Supporting Information

Plug-and-Play Nanorization of Coarse Black Phosphorus for Targeted Chemo-Photo-Immunotherapy of Colorectal Cancer

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FIGURE S1

Schematic of ultrasonic bubble-bursting system for plug-and-play nanorization of coarse BP flakes (digital image, left side). Probe sonication under continuous supply of coarse BP dispersed solution and Ar gas simultaneously induced pulverization of coarse BP flakes near the sonication probe and fine bubble bursting at the free surface of solution. The floated droplets containing pulverized BPs were induced to flow by Ar gas, and the PB-laden flow was diluted with positively ionized Ar gas to prevent agglomeration of BPs and subsequently passed through a diffusion dryer to remove water from the droplets. The dried BP powders (digital image, right side) with a nano-size distribution (41.4 nm GMD and 1.37 GSD) were collected on a substrate for use in characterizations and bioassays.
Characterizations to confirm the production of uniform nanoscale BP and loadings of D, DcF, and DcF@sPL on the BP. (A) Changes in PDI of BP by increasing the cF content ($N = 6$). (B–D) Changes in UV-vis spectrum, dispersion color, and loading capacity of BP by increasing D content ($N = 6$). (E) D loading capacities of BP for the incorporation of D, DcF, and DCF@sPL ($N = 6$).
Loading of cF and DcF@sPL on BP. (A, B) FTIR and XRD spectra of BP-cF and BP-DcF@sPL, including individual D, cF, and BP, for the assessment of cF and DcF@sPL loadings.

EDS profile of the BP-DcF@sPL NSs. To examine the incorporation between BP and DcF@sPL, carbon (C), nitrogen (N), oxygen (O), and phosphorus (P) were selected to acquire their atomic fractions.
FIGURE S5

Loading capacity profile of D in BP-DcF@sPL with time followed its incubation in the mouse serum for 24 h ($N = 6$). Concentrations of D and s were 5.0 µg/mL and 50 nM, respectively.

FIGURE S6

Expression of folate receptor (FR) $\alpha$ in HCT116, HT29, and MC-38 cells.
FIGURE S7

Confocal images of endosomal escape of s in HCT116, HT29, and MC-38 cells upon their incubation with BP-DcF@sPL. Lysotracker red and DAPI were used for lysosome and nuclear staining, respectively.

FIGURE S8

Quantified results of apoptosis assays in MC-38 cells upon their incubation with BP-DcF@sPL in the absence and presence of F treatment or NIR irradiation (808 nm, 1.5 W/cm², 5 min), including PBS, D, and BP-Dc for comparison purposes (N = 3).
FIGURE S9

Expression of PL in HCT116, HT29, and MC-38 cells at 0, 12, 24, and 48 h incubation stages upon their post-incubation with IFN-γ (25 ng/mL) for 4 h.

FIGURE S10

Co-culture approach in a Transwell system and intracellular uptake of BP-Dc@s with and without F. (A) Schematic of the co-culture of MC-38 and CD8^+ T cells to simulate the tumor microenvironment. Competitive uptake of MC-38 (B), CD8^+ T (C), dendritic (D), and RAW264.7 (E) cells in the co-culture system for 6 h of incubation with BP-Dc@s and BP-DcF@s.
FIGURE S11

Quantified *in vivo* biodistribution of D in BP-DcF@sPL compared with free D in tumors and major organs 24 h post-injection (*N* = 6).

FIGURE S12

*In vivo* DCFDA-specific ROS generation (forward scatter, FSC) (A), IFN-γ-specific CD8+ T cells (B), and annexin V/PI staining-specific apoptotic tumor cell levels (C) upon treatments with BP-DcF and BP-DcF@sPL in the absence and presence of NIR irradiation, including individual PBS, NIR, free D, and BP-DcF+aPL (NIR) for comparison purposes (*N* = 6 for each group, **p < 0.01, ***p < 0.001).
Digital images of MC-38 tumor-bearing C57BL/6 mice treated with BP-DcF and BP-DcF@sPL in the absence and presence of NIR irradiation, including individual PBS, NIR, and D with BP-DcF+aPL (in the presence of NIR) for comparison purposes.
Histopathological observations in heart, liver, spleen, lungs, and kidneys collected from mice treated with BP-DcF and BP-DcF@sPL in the absence and presence of NIR irradiation, including individual PBS, NIR, and D with BP-DcF+aPL (in the presence of NIR) for comparison purposes.
Digital images of HCT116 tumor-bearing Balb/c nude mice treated with BP-DcF and BP-DcF@sPL in the absence and presence of NIR irradiation, including individual PBS, NIR, and D for comparison purposes.
Potent therapeutic efficacies of BP-DcF and BP-DcF@sPL in the absence and presence of NIR irradiation, including individual PBS, NIR, and D for comparison purposes against HCT-116 tumor via the monitoring of tumor volume, body weight, and survival rate (N = 6 for each group, **p < 0.01, ***p < 0.001). The sample injections were performed and continued on days 10, 13, 16, 19, 22, and 25 when the tumor size reached 100 mm$^3$. The NIR irradiation (808 nm, 1.5 W/cm$^2$, 5 min) was applied 8 h after each injection. (A, B) Average tumor volumes and body weights of the treated mice. (C) Percentages of surviving mice with different treatments.
In vivo analyses of CD4⁺ or CD8⁺ T cell blocking upon injection of anti-CD4⁺, -CD8⁺, and -CD4⁺–CD8⁺, including mouse IgG on day 0 and day 5 (10.0 mg/kg for each dose).

Potent therapeutic efficacies of BP-DcF@sPL in the presence of NIR irradiation (808 nm, 1.5 W/cm², 5 min) for CD4⁺, CD8⁺, or CD4⁺–CD8⁺ T cell depletion in MC-38 tumor-bearing immunocompetent mice, including PBS- and IgG-treated groups for comparison purposes ($N = 6$). Changes in average tumor volume (A) and body weight (B) ($N = 6$, **$p < 0.01$, ***$p < 0.001$). (C) Digital tumor images of the treated mice.
**TABLE S1**

Histomorphometrical analysis of tumor mass, taken from MC-38 colon adenocarcinoma cell-allograft mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tumor cell volumes (%/mm²)</th>
<th>Immunoreactive cell percentages (%/mm² of tumor mass)</th>
<th>Immunoreactive cell numbers (cells/mm² of tumor mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ki-67</td>
<td>CD31 (PECAM-1)</td>
</tr>
<tr>
<td>Control (G1)</td>
<td>79.66±11.35</td>
<td>82.51±10.51</td>
<td>61.63±4.37</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>79.69±10.68</td>
<td>79.56±10.86</td>
<td>61.28±6.55</td>
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<tr>
<td>G3</td>
<td>62.01±4.88&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>60.51±3.98&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>49.90±4.59&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td>G4</td>
<td>51.89±5.80&lt;sup&gt;ace&lt;/sup&gt;</td>
<td>49.69±4.90&lt;sup&gt;ace&lt;/sup&gt;</td>
<td>38.81±3.45&lt;sup&gt;ace&lt;/sup&gt;</td>
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<tr>
<td>G5</td>
<td>30.02±3.11&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>31.41±2.50&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>22.26±3.87&lt;sup&gt;abcd&lt;/sup&gt;</td>
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<td>G6</td>
<td>40.75±2.53&lt;sup&gt;aceg&lt;/sup&gt;</td>
<td>40.74±2.90&lt;sup&gt;aceg&lt;/sup&gt;</td>
<td>29.79±2.03&lt;sup&gt;aceg&lt;/sup&gt;</td>
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<td>G7</td>
<td>22.52±2.97&lt;sup&gt;abcde&lt;/sup&gt;</td>
<td>21.81±3.74&lt;sup&gt;abcde&lt;/sup&gt;</td>
<td>14.60±2.79&lt;sup&gt;abcde&lt;/sup&gt;</td>
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<td>G8</td>
<td>14.12±3.36&lt;sup&gt;acegij&lt;/sup&gt;</td>
<td>11.89±4.29&lt;sup&gt;acegij&lt;/sup&gt;</td>
<td>7.67±1.97&lt;sup&gt;acegij&lt;/sup&gt;</td>
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Values are expressed as mean ± SD of six tumor mass histological fields.

Treated groups: G1 = PBS; G2 = NIR; G3 = D; G4 = BP-DcF without NIR irradiation; G5 = BP-DcF with NIR irradiation; G6 = BP-DcF@sPL without NIR irradiation; G7 = BP-DcF@sPL with NIR irradiation; G8 = BP-DCF+aPL with NIR irradiation

<sup>a</sup> p < 0.01 and <sup>b</sup> p < 0.05 as compared with G1 by MW test; <sup>c</sup> p < 0.01 and <sup>d</sup> p < 0.05 as compared with G2 by MW test; <sup>e</sup> p < 0.01 as compared with G3 by MW test; <sup>f</sup> p < 0.01 as compared with G4 by MW test; <sup>g</sup> p < 0.01 and <sup>h</sup> p < 0.05 as compared with G5 by MW test; <sup>i</sup> p < 0.01 as compared with G6 by MW test; <sup>j</sup> p < 0.01 as compared with G7 by MW test