2294
doi:10.1017/S1431927620021108

Microsc. Microanal. 26 (Suppl 2), 2020
© Microscopy Society of America 2020

STEM-EDS and STEM-EELS Studies of Antibacterial Behavior of Phosphorene

Abhijit H. Phakatkar, Reza Shahbazian-Yassar and Tolou Shokuhfar

University of Illinois at Chicago, Chicago, Illinois, United States

The evolving antimicrobial resistance (AMR) levels for pathogenic bacteria have been a compelling global challenge.[1,2] To counteract AMR mutating abilities of bacteria, in the past two decades, the research has been more focused on evaluating the antibacterial activities of various advanced nanomaterials against Gram-positive and Gram-negative bacteria by considering their advantages with material properties, surface charge and characteristics, shape, size, aspect ratio, dispersion abilities and reactivity with surrounding environmental conditions.[3] Among the family of 2D nanomaterials, phosphorene (two-dimensional black phosphorus) nanosheets have demonstrated promising potential for biomedical applications. Herein, for the first time, we investigate the interaction of phosphorene nanosheets against Gram-negative *Escherichia coli* (*E. coli*) bacteria by using transmission electron microscopy technique.

Our results provide nanoscale insights of structural damage caused by phosphorene nanosheets to the bacterial cell membrane. Primarily, phosphorene nanosheets were synthesized by the liquid exfoliation method in the ecofriendly, non-toxic and biocompatible deoxygenated water media. Synthesized micron size phosphorene nanosheets were characterized by using low-magnification TEM (**Figure 1a**)[4] and their chemical stability was evaluated by using EDS and electron energy loss spectroscopy (EELS) techniques by using aberration corrected ARM-200CF atomic resolution transmission electron microscope in the scanning transmission electron microscope (STEM) mode (**Figure 1b to 1d**).[4] Various events of phosphorene nanosheets interacting with bacterial cell wall and the cytoplasmic leakage, detachment of cytoplasm from the cell membrane, distorted bacterium morphology, reduced density of lipid bilayer and agglomerated DNA structure leading to the loss of cellular metabolism are evaluated by using TEM micrographs (**Figure 2a to 2f**).[4] The lost cellular integrity, the disrupted membrane permeability and the loss of cytoplasmic and cell membrane components are confirmed by analyzing the presence of diagnostic phosphorus, sulfur, chlorine and calcium ions evaluated with the EDS elemental line mapping of high angle annular dark field (HAADF) STEM image of damaged bacterium (**Figure 2g to 2j**).[4] Further TEM studies include STEM-EELS analysis of damaged *E. coli* bacterium providing elemental bonding details bacterial cell membrane components interacting with phosphorene nanosheets. By attributing to the biocompatibility and biodegradability properties of phosphorene nanosheets, we believe our efforts will promote the use of phosphorene nanosheets as an antimicrobial platform in the biomedical field.

Downloaded from https://www.cambridge.org/core. IP address: 198.54.106.171, on 04 Jan 2021 at 03:07:14, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S1431927620021108

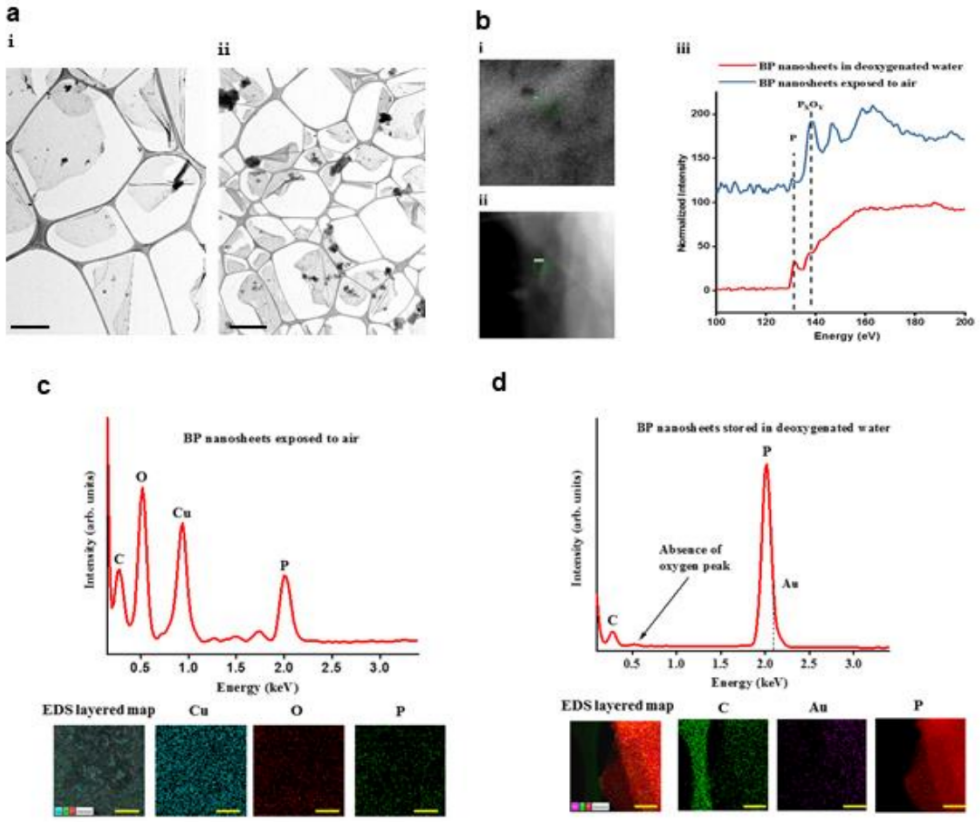

Figure 1. Morphological characterization and elemental analysis of phosphorene nanosheets. (a) Low-magnification TEM images of exfoliated phosphorene ultra-large nanosheets. In image i, the scale bar represents 1 μm, and image ii (scale bar is 2 μm). (b) EELS analysis: i. The high angle annular dark field (HAADF)-STEM image of phosphorene nanosheet exposed to air, acquired at 80 kV (scale bar is 20 nm). ii. The HAADF-STEM image of phosphorene nanosheet in deoxygenated water (scale bar is 0.2 μm). iii. The EELS spectra corresponding to the selected area in i and ii shows the P-L2,3 edge of phosphorene nanosheet, confirming the phosphorene nanosheet stored in the deoxygenated water were not oxidized. (f) The EDS analysis of phosphorene nanosheet exposed to air along with elemental mapping confirming the presence of oxygen (scale bar is 25 nm). (g) The EDS analysis of phosphorene nanosheet stored in deoxygenated water along with elemental mapping confirming the absence of oxygen (scale bar is 250 nm).

Downloaded from https://www.cambridge.org/core. IP address: 198.54.106.171, on 04 Jan 2021 at 03:07:14, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/S1431927620021108

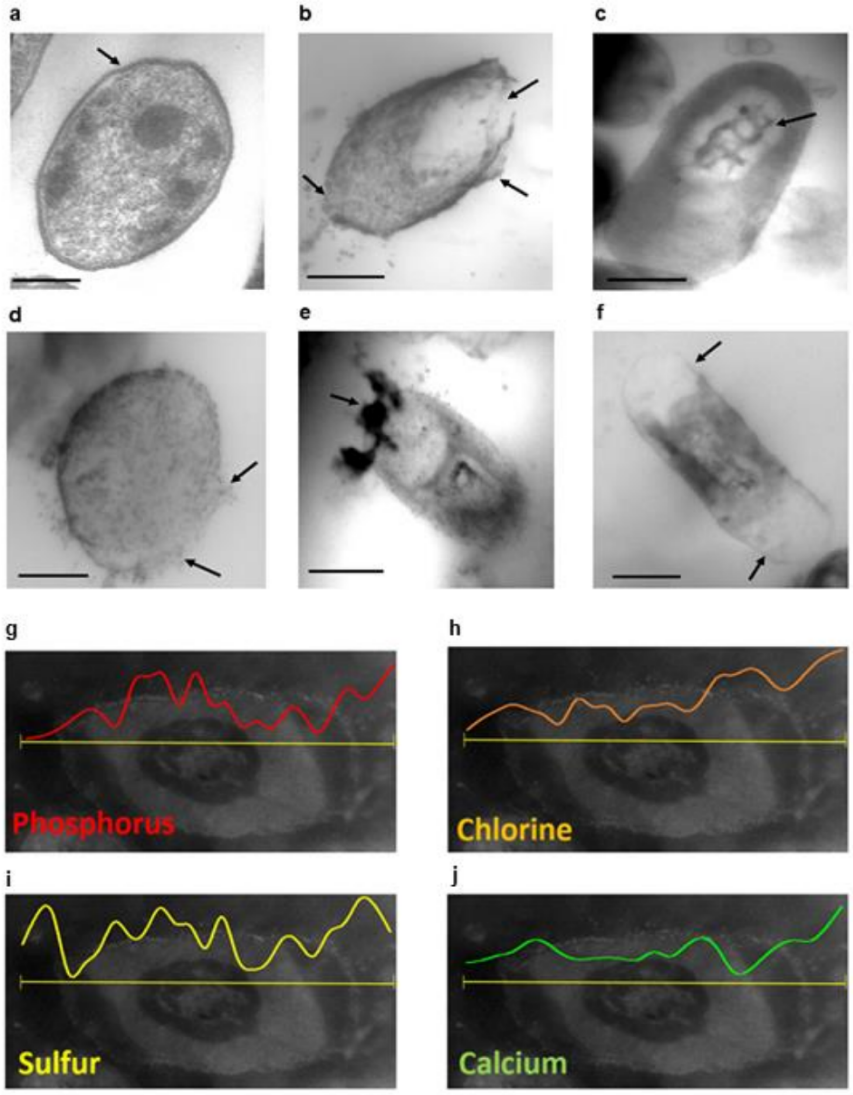

Figure 2. TEM micrographs and HAADF STEM image with EDS elemental line mapping of damaged E. Coli bacteria upon treating with phosphorene nanosheets for 3 hours. (a) Internal cell structure of untreated *E. coli* bacterium – control sample (Scale bar is 200 nm). (b) Ruptured cell membrane and the event of cytoplasmic leakage from the cell membrane (Scale bar is 500 nm). (c) Central large electron light area indicating accumulation of DNA molecules confirming disturbed cellular metabolism (Scale bar is 400 nm). (d) Reduced density of phospholipids upon interacting with BP nanosheets (Scale bar is 200 nm). (e) The event of BP nanosheets interacting with cell membrane and detached cytoplasm from the cell membrane (Scale bar is 500 nm). (f) Ruptured cell wall and separation of cytoplasm from the cell wall (Scale bar is 500 nm). (g) EDS elemental line map indicating the concentration of P across the damaged *E. coli* bacterium. (h) EDS elemental line map indicating the concentration of Cl across the damaged *E.*

Downloaded from https://www.cambridge.org/core. IP address: 198.54.106.171, on 04 Jan 2021 at 03:07:14, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/S1431927620021108

coli bacterium. (i) EDS elemental line map indicating the concentration of S across the damaged *E. coli* bacterium. (j) EDS elemental line map indicating the concentration of Ca across the damaged *E. coli* bacterium. (The horizontal yellow line across bacterium represents elemental line scan region.)

References

1 Gupta, A., Mumtaz, S., Li, C.-H., Hussain, I. & Rotello, V. M. Combatting antibiotic-resistant bacteria using nanomaterials. *Chemical Society Reviews* **48**, 415-427 (2019).

2 Germe, T. *et al*. A new class of antibacterials, the imidazopyrazinones, reveal structural transitions involved in DNA gyrase poisoning and mechanisms of resistance. *Nucleic acids research* **46**, 4114-4128 (2018).

3 Xu, J. W., Yao, K. & Xu, Z. K. Nanomaterials with a photothermal effect for antibacterial activities: an overview. *Nanoscale* **11**, 8680-8691, doi:10.1039/c9nr01833f (2019).

4 Phakatkar, A. *et al*. Transmission electron microscopy studies of chemically stable black phosphorus nanosheets against Escherichia Coli bacteria. *International journal of nanomedicine (submitted)*.

Downloaded from https://www.cambridge.org/core. IP address: 198.54.106.171, on 04 Jan 2021 at 03:07:14, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S1431927620021108