Synthetic method may lead to different
characterized?			physicochemical properties of GFMs and thus should
			be described
	Impurities/ doping or	XPS, ICP-MS	Impurities such as sulphur per se can induce
	composites		antibacterial effect, or doping such as nitrogen
			affects the antibacterial effects, and thus should be
			quantified, including potentially their release kinetics
			in the exposure medium
	Lateral size or surface	TEM, SEM,	Larger-sized GFMs have higher antibacterial activity
	area	AFM	while the effects can be affected by other factors
			simultaneously
	Thickness or number	AFM	Thickness affects the edgewise contact of GFMs with
	of layers		the bacteria, which may reduce the "cutting" effect
	Surface oxygen	XPS	Surface oxygen content affects the properties of
	content or C/O ratio		GFMs (e.g. rigidity, electric conductivity,
			hydrophilicity) thus the interaction of GFM with
			bacteria
	Surface charge	DLS / zeta	Surface charge affects the interaction of GFMs with
		potential	the bacterial surface
Are bacterial	Bacterial species and	N/A	Different species respond differentially to the same
species accurately	strains		GFMs
reported?	Growth stage	N/A	At different stages in their growth curve the bacteria
			respond differentially to GFMs
Are culture media	Name and	N/A	Different culture media affect the agglomeration
compositions	compositions of the		state of GFMs. Rich media change the surface by
reported clearly?	media		forming a biocorona on the GFMs surface
Are exposure	Exposure method and	N/A	Results can be different due to the variation in the
method and	duration		test method (suspension assay, biofilm assay, colony
duration clearly			counting test) and duration (3h, 24h or more)
described?			
Are relevant	GFMs properties,	Computational	One parameter (e.g. size, surface oxygen content of
parameters	model cell membrane	simulation	GFMs, or medium pH, organic matter content) may
clearly reported	composition, medium		change the interaction of GFMs with the cell
for computational	composition,		membrane
modelling?	interaction duration		
	etc		

313

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