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Supporting Information

Modified Graphene Oxide (GO) Particles in Peptide Hydrogels: A Hybrid System Enabling Scheduled Delivery of Synergistic Combinations of Chemotherapeutics

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S1. Size determination of GO nanoparticles

The size of GO nanoparticles was characterized by scanning electron microscopy (SEM). The SEM image of GO nanoparticles presented in Fig. S1 indicated an average size of 800 nm.

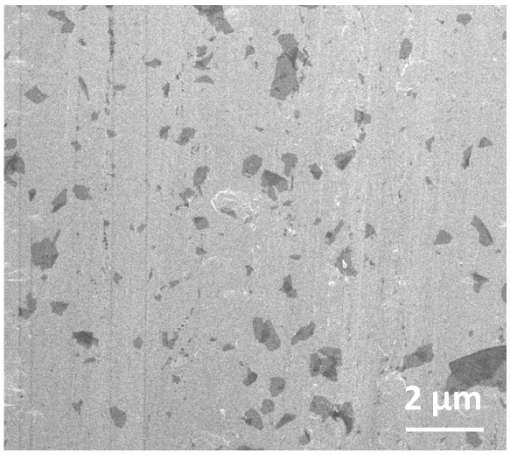

Fig. S1 SEM of GO particles with an average size of 800 nm.

S2. Evaluation of doxorubicin (DOX) loading to and release from tGO particles

The initial screening of tGO particles was performed in PBS at pH 7.4 over 500 hr. Fig. S2 presents the values of DOX % release through the first 100 hr. Similar to prolonged release, the TREN and EDA moieties provided the most favorable release characteristics relative to native GO, releasing 2.63 ± 0.3% and 1.74 ± 0.2% of the loaded DOX after 72 hrs, respectively. Comparatively, native GO released only 1.38 ± 0.2% after 72 hrs. As mentioned, we hypothesize that the electrostatic repulsion between DOX molecules and EDA/TREN moieties, both being positively charged, promotes the release of DOX relative to native GO; the other modifications considered in this work, none of which possess positively charged moieties, provide no electrostatic repulsion, and may rather promote DOX retention, through electrostatic attraction (succinyl) and hydrophobic interaction (benzyl and acetyl).

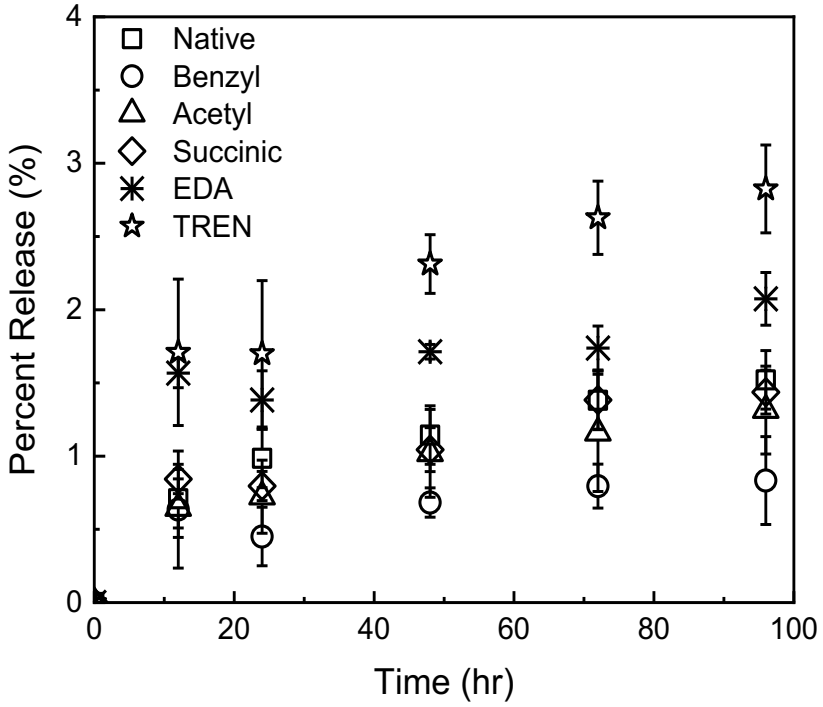

Fig. S2 DOX release from modified GO particles within 100 hrs. Error bars represent mean ± 99% CI (n ≥ 3).

S3. Characterization of tGO particles

To further study the impact of GO surface modification on DOX loading and release, we sought to modify the surface of GO with TREN (tGO) at different degrees of modification. Fig. S3 presents the results describing the relationship between TREN equivalents used in the reaction and the ζ potential measured on the resulting tGO particles, as well as a quantitative relationship to surface density of amine groups. We later utilized this information to guide the *in silico* design of tGO surfaces. The tGO particles featured values of ζ potential ranging from -36.5 mV (native GO) to +20 mV. We resolved that this range of modification levels was sufficient to interrogate the impact of surface charge (TREN density) on DOX loading and release on tGO systems. Furthermore, from Fig. S2, we were able to estimate the molar equivalents of TREN needed to ensure a obtain a tGO sample with desired ζ potential. If is finally observed

that, as molar ratio TREN increases, the ζ potential asymptotically approaches 20-30 mV, prompting the conclusion that the range of modifications utilized in this work is sufficient to comprehensive sample the behavior of tGO.

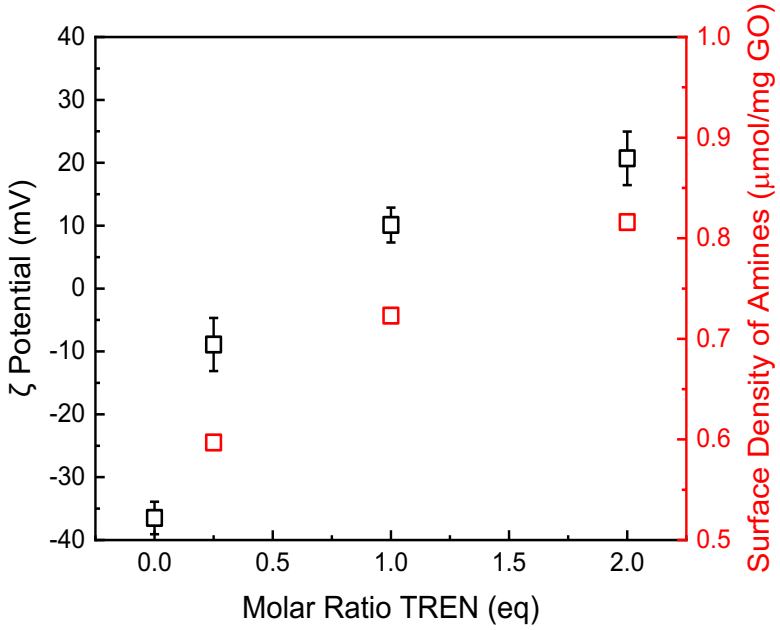

Fig. S3 The effect of molar ratio of TREN used for tGO reactions on ζ potential (black) and the corresponding concentration of amine groups (red). Error bars represent mean ± S.D (n ≥ 3).

S4. Experimental and in silico evaluation of DOX adsorption on tGO

To study the effect of pH and ionic strength on DOX loading onto tGO particles, we utilized four separate loading conditions: low salt concentration at pH 4, 7, and 9, and PBS at pH 7.4. tGO particles with different ζ potentials were incubated with aqueous DOX solutions for 48 hrs in the various conditions. The values of these loading experiments are presented in Table S1.

Table S1. Loading of DOX on GO and tGO particles (mg DOX mg^{-1} GO). Error represents 99% CI (n ≥ 3).

Zeta Potential (mV)	Acidic	Neutral	Basic	PBS
-36.5	0.595 ± 0.008	0.581 ± 0.004	0.576 ± 0.002	1.048 ± 0.009
-18.8	0.248 ± 0.050	0.257 ± 0.030	0.262 ± 0.040	0.613 ± 0.015

-8.9	0.231 ± 0.004	0.266 ± 0.028	0.280 ± 0.017	0.570 ± 0.001
0	0.169 ± 0.009	0.257 ± 0.015	0.208 ± 0.013	0.524 ± 0.024
6.8	0.150 ± 0.002	0.221 ± 0.002	0.225 ± 0.011	0.521 ± 0.029
19.3	0.202 ± 0.051	0.163 ± 0.012	0.176 ± 0.002	0.367 ± 0.104

The *in silico* modeling provided further molecular-level insight in the mechanism governing DOX adsorption; snapshots of the molecular configurations at DOX-tGO equilibrium are presented in Fig. S4. Three main phenomena are observed: *(i)* DOX molecules always adsorb onto the native GO surface, in between TREN moieties; *(ii)* as they adsorb onto the GO surface, DOX molecules tend to form aggregates; and *(iii)* at higher degree of modification, the higher density of TREN moieties prevents DOX adsorption by a combination of electrostatic repulsion and steric hindrance, lowering the density of adsorbed DOX molecules.

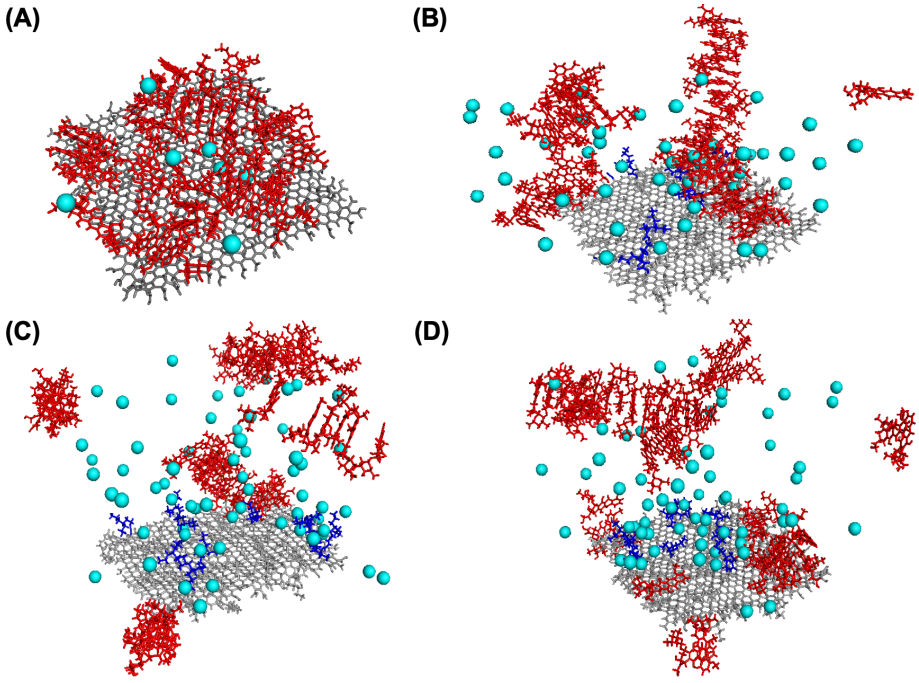

Fig. S4 Snapshots from DOX (red frame) loading on TREN- (blue frame) modified GO (grey frame) and the dispersion of Cl^- ions (cyan balls) on **(A)** native GO (ζ = -36.5 mV), **(B)** tGO (ζ = -8.9 mV, **(C)** tGO (ζ = 10.1 mV), and **(D)** tGO (ζ = 20.7 mV).

S5. Evaluation of DOX Release from tGO

In addition to release experiments performed at cancer-mimetic environment (pH 5), we resolved to perform release experiments at physiological condition (pH 7.4). In these release studies, the prolonged time scale (> 600 hr) is typical of a maintenance chemotherapy regimen. The resulting release profiles, presented in Fig. S5, are similar to those obtained at pH 5 (Fig. 4), in that DOX release is the highest at values of ζ potential of -18.8 mV and -8.9 mV, and is lower in native GO (ζ potential = -36.5 mV) and tGO particles with higher values of ζ potential. The overall magnitude of release, however, is statistically different and lower at pH 7.4 than pH 5, likely due to lower DOX-DOX and DOX-TREN electrostatic repulsion. The values of release at 72 hrs and 650 hrs are reported for all loading conditions for pH 5 (Table S2) and pH 7.4 (Table S3).

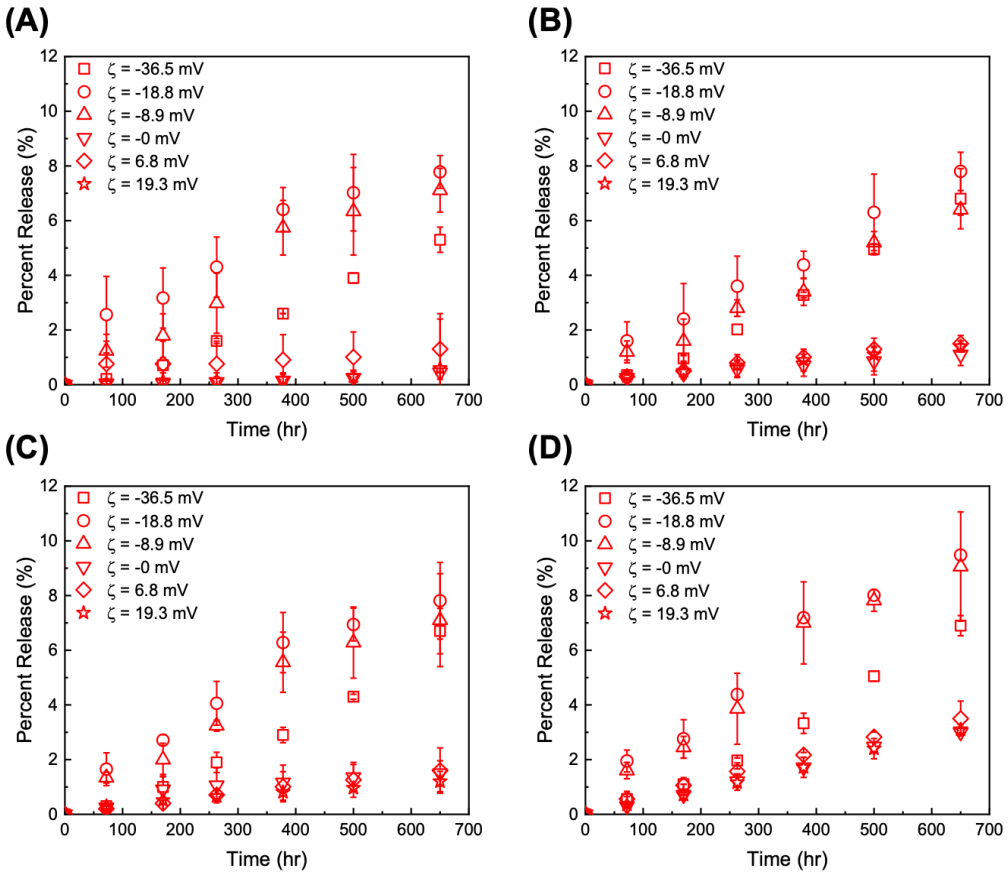

Fig. S5 Release profiles from DOX loaded tGO at pH 7.4 for tGO loaded in **(A)** acidic (pH 4), **(B)** neutral (pH 7), **(C)** basic (pH 9), and **(D)** PBS (10 mM pH 7.4). Error bars represent mean ± 99% CI (n ≥ 3).

Table S2 Percent release of DOX from GO and tGO in 10 mM PBS pH 5 after 72 hrs and 650 hrs, for particles loaded in acidic, neutral, basic, and high ionic strength (PBS) conditions. Error represents 99% CI (n ≥ 3).

ζ Potential (mV)	Acidic 72 hrs	Acidic 650 hrs	Neutral 72 hrs	Neutral 650 hrs	Basic 72 hrs	Basic 650 hrs	PBS 72 hrs	PBS 650 hrs
-36.5	6.21 ± 0.6	15.0 ± 1.7	6.47 ± 0.6	15.0 ± 0.5	6.01 ± 0.6	14.0 ± 0.1	7.42 ± 0.1	19.1 ± 0.9
-18.8	12.4 ± 0.4	19.2 ± 4.2	13.7 ± 2.1	22.7 ± 3.2	11.4 ± 0.8	19.8 ± 4.0	18.9 ± 2.0	31.4 ± 3.2
-8.9	9.14 ± 0.3	15.7 ± 3.8	8.99 ± 1.1	16.7 ± 4.0	10.6 ± 1.5	19.8 ± 3.9	18.0 ± 1.4	31.8 ± 2.8
0	2.68 ± 0.6	5.10 ± 1.0	2.11 ± 0.4	4.39 ± 0.3	3.08 ± 0.3	5.96 ± 0.2	15.3 ± 1.7	30.7 ± 4.1
6.8	3.03 ± 0.2	5.46 ± 0.01	3.11 ± 0.5	6.80 ± 1.3	3.94 ± 0.9	8.08 ± 0.9	16.1 ± 0.6	32.0 ± 1.3
19.3	1.48 ± 0.3	3.2 ± 0.4	0.68 ± 0.2	2.67 ± 0.1	2.01 ± 0.3	4.65 ± 0.1	14.3 ± 1.8	26.3 ± 4.7

Table S3 Percent release of DOX from GO and tGO in 10 mM PBS pH 7.4 after 72 hrs and 650 hrs, for particles loaded in acidic, neutral, basic, and high salt (PBS) conditions. Error represents 99% CI (n ≥ 3).

ζ Potential (mV)	Acidic 72 hrs	Acidic 650 hrs	Neutral 72 hrs	Neutral 650 hrs	Basic 72 hrs	Basic 650 hrs	PBS 72 hrs	PBS 650 hrs
-36.5	0.35 ± 0.1	6.80 ± 1.1	0.21 ± 0.1	5.30 ± 0.5	0.30 ± 0.1	6.70 ± 0.8	0.56 ± 0.1	6.90 ± 0.4
-18.8	1.60 ± 0.7	7.80 ± 0.7	2.56 ± 1.4	7.78 ± 0.6	1.66 ± 0.6	7.81 ± 1.4	1.95 ± 0.4	9.48 ± 0.5
-8.9	1.20 ± 0.4	6.40 ± 0.2	1.24 ± 0.6	7.11 ± 0.8	1.34 ± 0.2	7.10 ± 1.7	1.60 ± 0.3	9.06 ± 2.0
0	0.25 ± 0.1	1.10 ± 0.4	0.10 ± 0.1	0.50 ± 0.1	0.25 ± 0.1	1.50 ± 0.5	0.30 ± 0.1	3.00 ± 0.6
6.8	0.30 ± 0.1	1.50 ± 0.3	0.76 ± 0.8	1.30 ± 1.1	0.20 ± 0.1	1.60 ± 0.8	0.56 ± 0.3	3.50 ± 0.6
19.3	0.30 ± 0.1	1.40 ± 0.2	0.10 ± 0.1	0.60 ± 2.0	0.30 ± 0.1	1.20 ± 0.4	0.35 ± 0.1	3.10 ± 0.2

Analogous plots to those generated in Fig. 6 were generated for release at pH 7.4 and are presented in Fig. S6. While an extensive discussion of these phenomena is presented previously it is worth noting that both Fig. 6 and Fig. S6 present vertical shifts in percent release when DOX loading on tGO particles is performed at high ionic strength relative to low ionic strength, and a downward concave trend of percent release with ζ potential.

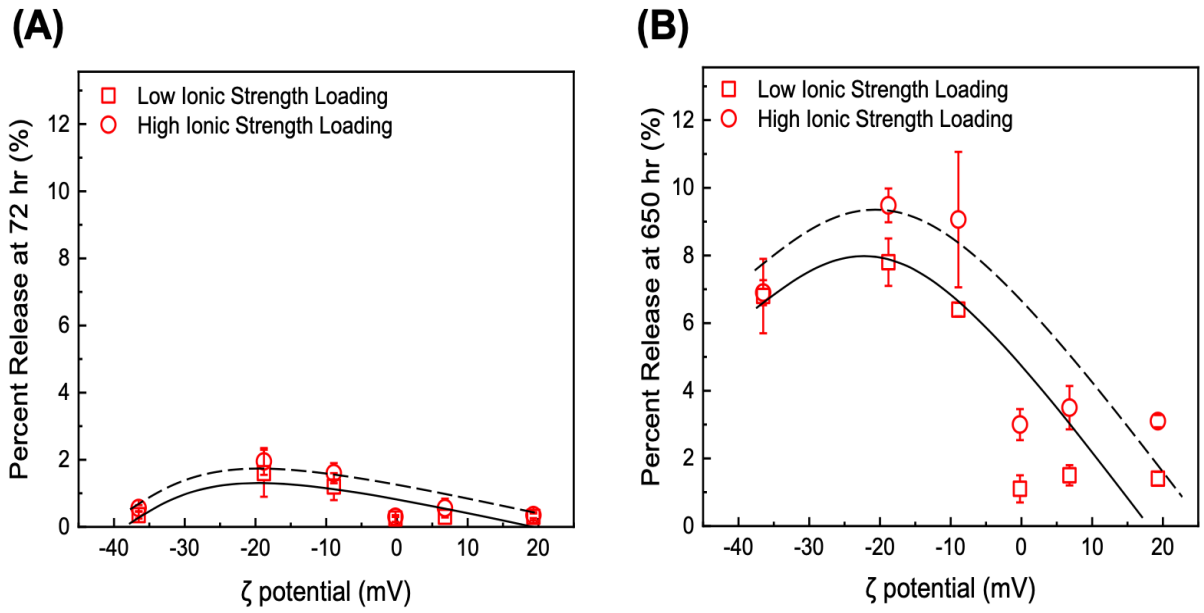

Fig. S6 Values of DOX percent release at pH 7.4 after (A) 72 hr and (B) 650 hr from GO and tGO particles loaded at neutral pH and low ionic strength (squares) or high ionic strength (circles). Hashed are added to guide the visualization of the results. Error bars represent mean ± 99% CI (n ≥ 3).

S6. tGO distribution in composite tGO-Max8 hydrogel

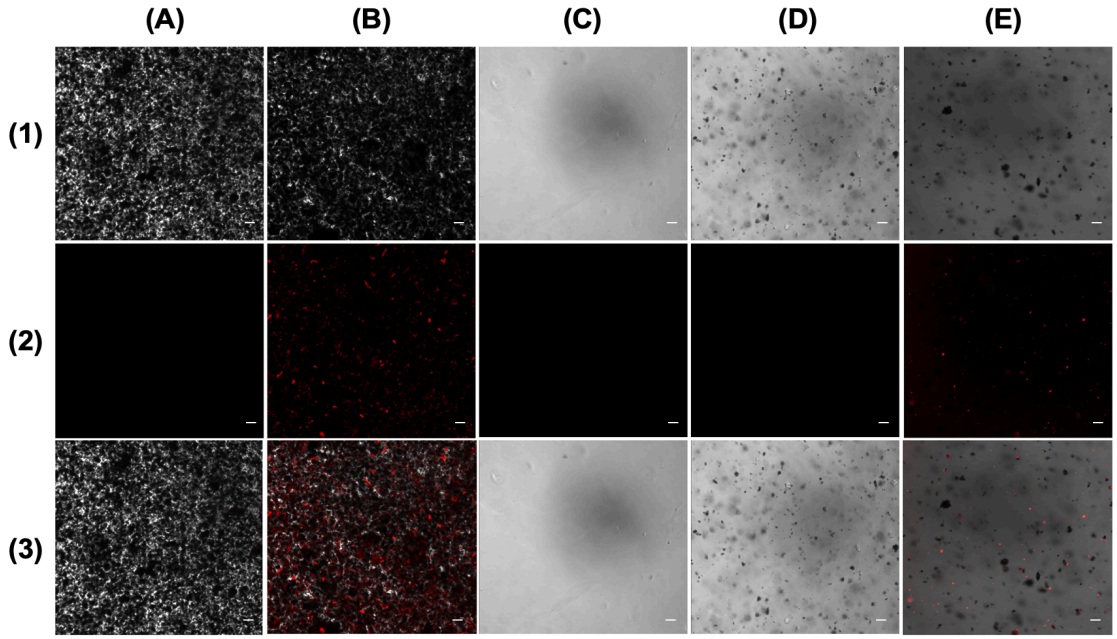

Fig. S7 Microscopy images of **(A)** tGO particles, **(B)** DOX/tGO particles, **(C)** Max8 hydrogel, **(D)** tGO particles dispersed in Max8 hydrogel, **(E)** DOX/tGO particles dispersed in Max8 hydrogel; **(1)** brightfield, **(2)** fluorescence (absorption: 590 nm – emission: 617 nm), and **(3)** combined brightfield-fluorescence overlay. Scale bar represents 20 µm.