Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supplementary information

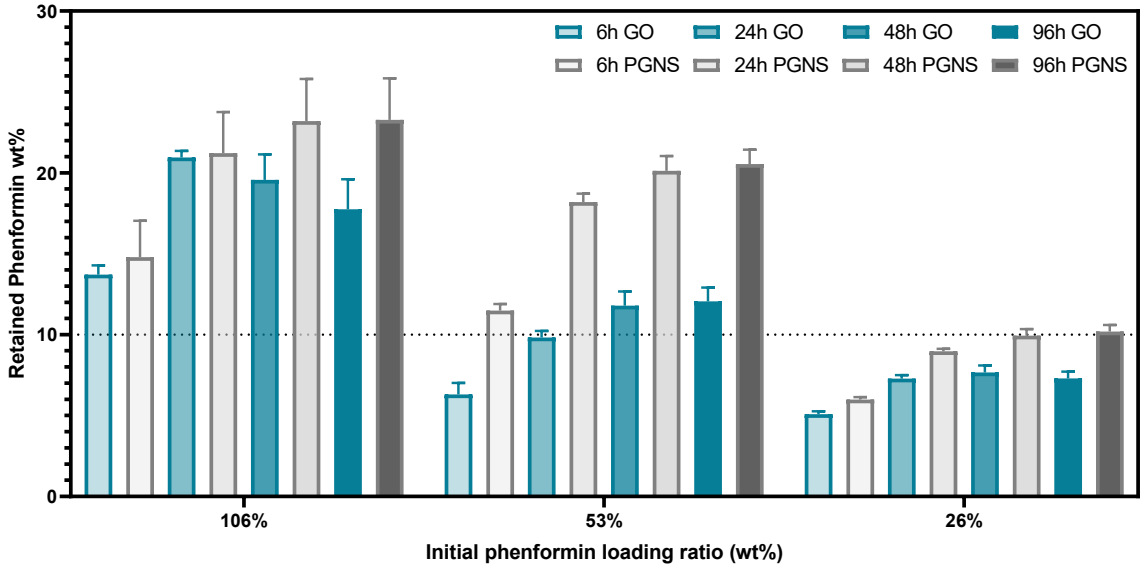

Figure S1 Retention efficiency of phenformin by GO and PGNS over time. Calculated amounts of retained phenformin are represented as phenformin/graphene $_{wt/wt}$% at measurement endpoint. Three initial starting concentrations of graphene of 12.5, 25 and 50 µg were tested responding to a loading wt% of 106, 53, and 26 wt%. The dotted line signifies the retention threshold of 10 wt% as measured up until 96hours post drug loading.

Table S1 Size and zetapotential stability in culture media of GO and PGNS using DLS.

Sample	**PGNS**		**GO**	
Day	0	5	0	5
Z-average (nm)	386.30	389.30	426.00	not possible
PdI (nm)	0.21	0.22	0.83	1.00
PdI Width (nm)	175.10	180.40	386.80	249.70
Peak 1 Mean (nm) (% of total)	438.1 ± 23.4 (100%)	430.4 ±6.116 (98.5%)	466.3 ± 22.49 (53%)	543.9 ± 20.22 (34%)
Peak 2 Mean (nm) (% of total)	0.00	5082 ± 108.9 (1.5%)	3351 ± 102.5 (17%)	2598 ± 58.69 (41%)
ZP	-19.9 ± 1.55	-20.2 ± 1.29	-6.88 ± 0.609	-9.22 ± 0.53

Measurements of z-average, Polydispersity index (PDI), Size peaks intensity distribution and Zeta potential (ZP) for GO and PGNS dispersed in culture media at day 0 compared with day 5.

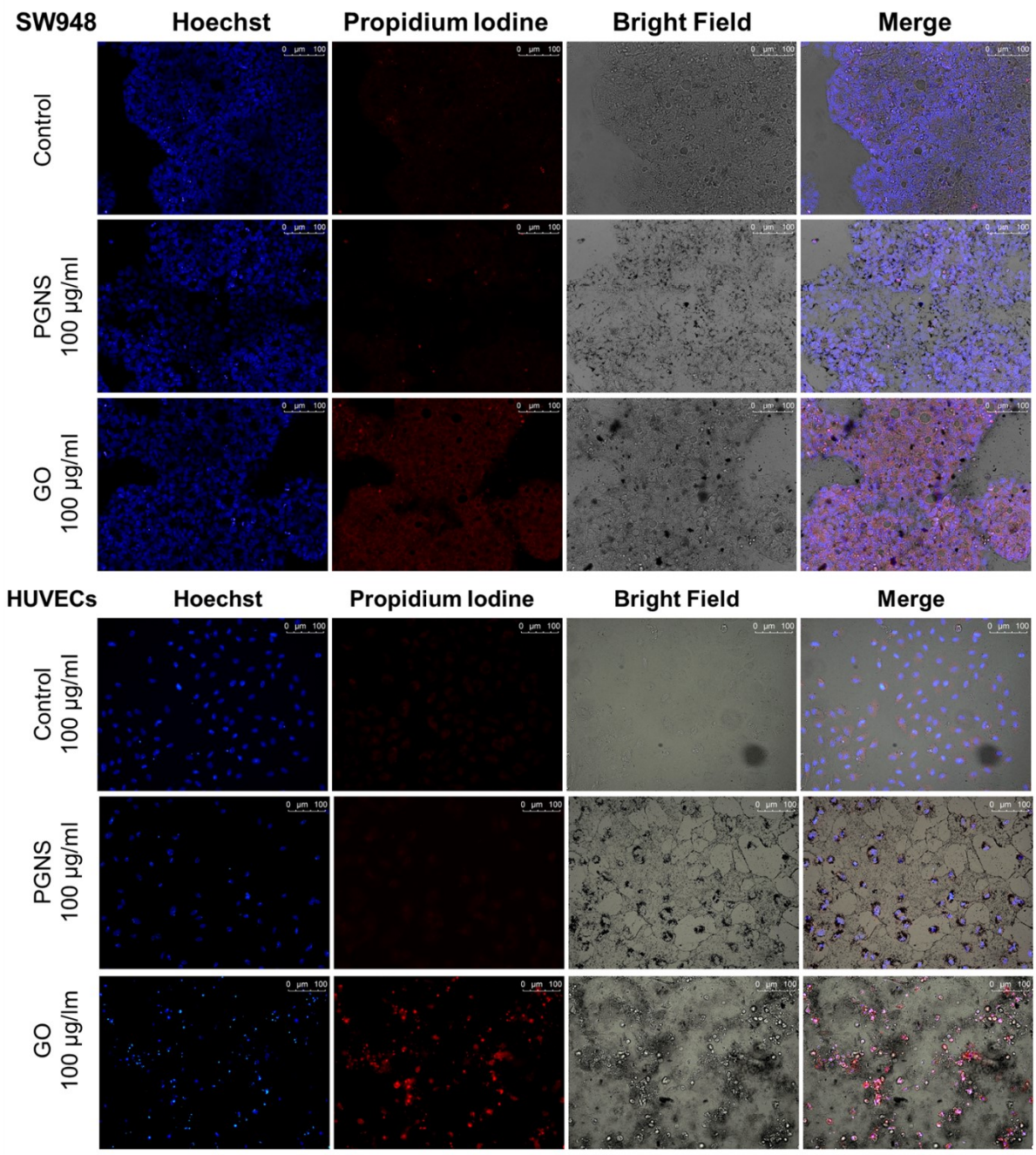

Figure S2 laser scanning confocal microscopy images of SW948 and HUVECs cells after GO 48 hours of GO or PGNS exposure labeled with propidium iodide (PI) for dead and membrane damaged cells with Hoechst counterstaining of the nuclei. The representative images show an increased PI internalization after GO exposure when compared to PGNS.

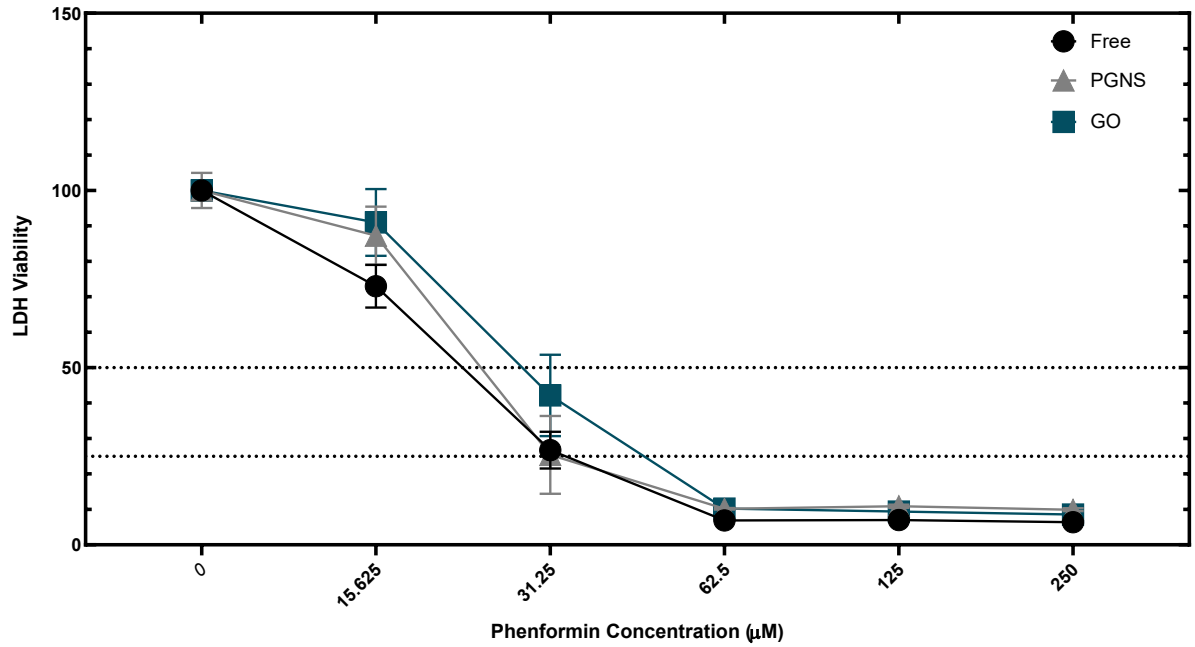

Figure S3 Cellular viability of SW948 cell lines based on their cellular content of LDH after 48 hours of exposure to free, PGNS-loaded and GO-loaded phenformin. The data supports the CCK8 assay results at 48 hours after treatment.

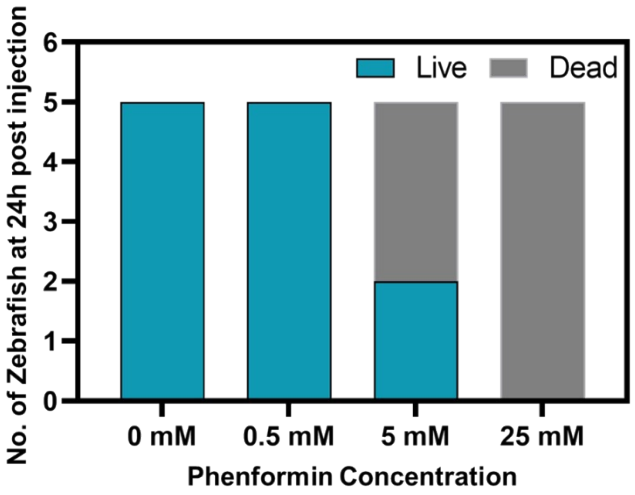

Figure S4 Mortality of zebrafish larvae 24h after phenformin injection. Yolk sack injection of increasing phenformin concentrations of 0.5mM (1.5ng total injected weight, corresponding with the graphene bound concentrations), 5mM (~15 ng), and 25mM (~75ng) induced dose-dependent mortality.

Table S2 Possible endogenous fluorescence sources after 470 nm excitation in zebrafish larvae and their reported lifetimes.

Fluorescent molecule	***Lifetime range (ns)***	***Notes***	***Refs***

Glutaraldehyde	0.9 - 1.4	An aldehyde-based fixation byproduct	[88]
Methylene blue	0.2 - 1	A common additive to zebrafish embryo water	[89–91]
Collagen	0.3 - 2	Depending on stiffness	[92–94]
Melanin	1.2 ()	“Casper” is a melanin-knockout Zebrafish larvae mutant	[95,96]