Polyethyleneimine-Modified Graphene Oxide Nanocomposites for Effective Protein Functionalization

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Endothelial Cells Viability Assay

The human umbilical vein endothelial cells (HUVECs) viability assay was performed according to previous studies[1,2] with minor modifications by using a cell counting kit-8 (CCK-8, Beyotime, China). Typically, ~2.0×10^4 cells were grown in a 96-well plate and cultured in DMEM medium with 10% fetal bovine serum (FBS) and maintained in a humidified 37 °C incubator with 5% CO_2. After culturing for 24 h, the cells were mildly washed with 150 μL of serum-free DMEM medium (SFM) for two times and then incubated with 150 μL of SFM with different sample concentrations. After contacting with the materials for 24 h, the cells were washed twice with SFM. Subsequently, 100 μL of SFM and 15 μL of CCK-8 solution was added into each well. After incubating for 1.5 h, 80 μL of the SFM was transferred to a new 96-well plate and the absorbance was measured at 450 nm with 650 nm as reference by using a Microplate reader (Synergy^{TM}H1, BioTek). The cell experiments were repeated three times and the statistical significance was calculated by Student's *t*-test. The cell viability was determined by the ratio of absorbance between sample groups and control groups.

Enrichment of Standard Glycoprotein

For selective capture of standard glycoprotein, 0.1 mg GO@BPEI@Con A composites were firstly dissolved in 1 mL binding buffer (0.15 M NaCl, 10 mM HEPES, 0.5 mM $MnCl_2$ and 1 mM $CaCl_2$, pH 7.5). Then, protein mixture contained 1.0 μg RNase B and 500 μg Myo were added. After incubating for 1 h at room temperature, the composites were washed five times with binding buffer to remove the unbounded proteins. Subsequently, 20 μL elution buffer (0.5 M NaCl, 10 mM HEPES, 0.5 mM $MnCl_2$, 1 mM $CaCl_2$, 0.2 M methyl-α-D-mannopyranoside and 0.2 M methyl-α-D-glucopyranoside, pH 7.0) was added into the composites and incubated for 15 min. Finally, the captured glycoprotein was specifically eluted with elution buffer and the supernatant was analyzed by MALDI-TOF MS.

The glycoprotein enrichment capacity of GO@BPEI@Con A is defined as the glycoprotein amount difference between the total glycoprotein used and the residual glycoprotein present in the supernatant after adsorption. To removal physical adsorbed glycoproteins as far as possible, the specific absorbed glycoproteins were washed another time and centrifuged, the supernatant was merged and the amount of glycoprotein was determined by BCA assay after supernatant was filtered by a 3 kDa filtration unit to remove the elution buffer.

Pretreatment and Analysis of Real Sample

Hepatocarcinoma ascites syngeneic cell lines (Hca) were presented by the Dalian Medical University. Hca cells were firstly washed three times with ice-cold PBS to remove blood and isolated by centrifugation (500 × *g*, 5 min) at room temperature. Then, the collected cells were homogenized in buffer composed of 8 M urea and 1% (v/v) protease inhibitor cocktail in an ice bath. The resulting homogenate was centrifuged at 20000 *g* for 30 min. Finally, the supernatant was collected and the protein concentration was determined by the BCA assay. All samples were stored at −80 °C pending further use.

For capture of glycoproteins extracted from Hca, the mixture of 0.5 mg GO@BPEI@Con A and 0.5 mg GO@BPEI@WGA were firstly dissolved in 1 mL binding buffer, then 50 μg proteins from Hca were added and incubated for 2 h at room temperature. The unbounded proteins were removed with 400 μL binding buffer for five times. The glycoproteins were recovered by washing two times with 300 μL elution buffer (0.5 M NaCl, 10 mM HEPES, 0.5 mM $MnCl_2$, 1 mM $CaCl_2$, 0.2 M methyl-α-D-mannopyranoside, 0.2 M methyl-α-D-glucopyranoside and 0.4 M *N*-acetyl-D-glucosamine, pH 7.0). Then, the collected proteins were concentrated by centrifugal ultrafiltration with 3 kDa filtration units to remove the monosaccharide and redissolved in 8 M urea. Then, the proteins were reduced in 20 mM DTT at 56 °C for 2 h and alkylated in 50 mM IAA at room temperature for 30 min in the dark. After that, the solution was diluted 10-fold with 50 mM NH_4HCO_3 (pH 8.0) to decrease the urea concentration below 1 M. Subsequently, trypsin was added at an enzyme/substrate ratio (m/m) of 1:30 and incubated at 37 °C for 16 h. After 2 μL of formic acid was added into the solution to terminate the reaction, the tryptic digests were desalted by homemade RP C18 precolumn and then dried down in a Speed Vac Concentrator (Thermo, CA). Next, the tryptic digests were redissolved in 20 mM NH_4HCO_3 and 100 units of PNGase F was added and incubated at 37 °C for 16 h. Finally, the solution was dried down and stored at −80 °C pending further analysis.

Compared with the enrichment of glycoprotein by the above-mentioned composites, the amount of commercial agarose bound WGA and agarose bound Con A was 5 mg, respectively, 10-fold amount more than GO composites. Other conditions were the same as described above.

MS Analysis and Database Searching

MALDI-TOF MS analysis was performed on Ultraflex III TOF/TOF (Bruker Daltonics, Bremen, Germany). Sinapinic acid (SA) matrix solution (20 mg/mL) was prepared in ACN/H_2O/TFA (50:50:0.1). one microliter amount of the sample and SA solution were sequentially spotted on the MALDI plate for MS analysis.

The tryptic digests were analyzed on a LTQ-Orbitrap Velos (Thermo, CA) mass spectrometer coupled with an Accela 600 HPLC system (Thermo, CA). The mobile phase A was 2% ACN with 0.1% formic acid and phase B was 98% ACN with 0.1% formic acid. The samples were automatically loaded onto a C18 trap column (150 μm i.d. × 5 cm) which connected to a homemade capillary separation column (75 μm i.d. × 15 cm). To achieve sufficient separation, an 85-min linear gradient (10% to 35% phase B) was employed with the flow rate at 280 nL/min. The LTQ-Orbitrap Velos was operated with a 2.0 kV spray voltage in positive mode, and the temperature of ion transfer capillary was 250 °C. The normalized collision energy was set at 35.0%. One microscan was set for each MS and MS/MS scan. All MS and MS/MS spectra were obtained in a data-dependent mode with one MS scan followed by 20 MS/MS scans in CID mode. The scan range was set from m/z 300 to m/z 1800. The dynamic exclusion function was set as follows: repeat count 1, repeat duration 40 s, and exclusion duration 40 s. All MS/MS spectra by nano-RPLC-ESI-MS/MS analysis were searched against the mouse IPI database (v3.68) in Mascot (version 2.3.2). Mass tolerances for LTQ-Orbitrap Velos were set as 10 ppm for parent ions and 0.5 Da for

fragments. Cysteine residues was searched as a fixed modification of +57.0215 Da. Oxidation (M) (+15.9949 Da) and deamidation (N) (+0.9840) were searched as variable modification, respectively. Peptides were searched using fully tryptic cleavage constraints, and up to 2 missed cleavages. The search results were filtered by pBuild to control the FDR ≤ 1%.

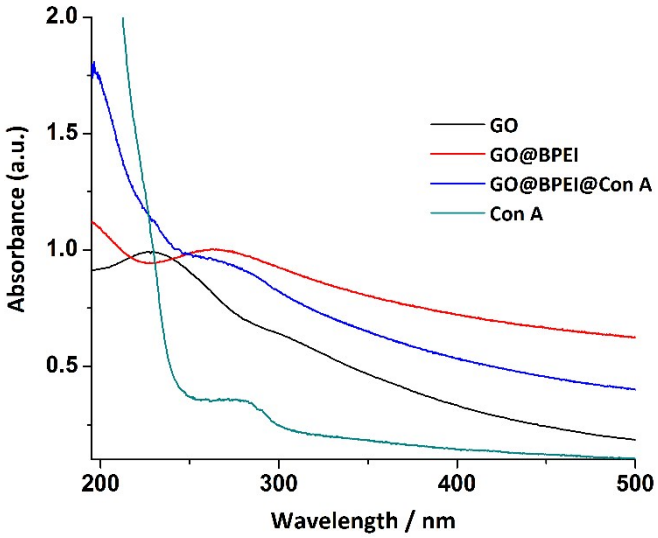

Fig. S1. UV-vis absorption spectra of GO, GO@BPEI, GO@BPEI@Con A and Con A

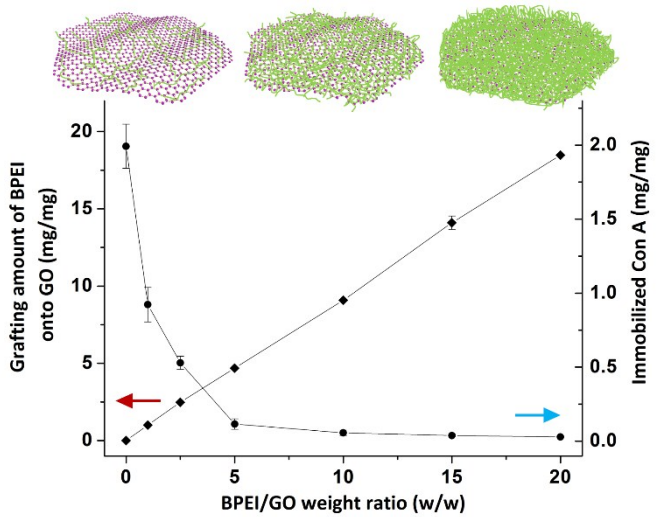

Fig. S2. Grafting amount of BPEI and immobilized Con A under different PEI/GO weight ratios (w/w).

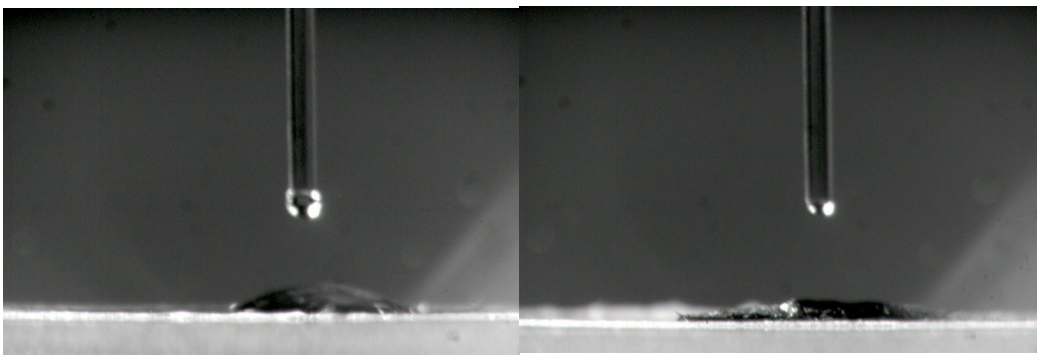

Fig. S3. The water contact angle test of (a) GO and (b) GO@BPEI

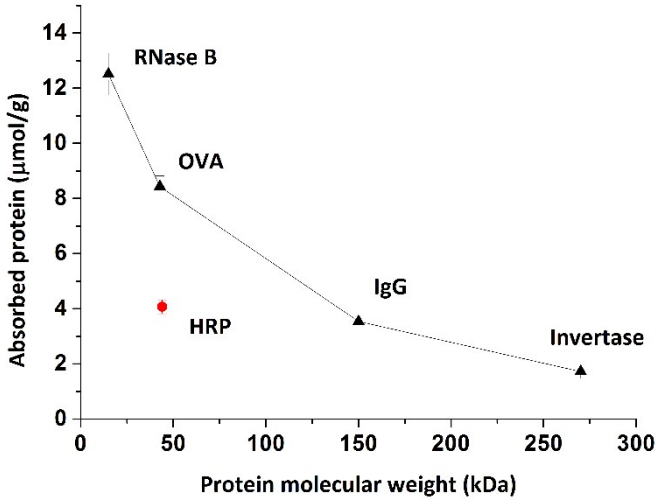

Fig. S4. The selective molar adsorption capacity of GO@BPEI@Con A for different glycoproteins.

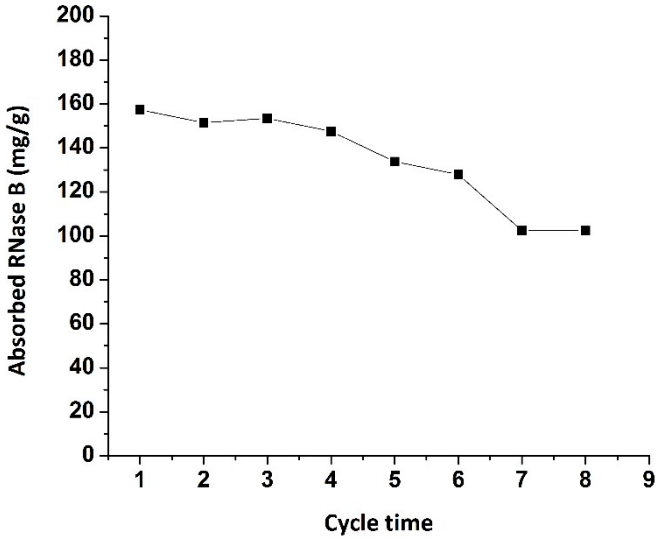

Fig. S5. Reusability of the GO@BPEI@Con A

Table S1. List of identified glycoproteins and their gene IDs captured by commercial agarose-based lectins and GO@BPEI@lectins composites, respectively

Number	Agarose-Based Lectins	Gene	GO@BPEI@Lectins	Gene
1	A1L0X5	Krt78	A1L0X5	Krt78
2	A2A6A1	Gpatch8	A6H694	Lrrc63
3	A2AQR0	Gpd2	B1AZP2	Dlgap4
4	A7RDN6	Rnls	B9EHJ2	Fbxw14
5	B1AZM2	Gm15091	O08573	Lgals9
6	O08756	Hsd17b10	O08756	Hsd17b10
7	O08795	Prkcsh	O35129	Phb2
8	O35129	Phb2	P04104	Krt1
9	O35379	Abcc1	P05202	Got2
10	O35887	Calu	P05784	Krt18
11	O35988	Sdc4	P07356	Anxa2
12	O88569	Hnrnpa2b1	P07901	Hsp90aa1
13	P02535	Krt10	P08003	Pdia4
14	P04104	Krt1	P08113	Hsp90b1
15	P05202	Got2	P08551	Nefl
16	P05213	Tuba1b	P08553	Nefm
17	P05784	Krt18	P08730	Krt13
18	P07356	Anxa2	P0C0A3	Chmp6
19	P07901	Hsp90aa1	P0C5E4	Ptprq
20	P08113	Hsp90b1	P10605	Ctsb
21	P08553	Nefm	P10852	Slc3a2
22	P08730	Krt13	P10853	Hist1h2bf
23	P09055	Itgb1	P11276	Fn1
24	P09405	Ncl	P11679	Krt8
25	P10605	Ctsb	P12960	Cntn1
26	P10852	Slc3a2	P17809	Slc2a1
27	P10853	Hist1h2bf	P18572	Bsg
28	P11679	Krt8	P20152	Vim
29	P15864	Hist1h1c	P20917	Mag
30	P17809	Slc2a1	P21956	Mfge8
31	P18572	Bsg	P24668	M6pr
32	P19001	Krt19	P27046	Man2a1
33	P20152	Vim	P29621	Serpina3c
34	P24668	M6pr	P32037	Slc2a3
35	P27046	Man2a1	P32883	Kras
36	P29621	Serpina3c	P35278	Rab5c
37	P32037	Slc2a3	P35456	Plaur
38	P35456	Plaur	P35550	Fbl
39	P39098	Man1a2	P46660	Ina

40	P43276	Hist1h1b	P46978	Stt3a
41	P46660	Ina	P47857	Pfkm
42	P46978	Stt3a	P51150	Rab7a
43	P47857	Pfkm	P51410	Rpl9
44	P51410	Rpl9	P52196	Tst
45	P52196	Tst	P53798	Fdft1
46	P55065	Pltp	P56480	Atp5b
47	P56480	Atp5b	P58242	Smpdl3b
48	P61979	Hnrnpk	P58281	Opa1
49	P62320	Snrpd3	P61027	Rab10
50	P62908	Rps3	P61979	Hnrnpk
51	P62960	Ybx1	P62320	Snrpd3
52	P68040	Gnb2l1	P62717	Rpl18a
53	P68134	Acta1	P62908	Rps3
54	P68369	Tuba1a	P68040	Gnb2l1
55	P70699	Gaa	P68369	Tuba1a
56	P84104	Srsf3	P70696	Hist1h2ba
57	P97311	Mcm6	P70699	Gaa
58	Q00896	Serpina1c	P97311	Mcm6
59	Q02257	Jup	Q00896	Serpina1c
60	Q03265	Atp5a1	Q02257	Jup
61	Q04857	Col6a1	Q03265	Atp5a1
62	Q3TDQ1	Stt3b	Q148Q7	4732456N10Rik
63	Q3U9G9	Lbr	Q3TCN2	Plbd2
64	Q3UKW2	Calm1	Q3TDQ1	Stt3b
65	Q5U462	Cdcp1	Q3THE6	N/A
66	Q61543	Glg1	Q3U9G9	Lbr
67	Q62261	Sptbn1	Q3UUQ7	Pgap1
68	Q62470	Itga3	Q4VBD0	Herc1
69	Q64133	Maoa	Q571E4	Galns
70	Q64478	Hist1h2bh	Q5U462	Cdcp1
71	Q64523	Hist2h2ac	Q60597	Ogdh
72	Q69Z23	Dnah17	Q60930	Vdac2
73	Q6DFW4	Nop58	Q61543	Glg1
74	Q6GQR8	Znf329	Q61548	Snap91
75	Q6IFZ6	Krt77	Q61762	Kcna5
76	Q6NXH9	Krt73	Q62261	Sptbn1
77	Q6PD26	Pigs	Q62470	Itga3
78	Q6PGB8	Smarca1	Q64133	Maoa
79	Q80T14	Fras1	Q64523	Hist2h2ac
80	Q8BHL4	Gprc5a	Q69ZL1	Fgd6
81	Q8BHN3	Ganab	Q6DFW4	Nop58
82	Q8BLF1	Nceh1	Q6IFZ6	Krt77
83	Q8BTM8	Flna	Q6NXH9	Krt73

84	Q8C129	Lnpep	Q6PB70	Ano8
85	Q8C7X2	Emc1	Q6PGB8	Smarca1
86	Q8CGY6	Unc45b	Q7TMS5	Abcg2
87	Q8K0E8	Fgb	Q80T14	Fras1
88	Q8K2T9	Tpr	Q80UM7	Mogs
89	Q8K385	FRRS1	Q80UX8	Abhd13
90	Q8R180	Ero1l	Q80Y83	Dixdc1
91	Q8R1M2	H2afj	Q8BHL4	Gprc5a
92	Q8VCM7	Fgg	Q8BHN3	Ganab
93	Q8VCN3	Ugt2b37	Q8BLF1	Nceh1
94	Q8VCW2	Krt25	Q8BQ93	N/A
95	Q8VED5	Krt79	Q8BTM8	Flna
96	Q8VHX6	Flnc	Q8BVP2	Ldhal6b
97	Q91VX9	Tmem168	Q8C129	Lnpep
98	Q91YQ5	Rpn1	Q8C7K6	Pcyox1l
99	Q99P88	Nup155	Q8C7X2	Emc1
100	Q9CQF9	Pcyox1	Q8CDM4	Ccdc73
101	Q9CQU0	Txndc12	Q8CG46	Smc5
102	Q9CQY5	Magt1	Q8CHK3	Mboat7
103	Q9CWF2	Tubb2b	Q8JZQ2	Afg3l2
104	Q9CY27	Tecr	Q8K0E8	Fgb
105	Q9CZ49	Klhl35	Q8K224	Nat10
106	Q9D024	Ccdc47	Q8K2I9	Fbxo18
107	Q9D771	Tmem206	Q8K385	FRRS1
108	Q9D8Z6	Atg101	Q8R143	Pttg1ip
109	Q9D9G7	1700074P13Rik	Q8R180	Ero1l
110	Q9DB25	Alg5	Q8R1M2	H2afj
111	Q9DBG6	Rpn2	Q8R2Q8	Bst2
112	Q9DBU0	Tm9sf1	Q8VBV3	Exosc2
113	Q9EQH2	Erap1	Q8VCM7	Fgg
114	Q9ERB0	Snap29	Q8VCN3	Ugt2b37
115	Q9JIS7	Cacna1f	Q8VCW2	Krt25
116	Q9QZD8	Slc25a10	Q8VED5	Krt79
117	Q9QZZ4	Myo15a	Q8VHX6	Flnc
118	Q9R0T7	Try4	Q91VC9	Ghitm
119	Q9WUA3	Pfkp	Q91VX9	Tmem168
120	Q9WUU7	Ctsz	Q91W50	Csde1
121	Q9WV02	Rbmx	Q91W96	Anapc4
122	Q9Z2G6	Sel1l	Q91XG0	Ly6c1
123			Q91YQ5	Rpn1
124			Q99K48	Nono
125			Q99LR1	Abhd12
126			Q99P88	Nup155
127			Q99PV0	Prpf8

128	Q9CQF9	Pcyox1
129	Q9CQW2	Arl8b
130	Q9CQY5	Magt1
131	Q9CRB9	Chchd3
132	Q9CY27	Tecr
133	Q9D024	Ccdc47
134	Q9D1L0	Chchd2
135	Q9D245	Cstad
136	Q9D2V8	Mfsd10
137	Q9D771	Tmem206
138	Q9DB25	Alg5
139	Q9DBG6	Rpn2
140	Q9DBU0	Tm9sf1
141	Q9DCE5	Pak1ip1
142	Q9EQH2	Erap1
143	Q9JIS5	Sv2a
144	Q9JKS4	Ldb3
145	Q9QZD8	Slc25a10
146	Q9QZM4	Tnfrsf10b
147	Q9QZZ4	Myo15a
148	Q9R0E2	Plod1
149	Q9R0T7	Try4
150	Q9R233	Tapbp
151	Q9WTR5	Cdh13
152	Q9WTS6	Tenm3
153	Q9WUA3	Pfkp
154	Q9WUU7	Ctsz
155	Q9Z127	Slc7a5
156	Q9Z2B1	Il18rap
157	Q9Z2G6	Sell1

Reference

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