



Supplementary Materials

Chitosan-functionalized Graphene Nanocomposite Films: Interfacial Interplay and Biological Activity

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Experimental Section: Preparation of Functional Graphene Fillers

Graphene oxide (**GO**) was obtained from graphite flakes using the Hummers method [19]. In a typical procedure, graphite flakes (5 g) and NaNO₃ (2.5 g) are mixed in 150 mL of H₂SO₄ (98%) in a 1000 ml volumetric flask kept under at ice bath (0 °C) with continuous stirring. The mixture was stirred for 4 h at this temperature, and potassium permanganate (15 g) was added to the suspension very slowly. The mixture is diluted with the very slow addition of 200 ml water and kept under stirring for 2 h. Then, the ice bath was removed, and the mixture was stirred at 35 °C for 2 h. The above mixture is kept in a reflux system at 98 °C for 10–15 min. After cooling, the mixture kept under stirring for 2 h at 25 °C. The solution is finally treated with 40 ml of H₂O₂ and then 200 mL of water. Then, it is kept without stirring for 3–4 h, where the particles settle at the bottom and the remaining water is poured through a filter. The resulting mixture is washed repeatedly by centrifugation with 10% HCl and then with deionized (DI) water several times until it forms a gel-like substance (pH-neutral). After centrifugation, the gel-like substance is vacuum dried at 60 °C for more than 6 h to GO powder.

Phosphorylated GO (**PGO**) was obtained through the phosphorylation of graphene oxide using POCl₃ as the phosphorus source. The details can be found in the supporting information. In a typical procedure using POCl₃ as the phosphorylating agent, 80 mg of lyophilized graphene oxide is dispersed in 250 mL of THF. The mixture is sonicated until the formation of a homogeneous suspension. Then, 42.9 mmol of K₂CO₃ is added to the suspension, and the mixture is kept under stirring for 3 h. Subsequently, 42.9 mmol of POCl₃ is dropped into the suspension, and the mixture is kept under stirring for 4 days. The phosphorylated product is dispersed in deionized water and kept

under stirring for 18 h to hydrolyze the residual Cl that did not react with GO. Finally, the product is collected through filtration and is thoroughly washed/rinsed with DI water and ethanol.

Sample	Contact Angle	TGA (wt%)ª			DSC		Mechanical Properties			
		300 °C	500 °C	700 °C	Т1 (°С)ь	T2 (°C)¢	Tensile Modulus (MPa)	Tensile Strenght (MPa)	Elongation at break	Toughness (MJ/m³)
CS-f	73.3 ± 2.18	40%	73.5%	100	105	247	1049	35	36%	9.78
CS-GO-f	70.6 ± 1.22	44%	82%	99%	101	296	1926	63	21%	9.90
CS-PGO-f	76.7 ± 1.14	37%	71%	100%	102	295	1677	53	24.5%	10.30
CS-rGO-f	101.1 ± 3.67	36%	75%	97%	97	296	1411	52	31%	12.96
CS- SiMe3GO-f	100.5 ± 2 27	34.5%	57%	62%	97	297	1245	42	33%	10.22

Table S1. Contact angle, thermal analysis, and mechanical properties of modified chitosan films.

^a decomposition wt % at a corresponding temperature. ^b endothermic peaks for water evaporation temperature. ^c exothermic peaks of chitosan decomposition.

Table S2. Propidium iodide (PI) permeability (%) of cell membranes of S. *aureus* after incubation with modified chitosan films.

Sample	PI Permeability (%)
Control	4.5 ± 1.94
CS-GO-f	81.0 ± 10.79
CS-PGO-f	98.48 ± 4.02
CS-SiMe ₃ GO-f	96.98 ± 4.87

Solid-state ¹³C SSNMR of graphene oxide reveals the presence of epoxide groups evidenced by the signal at 60 ppm and the presence of alcohols (signal at 68 ppm), and the signal at 131 ppm is attributed to aromatic carbons of graphitic domains.

Solid-state ¹³C SSNMR of phosphorylated graphene oxide (PGO) reveals the presence of epoxide groups evidenced by the signal around 63 ppm; the formation of C-O-P bonds might be evidenced by the signal at 69 ppm, and the signal at 127 ppm is attributed to aromatic carbons of graphitic domains.



Figure S1. ¹³C Solid-State NMR Spectrum of Graphene Oxide. (**a**) ¹³C Solid State NMR spectrum of graphene oxide; (**b**) ¹³C Solid-state NMR spectrum of **PGO.**

In GO's Raman spectrum, two bands corresponding to the D band and the G band appear at 1354 cm⁻¹ and 1601 cm⁻¹, respectively. The I_D/I_G found for GO is 0.87.

In the case of PGO, these two bands are shifted to lower values (1348 cm⁻¹ for the D band and 1586 cm⁻¹ for the G band). An increase of the I_D/I_G value is observed (0.91). This increase and the shift of the two bands are a consequence of the creation of more defective sites on the sheets or a decrease of the graphitic domains due to sonication.

In the case of rGO, these two bands are shifted to lower values (1349 cm⁻¹ for the D band and 1594 cm⁻¹ for the G band). An increase of the I_D/I_G value is observed (1.26).



Figure S2. Raman spectrum of GO and PGO. (a) Raman spectrum of GO; (b) Raman spectrum of PGO; (c) Raman spectrum of rGO.

The integral C1s spectrum of GO can be deconvoluted into three components at 284.5 eV, 286.5 eV, and 288.5 eV corresponding to carbon atoms of graphitic domains, carbon atoms of epoxides and tertiary alcohols, and carboxyl and ester groups carbon O-C=O respectively.

The O1s spectrum of GO evidences the presence of oxygenated functional groups.

The C1s spectrum of PGO is different from the one found for GO. The integral C1s spectrum of PGO can be deconvoluted into four components at 284.5 eV, at 286.3 eV, 287.2 and 288.7 eV, corresponding to carbon atoms of graphitic domains, carbon atoms of tertiary alcohols, carbon atoms of epoxides, and carboxyl and ester groups carbon O-C=O respectively.

The O1s spectrum of GO evidences the presence of oxygenated functional groups.

The P2p spectrum is deconvoluted to one component mainly due to the presence of orthophosphate groups, since the binding energy observed indicates that phosphorus is linked to oxygen atoms.

The integral C1s spectrum of PGO can be deconvoluted into five components at 284.7 eV, 286.2 eV, 286.9, 288.3 eV, and 289.2 eV corresponding to carbon atoms of graphitic domains, carbon atoms of tertiary alcohols, and the carbon atoms of epoxides, carboxyl, and ester groups carbons O-C=O and C-Si, respectively. The 289.2 eV might be due to the subsistence of residual Si–CH₃ groups upon the functionalization of GO with HMDS.

The Si2p spectrum of SiMe₃GO can be deconvoluted to one component, and this is mainly due to the presence of Si–O bonds.

The N1s peak at 401.3 indicates the formation of C-NH and the peak around 399.8 eV suggests the formation of C-N⁺ bonds.²





Figure S3: XPS Analysis of GO, PGO, and SiMe₃GO. (**a**) XPS analysis of **GO**; (**b**) XPS analysis of **PGO**; (**c**) XPS analysis of SiMe₃GO.



Figure S4. Water dispersion of functionalized graphene fillers.



Figure S5. TGA of GO, PGO, rGO, SiMe₃GO, and CS-SiMe₃GO-*f* (N₂).









Figure S6. DRIFT analysis of graphene-reinforced chitosan films. (a) DRIFT analysis of **CS-GO***-f;* (b) DRIFT analysis of **CS-PGO***-f;* (c) DRIFT analysis of **CS-rGO***-f;* (d) DRIFT analysis of **CS-SiMe**₃**GO***-f.*



Figure S7. Stress-strain curves of chitosan and chitosan-modified graphene nanocomposites.



Figure S8. Permeability of *S. aureus* cell membrane after the treatment with chitosan films: (**a**) control, (**b**) CS-GO-*f*, (**c**) CS-PGO-*f*, (**d**) CS-SiMe₃GO-*f*.



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