Supplementary Information

Gene delivery to mammalian cells using a graphene nanoribbon platform

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Keywords

Graphene oxide, gene delivery, transfection, gene expression, cytotoxicity

Table S1:

Name	Primer sequences
For-0.7 kb	CCTAAAGCTTGTGAGCAAGGGCGAGGAG (Template: pBY2982)
Rev-0.7 kb	TAGGAAGCTTGTACAGCTCGTCCATGCC (Template: pBY2982)
For-1 kb	GCCAAGCTTGGCCCATTG (Template: pBS185 CMV-Cre)
Rev-1 kb	CCATTTCCGGTTATTCAACTTGCACC (Template: pBS185 CMV-Cre)
For-2 kb	GCCAAGCTTGGCCCATTG (Template: pBS185 CMV-Cre)
Rev-2 kb	CAGGCATTCTATCGCTCAGC (Template: pBS185 CMV-Cre)
For-3 kb	GCCAAGCTTGGCCCATTG (Template: pBS185 CMV-Cre)
Rev-3 kb	GCCCTGGGCACATTTGGAG (Template: pBS185 CMV-Cre)
For-4 kb	GCCAAGCTTGGCCCATTG (Template: pBS185 CMV-Cre)
Rev-4 kb	GGCCATCTTCTGCTACATACGAAG (Template: pBS185 CMV-Cre)

For-5 kb	GCCAAGCTTGGCCCATTG (Template: pBS185_CMV-CRE)
Rev-5 kb	CCGGATAAGGCGCAGCG (Template: pBS185 CMV-Cre)
For-6 kb	GCCAAGCTTGGCCCATTG (Template: pBS185 CMV-Cre)
Rev-6 kb	GCATCTTACGGATGGCATGACAG (Template: pBS185 CMV-Cre)

Figure Legends:

Figure S1. Characterization of DNA length that enable DNA interactions with O-GNR. Binding is independent of dsDNA length. A cocktail of dsDNA fragments ranging in size from 0.7 to 6.0 kb was incubated with O-GNR overnight at 4^{o}C.

Figure S2. O-GNR complexes are not cytotoxic to a murine B cell line. A20 B lymphocytes were incubated with the indicated concentrations of O-GNR for 24 or 48 hrs. A. Trypan Blue dye exclusion was used to examine the mean (-/+ SD) percentage of viable cells at the indicated time points. B. Lactate dehydrogenase (LDH) release in the culture media assay was used to assess O-GNR cytotoxicity at the indicated time points. Percentage lysis was calculated relative to the positive control treatment. Data represents the mean (+/- SD) fold change in the percentage of lysis of six replicates compared to the untreated sample. No significant difference was observed.

Figure S3. EGFP gene expression in cells upon delivery of DNA:O-GNR complexes. HEK 293T were overlaid with the linear pEGFP DNA:O-GNR complex, input O-GNR alone, the matched void volume of third wash, and 2 μg pEGFP. Cells were imaged at 24 hrs and 48 hrs. Live cell images by phase or fluorescence were obtained with the same exposure.

Figure S4. Quantitation of EGFP gene expression in HEK 293T. A. Linear pEGFP:O-GNR complex as described in Figure 6B-D and linear pEGFP DNA standards (0.1μg - 2.0μg) were analyzed by gel electrophoresis. Their pixel intensities were measured using ImageJ 1.49v software. The bound pEGFP (Bound) amount was determined to be 0.38 μg by linear regression analysis of the DNA standards. B. Histogram plot representing the percentage of HEK 293T cells (+/-SD) positive for GFP expression by the flow cytometric analysis of cells upon transfection with 0μg (mock), 0.1μg and 2.0μg linear pEGFP performed in parallel with the pEGFP:O-GNR complex described in Figure 6B.

Figure S1:

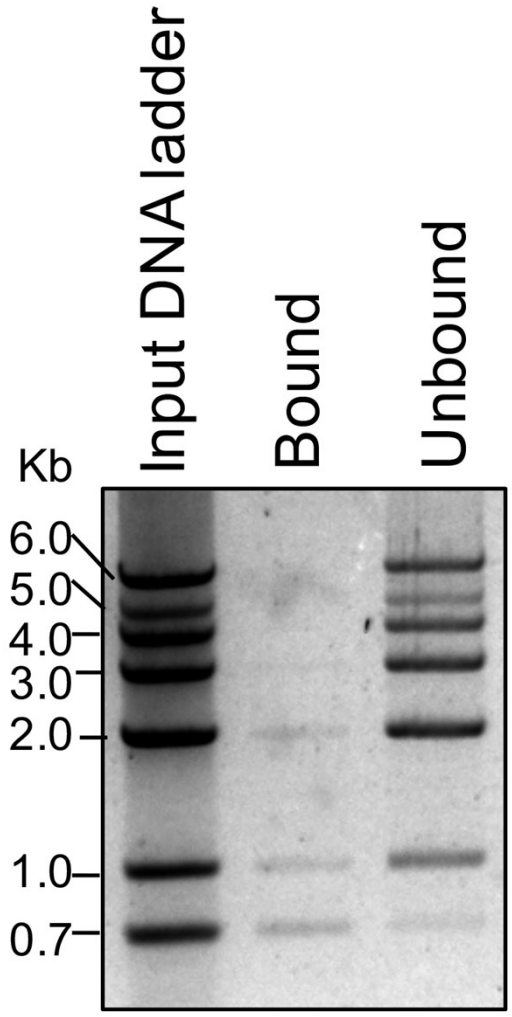


Figure S2:

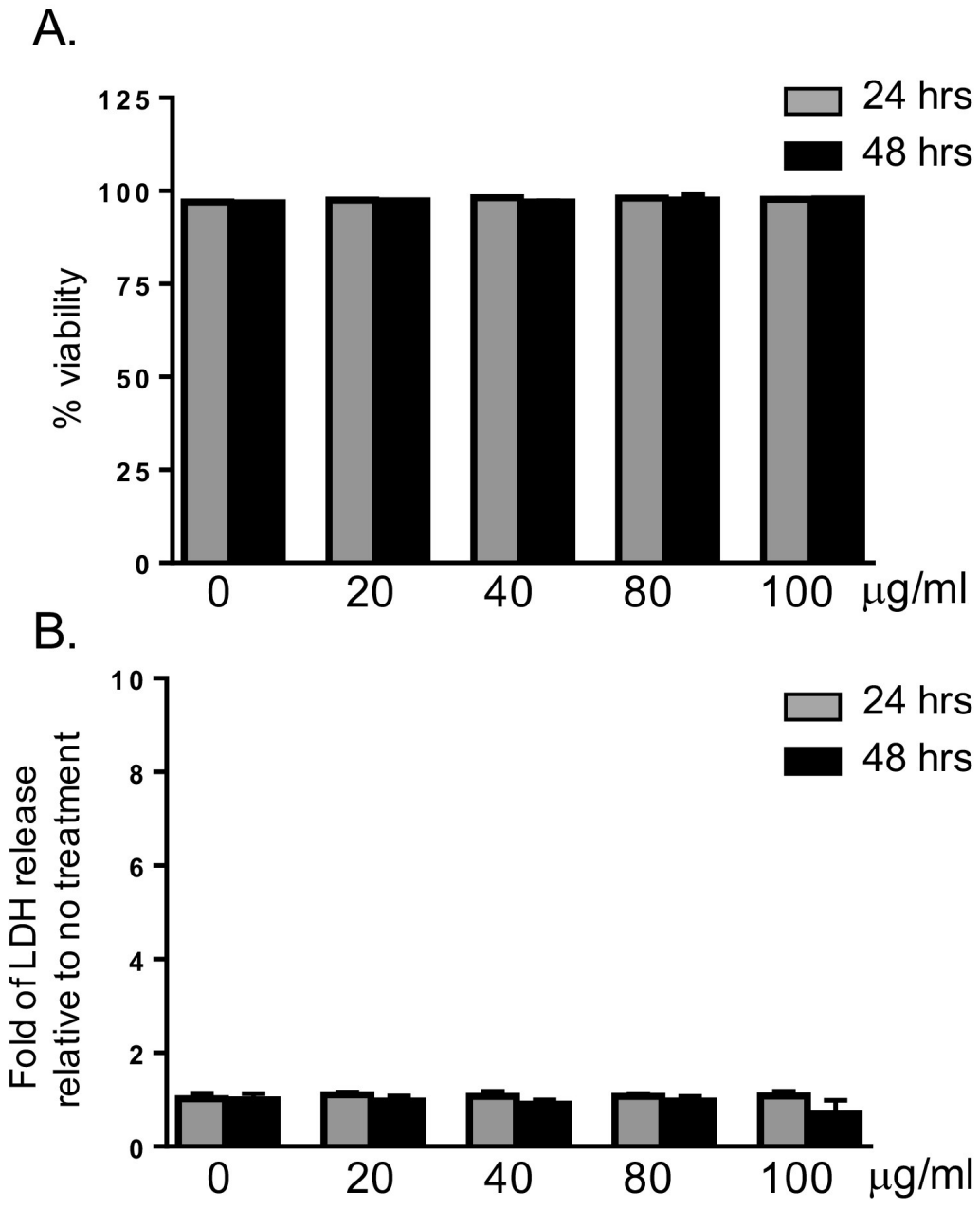

A.
24 hrs
48 hrs
% viability
125
100
75
50
25
0
0
20
40
80
100
μg/ml
B.
24 hrs
48 hrs
Fold of LDH release relative to no treatment
10
8
6
4
2
0
0
20
40
80
100
μg/ml

Figure S3:

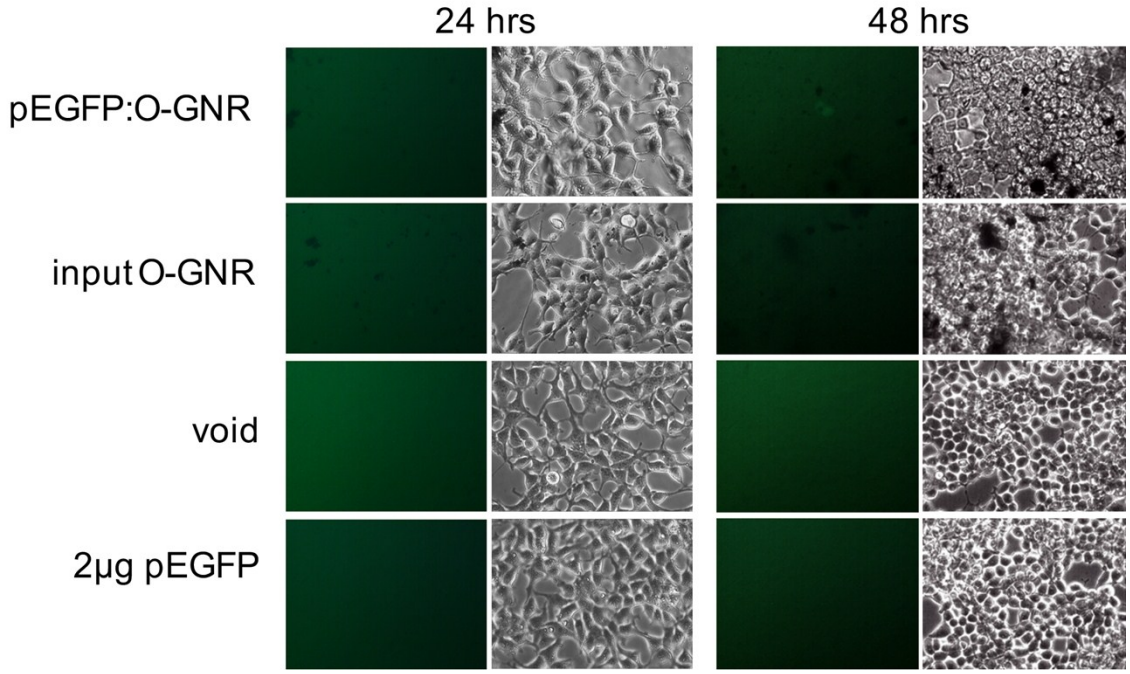
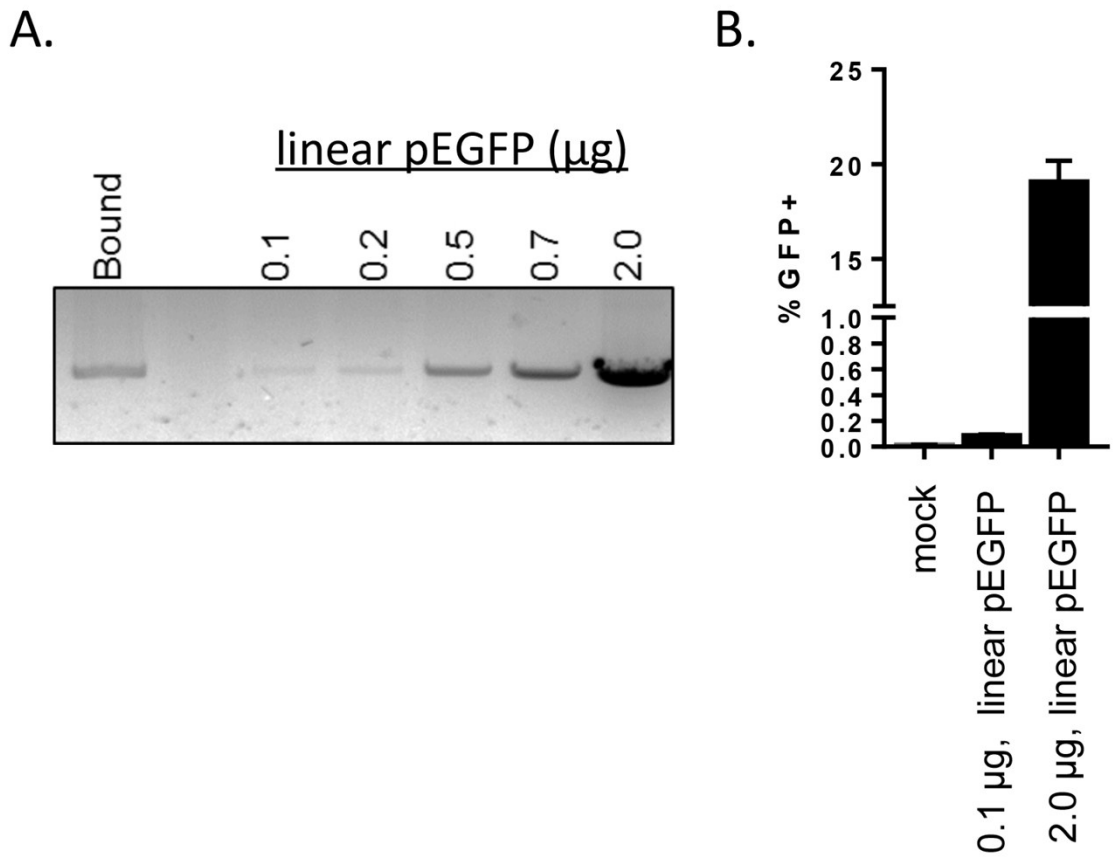


Figure S4:


A.
linear pEGFP (µg)
Bound
0.1
0.2
0.5
0.7
2.0
B.
% GFP+
25
20
15
1.0
0.8
0.6
0.4
0.2
0.0
mock
0.1 µg, linear pEGFP
2.0 µg, linear pEGFP