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Graphene–Curcumin Coatings Resistant to SARS-CoV-2 and Mycobacteria for the Production of Personal Protective Equipment

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ABSTRACT

Respiratory tract infections represent the main cause of death from infectious diseases worldwide. SARS-CoV-2 infection (i.e. COVID-19) added to the existing global burden of respiratory tract infections, including tuberculosis. Among nanomaterials for fabric functionalization, graphene, in combination with hydrophobic molecules such as phytochemicals, represents a promising low-cost alternative to antibiotics. In this work, we used graphene and curcumin to create fabric coatings on cotton and polyester for the production of personal protective equipment resistant to infective agents. These coatings ensure the trapping of microorganisms via interaction with SARS-CoV-2 or mycobacteria surface and inhibit microbial infections.

摘要

呼吸道感染是全球传染病死亡的主要原因。SARS-CoV-2感染（即新冠肺炎）增加了包括结核病在内的现有呼吸道感染的全球负担。在用于织物功能化的纳米材料中，石墨烯与疏水分子（如植物化学物质）结合，是一种有前途的低成本抗生素替代品。在这项工作中，我们使用石墨烯和姜黄素在棉花和聚酯上制造织物涂层，用于生产抗感染剂的个人防护设备。这些涂层通过与SARS-CoV-2或分枝杆菌表面的相互作用确保捕获微生物，并抑制微生物感染。

KEYWORDS

Graphene; curcumin; coatings; PPE; tuberculosis; SARS-CoV-2; COVID-19

关键词

石墨烯; 姜黄素; 涂层; 肺结核


Introduction

Respiratory infections and pneumoniae remain a significant public health concern on a global scale and the top cause of morbidity and mortality from infectious diseases. SARS-CoV-2 infections rapidly went on top of the World Health Organization (WHO) Blueprint priority list, because of the morbidity and mortality associated with the disease (COVID-19). SARS-CoV-2 transmission was rapid and abrupt worldwide, posing a major threat that forced governments in most countries to impose several public health measures (Brauner et al. 2021; Talic et al. 2021).

Besides, other airborne-transmitted infectious diseases with epidemic or pandemic potential pose major challenges to public health authorities. Tuberculosis (TB), for example, is an ancient disease that kills approximately 1.8 million years and killed more than 1 billion people in the last 2000 years

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(Barbier and Wirth 2016) and its etiologic agent, *Mycobacterium tuberculosis* (*Mtb*) is usually transmitted in domestic and workplace settings and use of effective preventive measures may reduce contagion. Recently, the emergence of multidrug and extensively drug-resistant *Mtb* strains represent a global challenge that requires the adoption of urgent, effective and innovative preventive measures (WHO 2021).

The need for protection against SARS-CoV-2 and other airborne pathogens boosts the design of innovative personal protective equipment (PPE) (Palmieri et al. 2021). Nanoparticles, nanofibers, and other pioneering technologies based on nanomaterials have been introduced in PPE production chains to improve performance and confer properties that would limit microbial spreading through clothing. As an example, it has recently been demonstrated how Cu and Zn cotton functionalization can create human breath-activated fabric against *Escherichia coli*, *Staphylococcus aureus*, and *Enterovirus 71* (Zhang et al. 2022). Similarly, chitosan and silver have been used to functionalize PPE (Zhang, Zhang et al. 2022; Zhu et al. 2022).

Among nanomaterials, graphene, the two-dimensional carbon-based material with exceptional light absorption and biological properties, has been studied recently as an antimicrobial agent for its ability to interact mechanically and chemically with lipid membranes, breaking the membranous ultrastructure of bacteria and enveloped viruses. Furthermore, graphene can be easily functionalized with hydrophobic molecules such as phytochemicals (Bugli et al. 2018; De Maio, F., V. Palmieri, A. Salustri et al. 2019; Palmieri and Papi 2020; Palmieri et al. 2018) plant derivatives that represent a promising alternative to synthetic chemical compounds, with antibacterial, antiviral, and immunomodulatory properties (Ayaz et al. 2019).

Curcumin, the principal curcuminoid of turmeric (*Curcuma longa*) alone or in combination with antibiotics, is known for its antibacterial properties for a long time both as coating and in soluble form (Bugli et al. 2018; Palmieri et al. 2018). Curcumin protects against pulmonary inflammation and acute lung injury generated during *Klebsiella pneumoniae* induced lung infection in vivo (Bansal and Chhibber 2010) and inhibits 3CL protease of SARS-CoV (Wen et al. 2007). Furthermore, curcumin is known to enhance human macrophage control of *Mtb* infection (Bai et al. 2016).

In this work, we used graphene and curcumin to create fabric coatings for PPE resistant to infective agents for the management of pandemic emergencies. Here, we report the functionalization of two commonly used fabrics for PPE production, i.e. cotton and polyester (PES), and first analyzed the effects on SARS-CoV-2 (wild-type clinical isolate) infectivity using African green monkey kidney cells (VERO cells, ATCC CCL-81) in biosafety laboratory level 3 (BCL3). Subsequently, we measured the efficacy of coatings on *Mycobacterium smegmatis* (Ms, mc2 155) as a model for *Mtb* infection. We demonstrate how the coating of graphene and curcumin represents a feasible functionalization for a broad-spectrum antimicrobial effect.

Curcumin–graphene coating on textiles inhibits SARS-CoV-2 infectivity

Fabric coatings have been prepared using graphene alone (G), curcumin alone (C), or a combination of the two (G/C); the resulting materials are shown in Figure 1a. Graphene nanoplatelets (G+, Directa-Plus) have been produced according to a proprietary patented technology that involves three different steps: expansion, exfoliation, and drying (Bonetti et al. n.d.). A representative image of G+ obtained by atomic force microscopy and the corresponding height profile is reported in Figure S1. Full characterization of this nanomaterial is reported elsewhere (Cesareo, Parrini, and Rizzi 2020). Non-woven (200 gsm, 100% Polyester PES) and cotton fabrics (110 gsm, 100% cotton) were functionalized with G+ using impregnation method. In Figure 1b and 1c, we report experimental FTIR spectra for cotton and PES showing the functionalization of fabrics. Data indicate a successful coating for both samples. Coated cotton depicted two peaks in the range between 700 and 800 cm^{-1} , which are associated with the stretching vibration of C–H in curcumin (Figure 1b) (Ching et al. 2019). Two peaks (1270 and 1560 cm^{-1}) can be observed, which correspond to C–O vibrations and C=N vibrations of curcumin, respectively (Ismail et al. 2014). A peak at 1603 cm^{-1} is associated with the stretching of the two

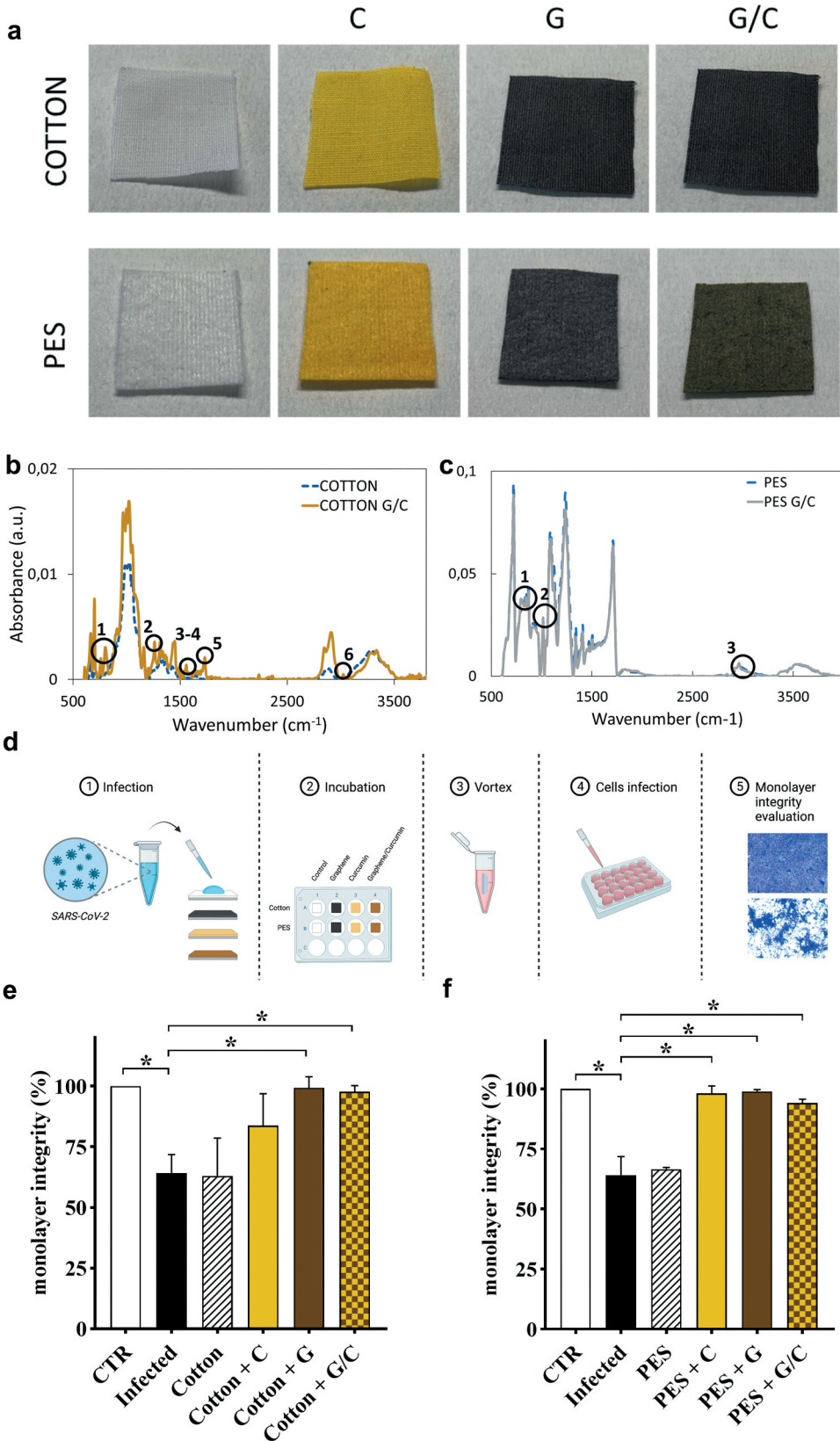


Figure 1. (a) Representative images of coated cotton and PES fabrics functionalized with curcumin C graphene G (G+ Directa-Plus) and graphene/curcumin G/C. Functionalization with curcumin (CAS: 458-37-7, TCI Zentek), graphene or G/C has been performed

phenolic groups of curcumin, and a peak at 1730 cm^{-1} indicates the stretch of the amide I band (Banerjee 2014). Finally, a peak at 3000 cm^{-1} indicates the presence of asymmetric and symmetric stretches typical of graphene (Hayyan et al. 2015). In Figure 1c, as visible for coated cotton, a clear peak at 800 cm^{-1} indicates stretching vibrations of C–H in curcumin on PES samples (Ching et al. 2019). Another peak, which is present only on coated PES, is visible at 1020 cm^{-1} and is associated with enhanced C–O–C stretching vibrations of curcumin (Chen et al. 2015). Similar to cotton, a peak at 3000 cm^{-1} indicates CH_2 asymmetric and symmetric stretch of graphene.

In Figure 1d, the experimental setting used to evaluate the effects of coatings on the virus is shown. The quantification of the effects on SARS-CoV-2 has been performed by evaluating the viability of VERO epithelial cells, by measuring monolayer integrity, after exposure to solutions obtained from fabrics washed after contact (2 h) with SARS-CoV-2. Cell monolayer integrity has been evaluated on each image using ImageJ software using a threshold for image binarization and calculating the percentage of black pixels (uncoated petri) and white pixels (cells). ANOVA test was used to evaluate significant differences among infected cells and uninfected cells, or cells infected with SARS-CoV-2 exposed to fabrics. Values of $p < .05$ were considered as significant. In Figure S2, representative images of cell monolayers are reported.

Figure 1e and 1f displays how cotton or PES alone are not effective in blocking SARS-CoV-2. Concerning cotton, the addition of C slightly improves the ability of cotton to reduce SARS-CoV-2 infection.

Additionally, we then calculate the viral infectivity reduction from the following equation:

$$\frac{(\text{Treated} - \text{Infected})}{(\text{CTR} - \text{Infected})} * 100 \quad (1)$$

where Treated, CTR and Infected are respectively the percentage of monolayer integrity observed for cells infected treated with viruses recovered from fabrics, cells not infected and cells infected with the virus (De Maio et al. 2021, 2022). A $\approx 50\%$ reduction of viral load is visible for cotton C treatment, though a marked reduction in infectivity has been achieved only after coating cotton with G or G/C ($\approx 99\%$ and $\approx 98\%$ reduction) (Figure 1e). Interestingly, PES coating with C, G or G/C show a significant reduction in SARS-CoV-2 infectivity ($\approx 98\%$, 98% , and 94% , respectively) (Figure 1f).

It has been previously shown that graphene is efficient in inhibiting SARS-CoV-2 infectivity thanks to the trapping effect on viral particles (De Maio et al. 2021, 2022); viral particles are stacked to graphene flakes on fabric coatings and consequently are not released from fabrics (Ye et al. 2015). The interaction between the SARS-CoV-2 particle and graphene, which occurs via π – π stacking and hydrophobic interactions, significantly affects the secondary S protein structure (Du et al. 2021). The activity of curcumin against other coronaviruses is known since 2007 (Wen et al. 2007). Here we demonstrate the antiviral potential of curcumin-coated surfaces that, given curcumin insolubility in water, may result from the accumulation of the lipophilic molecule on the envelope membrane, thus affecting lipid order and overall viral stability (Duda, Cygan, and Wisniewska-Becker 2020). Our findings are in line with recent in silico studies showing that curcumin has a high-affinity for the S glycoprotein of SARS-CoV-2, ACE2 receptor and the transmembrane protein serine protease 2 (TMPRSS2), which facilitates the entry of SARS-CoV-2 in the host cells (Maurya et al. 2020; Rattis, Ramos, and Celes 2021).

according to literature (Cesareo, Parrini, and Rizzi 2020; De Maio et al. 2021). For the preparation of G/C fabrics, a ratio of 1:0.5 was used, corresponding to 1.4% of graphene and 0.7% of curcumin. (b) FTIR spectroscopy (OPUS spectrometer Bruker) of cotton and cotton G/C and (c) of PES and PES G/C is reported with characteristic peaks highlighted with dark circles. The material was directly laid on the ATR crystal and the spectra were recorded in the wavenumber range of 4000 – 5500 cm^{-1} according to the literature (De Maio et al. 2020). (d) Schematic representation of the experimental setting used to assay the antiviral activity of functionalized fabrics: 0.1 mL of SARS-CoV-2 solution (10^5 particles/mL) has been placed on the surface of each textile ($1 \times 1\text{ cm}$) in separate wells of a sterile plate. After 2 h of incubation, fabrics were transferred in a new tube containing 5 mL of fresh cell medium and vigorously vortexed. Then 0.1 mL of recovered supernatants were used to infect VERO cells. Cell viability was assessed by using Crystal violet staining (Supplementary Figure 2) and quantitative analysis presented in the graphs for cotton (e) and PES (f). Monolayer integrity has been normalized on not infected cells. All experiments were analysed by using one-way ANOVA comparison tests followed by Tukey's correction.

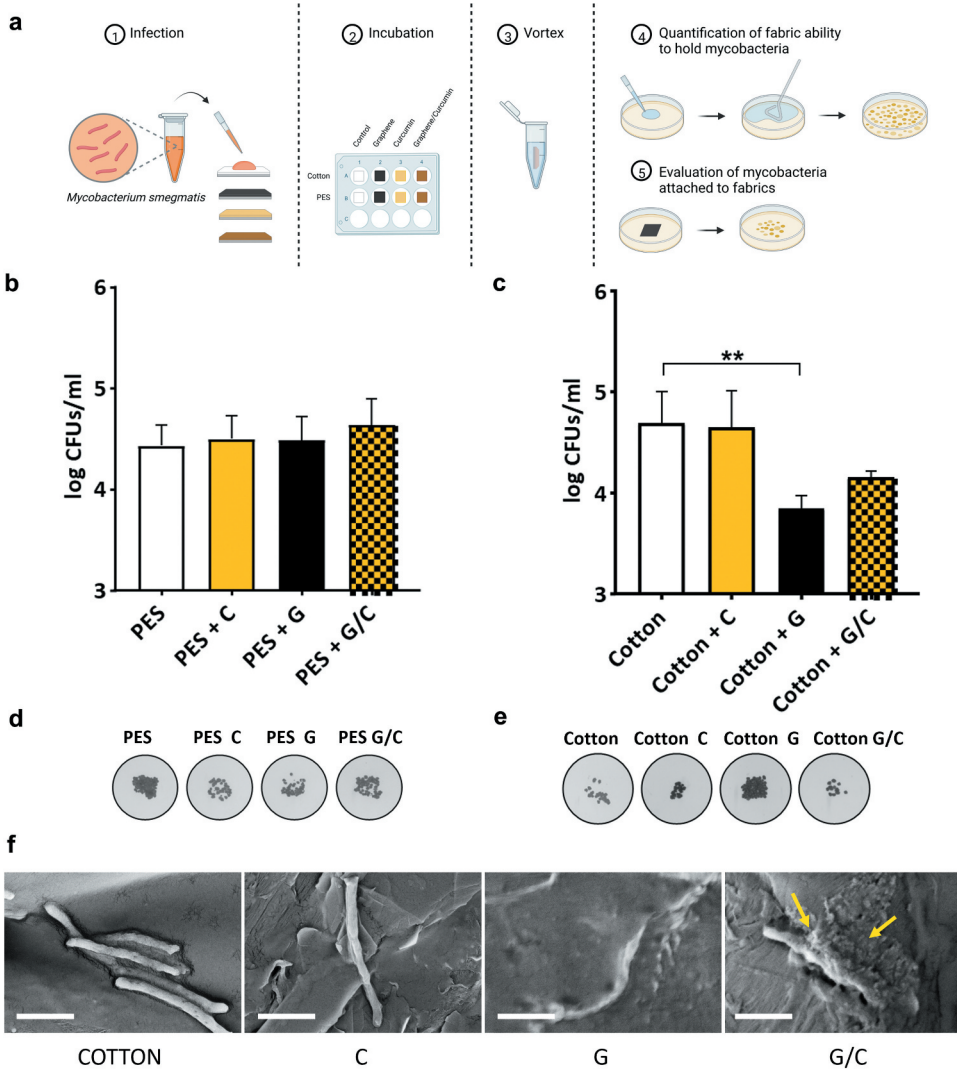


Figure 2. (a) Schematic representation of the experimental setting performed to test anti-mycobacterial activity of functionalized fabrics using *Mycobacterium smegmatis* (Ms). 0.1 ml of a solution containing $\approx 10^5$ CFU/ml of Ms were directly placed on the surface of each textile ($1 \times 1 \text{ cm}^2$) in separate wells of a sterile plate. After 4 h of incubation, textiles were transferred in a new tube containing 1 ml of sterile PBS. Tubes containing textiles were vigorously vortexed and serial dilutions of supernatants were plated on 7H11 solid medium to determinate colony forming units (CFUs) per mL and reported in (b) for PES and (c) for cotton. Furthermore, after vortexing, each fabric was placed on 7H11 solid medium with the infected functionalized side facing towards the plate. One hour post incubation at 37°C , textiles were removed from the medium and petri dishes were incubated at standard atmosphere conditions, results are in (d) for PES and (e) for cotton. (f) Representative SEM images of Ms on cotton fabric with different coatings have been acquired using SEM SUPRA 25 (ZEISS) after dehydration and sputter coating. Scale bar is 2 μm ; yellow arrows indicate membrane damages. All experiments were analysed by using one-way ANOVA comparison tests followed by Tukey's correction.

Curcumin–graphene coating disrupts the integrity of mycobacteria membrane and prevents spreading from contaminated fabric

Besides SARS-CoV-2, many viral and bacterial agents are airborne transmitted and are responsible for major infectious diseases, first and foremost Mtb. We analyzed the effects of coatings on Ms, which in this context can be considered an experimental model for Mtb infection and eventually other mycobacteria known to cause human disease following aerogenic exposure (Figure 2) (De Maio, F., V. Palmieri, M. De Spirito et al. 2019; De Maio, F., V. Palmieri, A. Salustri et al. 2019; De Maio et al.

2020). ANOVA test was used to evaluate significant differences among Ms exposed to fabrics and mycobacteria of the infecting solution. Values of $p < .05$ were considered as significant.

To measure the effect of coatings, we quantified the ability to hold bacteria after vortexing contaminated fabrics and evaluated the bacteria that remained attached (Figure 2a).

PES or PES coated with C, G or G/C displayed similar effects on bacteria with an overall reduced recovery of colonies after vortexing compared to bacteria in the infecting solution 10^5 CFU/mL (Figure 2b). By plating the colonies that remained on the fabric (Figure 2d), it is possible to observe that bacteria continued growing on PES during the incubation, while the functionalized fabric exerted a bacteria-killing effect similar for all conditions.

Unfunctionalized and C-coated cotton had a similar ability to retain bacteria (Figure 2c), yet CFU plating results shown in Figure 2e, demonstrate that bacterial loads are not reduced following contact with cotton or Cotton C.

Conversely, coating cotton with G led to a significantly stronger mycobacteria capture/trapping, as indicated by the reduced bacterial release after vortexing ($\approx 40\%$). The combined use of G/C did not provide any additive effect ($\approx 58\%$ of released mycobacteria) (Figure 2c). Interestingly, in Figure 2e, the plating results show that while G functionalized cotton fabric retained a high number of living bacteria, a small number of colonies remained on G/C-coated cotton. In summary, Cotton G/C can avidly capture bacteria cells without provoking cell damage that may affect bacterial viability.

Scanning electron microscopy images confirmed that the contact between G/C functionalized cotton and mycobacteria affected the Ms cell wall integrity, that was otherwise unaffected by other conditions (Figure 2f). Indeed, as highlighted by the yellow arrows in Figure 2f, the mycobacterial surface loses its typical ultrastructure when in contact with G/C coatings, with debris released from the bacterial cell. Bacterial cell walls appear also damaged following contact with G but not following contact with control fabrics or C-treated samples.

We need to consider that the effects of coating hydrophobicity may differ depending on microbe size. Specifically, since cotton is hydrophilic and tends to retain moisture, the overall release of bacteria compared to PES is lower (one order of magnitude) and the addition of graphene enhances microbial trapping, alone or when combined with curcumin. This effect is probably mediated by the increase in fabrics' hydrophobicity: hydrophobicity is an hallmark of *Mtb* and other pathogenic mycobacteria, suggesting that strong hydrophobic interaction may explain the ability of graphene-based fabrics to capture mycobacteria (Jankute et al. 2017). Hence, Cotton G/C or PES can properly expose curcumin, which is also a liposoluble drug, so to exert its known anti-mycobacterial and anti-biofilm properties (Barua and Buragohain 2021).

In summary, the G/C coating of fabrics is an effective option to reduce transmission of mycobacteria and SARS-CoV-2, and certainly other similar viruses and bacteria.

Conclusions

WHO constantly highlights the need for prioritization of personal protective equipment supplies for frontline health-care workers. Graphene–curcumin coatings can minimize the risk of transmission limiting pandemic spreading, with huge social and economic impact.

The poorest and most marginalized people across the world, who suffer most from TB and other infections, likely the most affected by the COVID-19 epidemic, can benefit from the use of PPE enriched with graphene–curcumin wide-spectrum antimicrobials.

Importantly, the prevention offered by graphene–curcumin coatings will avoid further transmission to health-care workers and limits pathogen spreading through waste in the environment. Furthermore, the combined mechanical and chemical effect of the two antimicrobial agents might reduce the appearance of resistant strains.










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Disclosure statement

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

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