Surface functionalization of graphene oxide with alkyl chains allows high rates of capture and release of viral particles in aqueous solutions

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Waterborne viral diseases can affect any population in the globe, but they are especially recurrent in poor rural regions of developing countries. Many of those affected by these diseases do not seek medical attention because of the existing limitations on the clinical detection of viral infections [1]. Moreover, further complications can arise from the fact that detecting the sources of infection when viruses are highly diluted, impedes their monitoring and successful identification. For this, in this study we intended to develop a new carbon-based material to increase virus capture and release performances beyond conventional methods, hoping it may be used as an alternative method to capture and detect pathogenic viruses in water.

Graphene oxide (GO) was prepared using a modified version of Hummer's method [2, 3] and exfoliated in water until obtaining a 1% w/v solution of GO. After that, we assessed the capacity of the produced pristine GO to capture viruses in aqueous solutions. For this, we prepared a solution of ~10^5 PFU/mL phage Q_β (NBRC, Tokyo, Japan) in 100 mM Phosphate Buffer (PB) solution and proceeded as follows. Briefly, 9 mL Q_β solutions were supplemented with 1 mL 1%, 0.5%, 0.1%, 0.05% or 0.01% w/v GO and were incubated at room temperature for 30 minutes. After that, the mixtures were centrifuged at 10,000 rpm for 30 min and 1 mL of the supernatant was assessed in duplicate for the presence of phages by plaque assay [4]. Next, to evaluate the capacity of GO to release the captured viruses, we resuspended the GO pellet in 10% beef extract solution and incubated the mixture at room temperature for 30 min. After that, the materials were centrifuged at 10,000 rpm for 30 min and 1 mL of the supernatant was cultured in duplicate by plaque assay to detect any phages present.

Results indicated that a minimal concentration of 0.01% w/v of pristine GO was able to capture phage Q_β to the order of 3 log10 (99.9%), and even lower concentrations, to the order of 0.005% and 0.001% w/v were able to capture more than 2 log10 (99.2%) of the suspended viruses (**Table 1**). When evaluating the release capacity of the material, our method could not release the virus attached to the surface of GO effectively (4.3%).

Concentration of GO (w/v)	Phage concentration [(PFU/mL) ± SD]	Adsorption (% ± SEM)
Initial phage	3.99×10^5 ± 2.12	-
0.1%	2.09×10^2 ± 1.13	99.96 ± 0.004[a]
0.05%	3.99×10^2 ± 4.63	99.92 ± 0.02[a]
0.01%	6.20×10^2 ± 4.12	**99.88 ± 0.01[a]**
0.005%	2.04×10^3 ± 1.06	99.27 ± 0.20[b]
0.001%	2.24×10^3 ± 1.30	99.28 ± 0.14[b]

Table 1. Capacity of different concentrations of pristine GO to reduce the amount of Q_β phage in a water-based solution. Data was obtained from at least 5 independent replicated experiments. [a,b]Different superscript letters indicate significant differences amongst values in the same column ($P < 0.01$).

Since it became clear that pristine GO was unable to release viruses once captured on its surface, we decided to change its properties by functionalization with other molecules. Since it is already known that viruses tend to attach to slightly hydrophobic surfaces [5], and that alkyl amines can be functionalized to the surface of GO [6], we added the following alkyl chains to GO: 1-butylamine (C_4NH_2) and 1-octylamine (C_8NH_2), but since C_8NH_2-GO displayed a subtle hydrophobicity, we further researched the influence of hydrophilic terminal groups such as NH_2 and OH by using 1,8-diaminooctane ($C_8(NH_2)_2$) and 8-amino-1-octanol (C_8NH_2OH) to improve the solubility of the materials. To prepare the functionalized GOs, a mixture of 0.5:1 w/w alkyl chains:GO was mixed in ethyl alcohol at room temperature for 1 h. After several washes to remove any non-attached compounds, the materials were either resuspended in water at a final concentration of 0.1% w/v or freeze-dried for further structural analysis.

Structural characterization was performed to assess the successful grafting of alkyl chains onto the GO sheets. FT-IR analysis of functionalized GO samples revealed the presence of alkyl chains around 2900 cm^{-1}, accompanied by a decreased C=O peak at 1732 cm^{-1}, indicative of a shift to approximately 1600 cm^{-1} due to amine neutralization (**Figure 1-A**). The degree of functionalization was further confirmed by TGA (**Figure 1-B**). GO exhibited a weight loss of 33.29% at 300 °C, whereas the functionalized materials exhibited a weight loss of 29.71%, 27.31%, 24.89% and 28.27% for C_4NH_2-GO, C_8NH_2-GO, $C_8(NH_2)_2$-GO and C_8NH_2OH-GO, respectively. The weight loss of pristine GO at a lower temperature is due to the degradation of the oxygen functional groups on its surface as opposed to the more resistant amine molecules present in all the other materials. XRD analysis (**Figure 1-C**) showed that the GO sheet distance increased with the addition of the longer chains from 0.86 nm in GO and C_4NH_2-GO to ~0.95 nm in the materials containing longer alkyl chains.

To further understand if the functionalized GOs could be used for the virus capture and release experiments, the materials were subjected to a dispersibility analysis of the supernatants after centrifugation by UV-Vis measurements. According to the results, we decided to exclude $C_8(NH_2)_2$-GO since complete precipitation could not be achieved even after 60 minutes.

Finally, we assessed the capacity of our materials to capture Q_β phage following the same protocol as with pristine GO. Since 0.01% GO was the minimal amount to obtain a 99.9% viral capture, we used this amount to compare the efficiency of our functionalized alkyl-GO materials. While no differences were observed amongst the adsorption rates for all the samples (> 98% reduction), significant differences were observed after phage release by 10% beef extract treatment, depending on the functionalized molecules (**Figure 2**). The lowest release rates (3.8%) were those of pristine GO, whose surface strongly interacted with the phages. Functionalization of GO with short alkyl chains (C_4NH_2-GO) slightly increased the release rates to 15.4%, and longer chains (C_8NH_2-GO), enhanced viral release performance to 36.2%. Remarkably, amongst all the materials analyzed, the presence of a terminal hydrophilic group at the end of a long alkyl chain (C_8NH_2OH-GO) showed the best release performance (55.8%).

This study allowed us to evaluate the capacity of GO to capture a model coliphage (Q_β phage) from aqueous solutions in controlled laboratory conditions. Furthermore, we successfully developed several GOs functionalized with alkyl chains with a simple protocol and evaluated their effectivity when capturing and releasing Q_β phages. All the alkyl-functionalized GOs were able to capture the virus to more than 2 log10, but the material with the best performance was C_8NH_2OH-GO, because of its ability to release the adsorbed phages to > 50%. Further evaluation on the practical applications of this material is required to determine its successful application for viral capture and release under field conditions.

References

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Figures

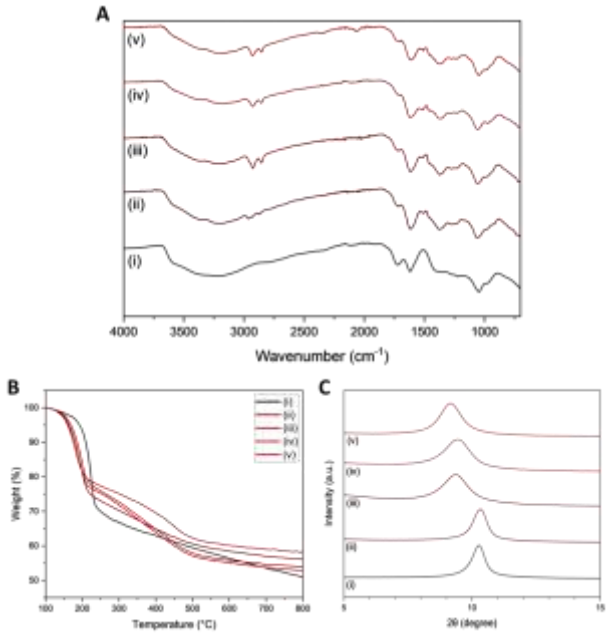

Figure 1. A. FT-IR spectra of (i) pristine GO, (ii) C_4NH_2-GO, (iii) C_8NH_2-GO, (iv) $C_8(NH_2)_2$-GO and (v) C_8NH_2OH-GO. The bands at ~ 2900 cm^{-1} indicate the presence of alkyl chains. **B.** TGA analysis of (i) pristine GO, (ii) C_4NH_2-GO, (iii) C_8NH_2-GO, (iv) $C_8(NH_2)_2$-GO and (v) C_8NH_2OH-GO. The samples containing alkyl chains show more stability than pristine GO at ~300 °C, confirmed by the lower weight loss. **C.** XRD analysis of (i) pristine GO, (ii) C_4NH_2-GO, (iii) C_8NH_2-GO, (iv) $C_8(NH_2)_2$-GO and (v) C_8NH_2OH-GO, the sheet interlayer space increases with the length of the alkyl chains attached to the surface of GO.

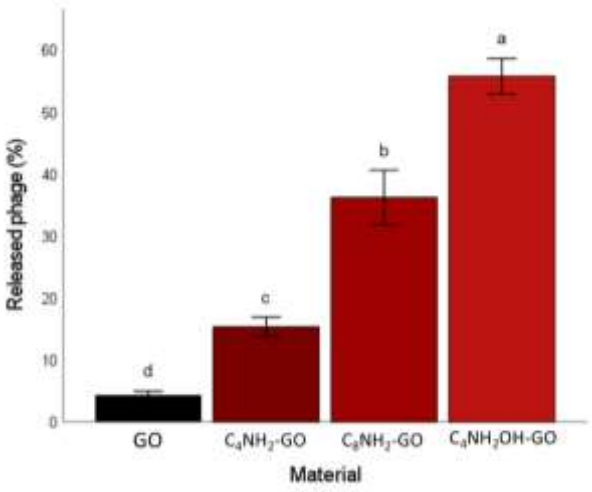

Figure 2. Average rates of Q_β phage release by functionalized GO after resuspension in 10% beef extract solution. Data was obtained from at least 4 independent replicates. $^{a\text{-}d}$ Different letters indicate significant differences amongst the values ($P < 0.01$).