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Cryoprotective Ice-Philic Black Phosphorus Nanosheets for Augmented Thawing of Frozen Cells

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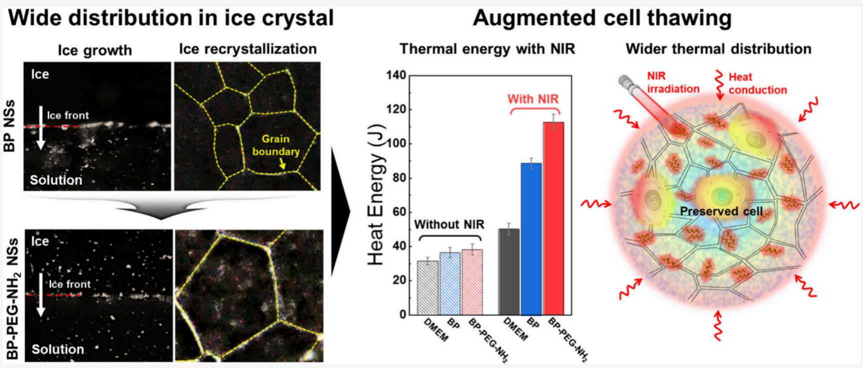

ABSTRACT: Controlling ice formation is critical in fields such as atmospheric science and biological cryopreservation. However, thermal heterogeneity during freezing and thawing in cryopreservation causes uneven ice crystallization and melting, leading to mechanical and thermal stress-induced damage. This study introduces biocompatible and biodegradable black phosphorus (BP)-polyethylene glycol-amine nanosheets (NS) to address this issue. BP NS primarily localize at ice grain boundaries, while amine groups of NH_2-PEG-NH_2 form hydrogen bonds with H_2O molecules, penetrating ice crystals. In situ cross-sectional observations confirmed that BP-PEG-NH_2 NS promotes uniform melting and facilitates ice cracks and boundaries. Heat transfer analysis using a bidirectional heating system revealed that the internal heat energy varies based on BP dispersion within the ice crystals. When applied to the cryopreservation of human tongue squamous cell carcinoma cells, BP-PEG-NH_2 NSs significantly improved post-thaw viability. It presents a promising strategy for designing thawing materials after cryopreservation of cells, tissues, and organs.

KEYWORDS: *black phosphorus nanosheets, polyethylene glycol-amine coating, ice-philic, photothermal thawing, cross-sectional in situ observation analysis*

Cryopreservation is employed across various fields, including food preservation, botany, medicine, and ecology, with applications in organ transplantation, gene banking, biodiversity conservation, and cell culture freezing.[1,2] However, its use is limited by ice recrystallization during freezing and thawing cycles, a process driven by Ostwald ripening, where small ice crystals enlarge to minimize total surface energy, causing physical damage to cells and challenging cell survival.[3−5] Moreover, intra- and extracellular ice formation creates osmotic gradients, increasing solute concentration and ultimately dehydrating cells.[6,7] Therefore, many developments have aimed to chemically inhibit ice crystal growth to mitigate cryo-injuries, including antifreeze (glycol) proteins (AF(G)Ps),[3] graphene oxide,[8] polymers,[9,10] and various nanomaterials.[11−13] Research has also been conducted to increase the heating effect during the thawing stage to regulate ice crystal formation.[14] The use of magnetic fields[15−17] and laser fields[18−22] during the thawing process rapidly and uniformly melts ice, with laser thawing offering the advantages of easy operability and high energy density.[18] In photothermal conversion under near-infrared radiation (NIR), laser exposure helps rapidly bypass the ice recrystallization zone. Photothermal agents—such as gold nanorods,[20] graphene oxide,[21] liquid metals,[22] and tungsten diselenide[18] — have also been utilized in laser thawing.

Black phosphorus (BP) is a novel 2D material containing a tunable layer-dependent band gap.[23] The band gap, which varies from 0.3 eV for bulk BP to 2.0 eV for monolayer BP, can be tuned to enable light absorption in a range from ultraviolet to near-infrared spectra. In particular, its high photothermal conversion efficiency at NIR wavelengths makes BP an attractive candidate for biomedical applications such as cancer

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