

## Supporting Information

### **Biodegradation of graphdiyne oxide (GDYO) in classically activated (M1) macrophages modulates cytokine production**

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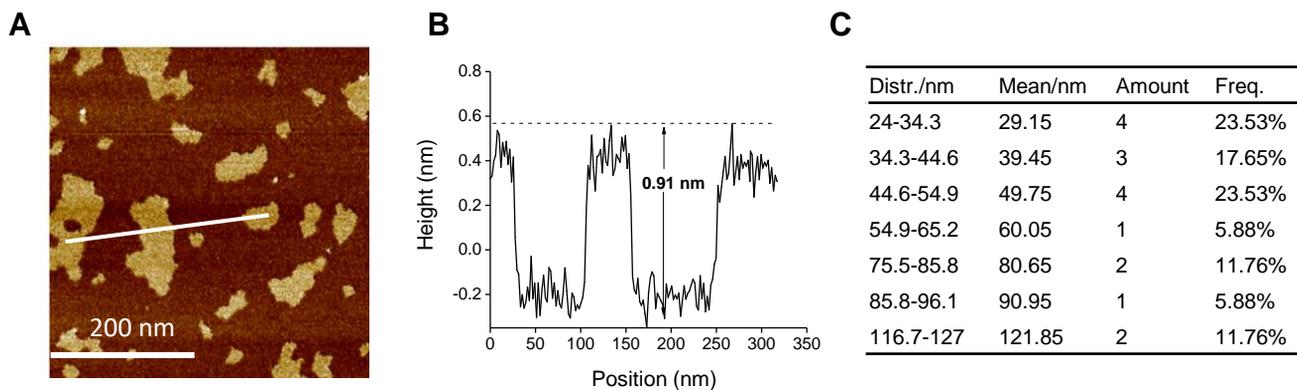
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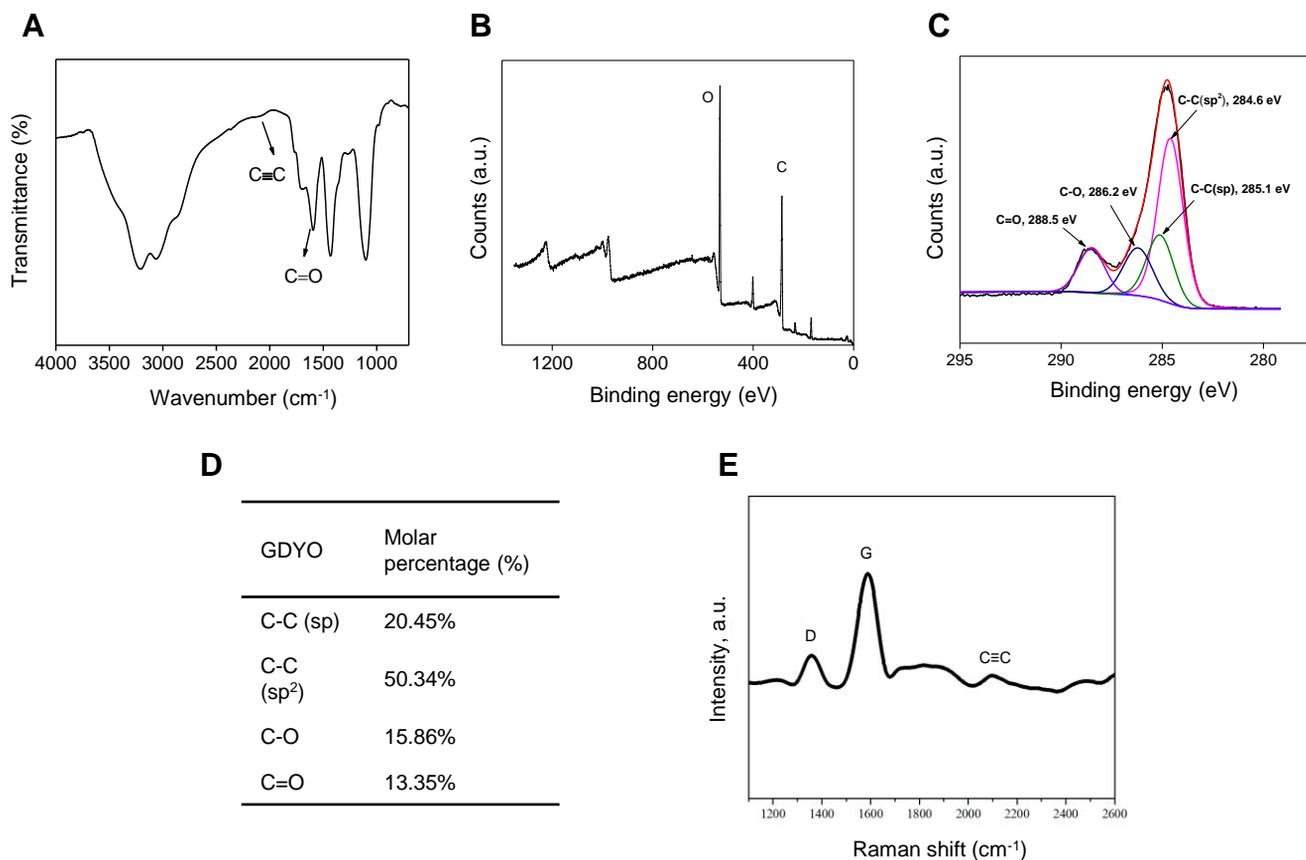
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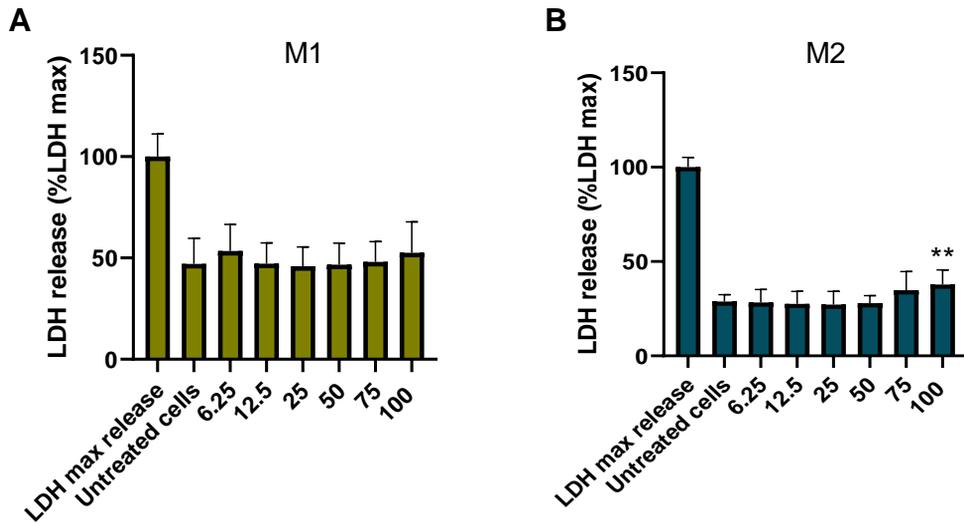
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**Figure S1** Physicochemical characterization of GDYO. (A) AFM analysis of GDYO. Scale bar: 200 nm. (B) Thickness analysis from the cross section in panel (A). (C) Lateral size distribution. The size was measured with Nano Measurer software v. 1.25 and the thickness of GDYO was processed with Bruker NanoScope Analysis software v. 1.80.



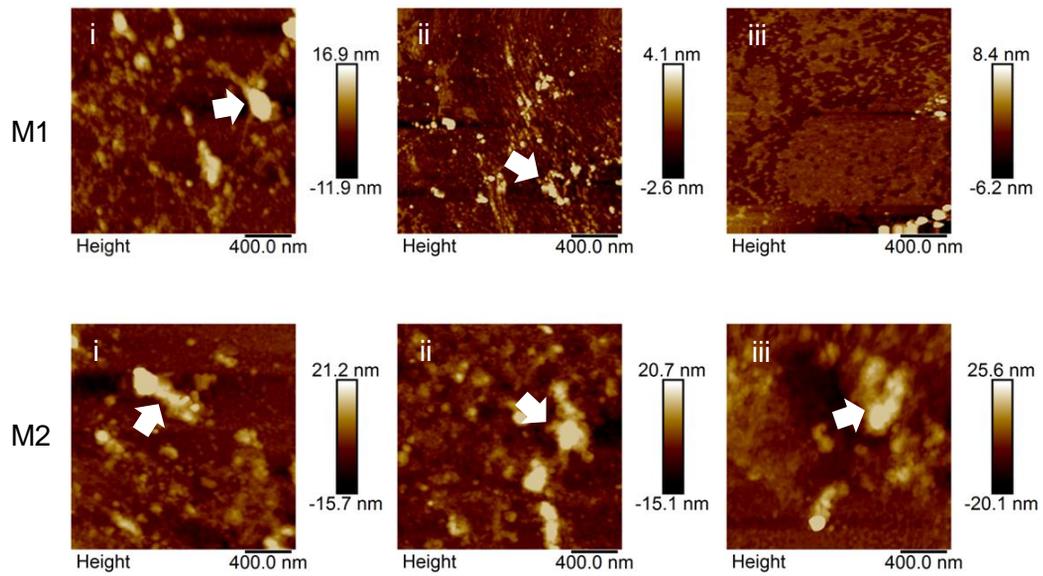
**Figure S2** Physicochemical characterization of GDYO. (A) The FTIR spectrum of GDYO shows a peak for the C=O stretching vibration at 1,596  $\text{cm}^{-1}$  and a peak for the C $\equiv$ C stretching vibration at 2090  $\text{cm}^{-1}$ . (B) The XPS survey scan of GDYO shows the peaks corresponding to C 1s and O 1s. (C) XPS scan of C 1s peaks. (D) Molar percentages of C-C  $sp^2$ , C-C  $sp$ , C-O, and C=O present in GDYO. (E) Raman spectrum of GDYO shows the characteristic D band (1360  $\text{cm}^{-1}$ ), G band (1582  $\text{cm}^{-1}$ ), and C $\equiv$ C band (2100  $\text{cm}^{-1}$ ).



**Figure S3** Cell viability of M1 (A) and M2 (B) macrophages following GDYO exposure as determined by LDH release. Results are presented as mean values  $\pm$  S.D. of results obtained from three independent human donors. \*\*  $p < 0.01$ . Student's t-test.

**Table S1** The intensity of D ( $I_D$ ) and G peak ( $I_G$ ) of the Raman signals of GDYO in M1 and M2 macrophages. The intensities at 48 h and 72 h were normalized to the values obtained at 24 h. Results are presented as the mean value  $\pm$  S.D. of 50 representative spectra.

Treatment	Intensity	48 h/24 h	72 h/24 h
M1	$I_D$	$21.2 \pm 2.4 \%$	$6.4 \pm 1.2 \%$
	$I_G$	$17.8 \pm 2.1 \%$	$5.8 \pm 1.1 \%$
M2	$I_D$	$96.8 \pm 2.1 \%$	$90.6 \pm 5.2 \%$
	$I_G$	$97.3 \pm 2.0 \%$	$93.4 \pm 3.6 \%$



**Figure S4** Biodegradation of GDYO in primary human M1 macrophages. Representative AFM images of M1 and M2 macrophages exposed to GDYO for 24 h (i), 48 h (ii) and 72 h (iii). For an overview of the entire scanning area, refer to the AFM images shown in Figure 4c.

**Table S2** The lateral size and thickness of GDYO in M1 and M2 macrophages as determined by AFM analysis. The results are presented as mean values  $\pm$  S.D. of 50 flakes.

Treatment		24 h	48 h	72 h
M1	Lateral size (nm)	44.0 $\pm$ 14.9	21.3 $\pm$ 9.4	10.7 $\pm$ 5.1
	Thickness (nm)	12.88 $\pm$ 5.93	7.58 $\pm$ 4.38	2.74 $\pm$ 1.66
M2	Lateral size (nm)	42.2 $\pm$ 10.6	46.6 $\pm$ 9.7	48.6 $\pm$ 9.6
	Thickness (nm)	10.64 $\pm$ 4.52	11.42 $\pm$ 3.65	9.84 $\pm$ 5.64

**Table S3** The lateral size and thickness of GDYO following incubation with the peroxyxynitrite donor SIN-1 for 5 days as determined by AFM analysis. The results are presented as mean values  $\pm$  S.D. of 50 flakes.

Treatment		5 days
GDYO	Lateral size (nm)	46.0 $\pm$ 21.5
	Thickness (nm)	6.14 $\pm$ 3.23
GDYO + SIN-1	Lateral size (nm)	25.7 $\pm$ 16.6
	Thickness (nm)	4.33 $\pm$ 2.18