Supporting Information

Surface Modification of Poly(styrene) 96-well Plates Using Aptamers via a Dendrimer-templated Strategy to Enhance ELISA Performances

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S1. Optimization of G7 concentration for surface engraftment.

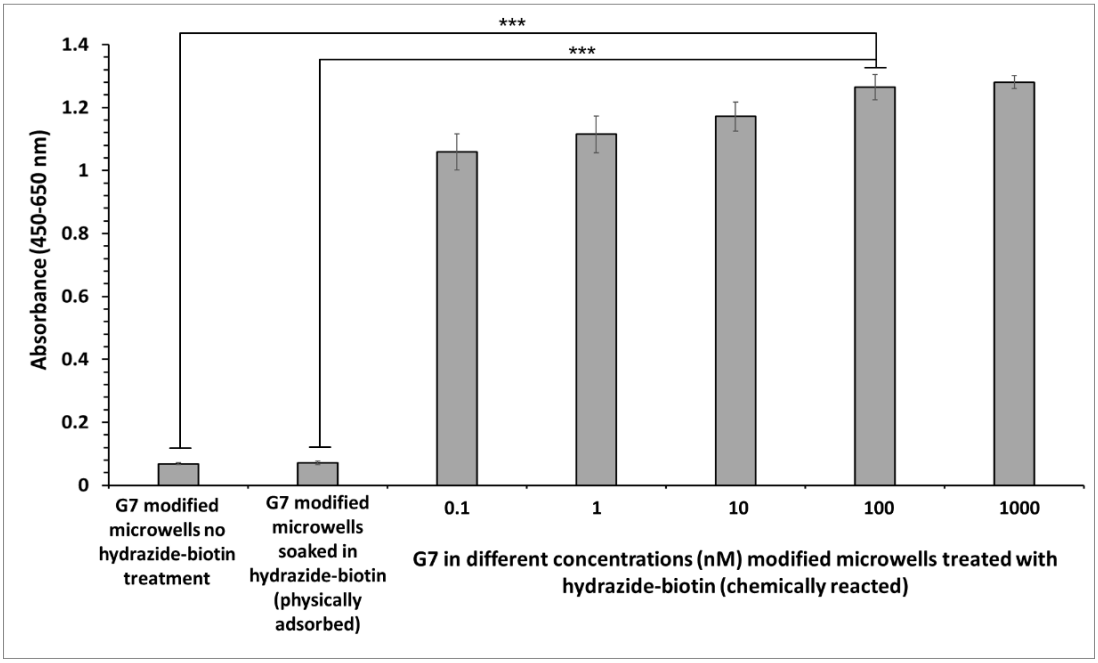


Figure S1. Optimization of G7 concentration for surface engraftment. Error bars indicate standard deviation, n=5; *p* values (***<0.001) were calculated by a Student's t-test, 2 tails.

S2. Optimization of aptamer concentration

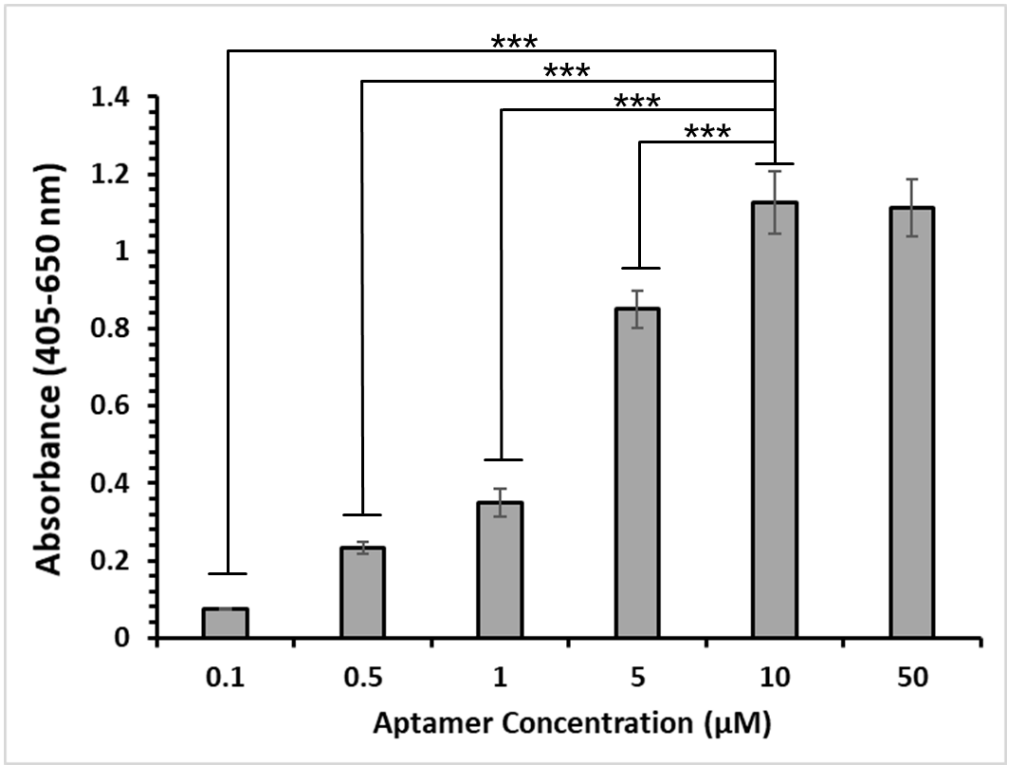


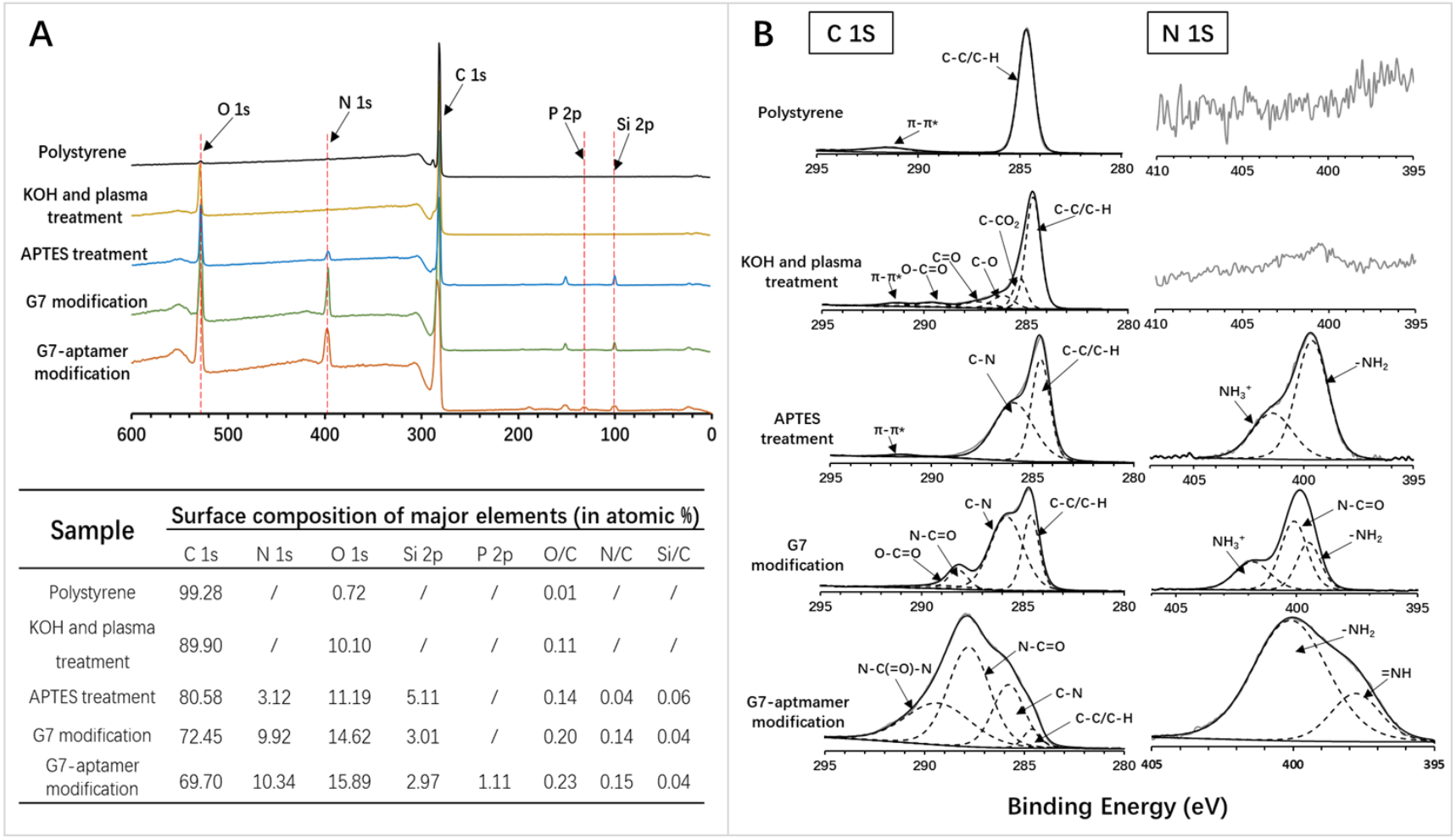
Figure S2. Optimization of aptamer concentration; error bars indicate standard deviation, n=5; P values (***<0.001) were calculated by a Student's t-test, 2 tails.

S3. X-ray photoelectron spectroscopy (XPS)

To study each step of the surface modification, surfaces of interest (*i.e.,* PS surfaces, KOH and plasma treated surfaces, APTES treated surfaces, G7-COOH modified surfaces, and G7-aptamer modified surfaces) were characterized using XPS. Specifically, XPS spectra were obtained using a Kratos Axis Ultra DLD spectrometer (Kratos Analytical Ltd, Manchester, UK) with monochromatized Al Kα X-rays. The take-off angles for all measurements were fixed at 45 °, and 284.6 eV was used as a reference position for the C-H(C) peak. Survey scan spectra and high-resolution spectra were analyzed by CasaXPS Version 2.3.19 PR1.0. (Casa Software Ltd., Teignmouth, UK).

As shown in Figure S3A, in comparison with PS surfaces, the surfaces treated with KOH and plasma showed a marked O 1s peak intensity increase, suggesting successful surface activation. Moreover, after the APTES modification, two new peaks for N 1s and Si 2p emerged, both of which can be attributed to the introduction of the APTES molecules to the surfaces. In addition, after surface modification with G7-COOH, the surface composition of N and O were increased to 9.92 % and 14.62 %, respectively, while Si dropped to 3.01 % when compared to APTES modified surfaces, suggesting the successful introduction of G7-COOH to the surfaces. Furthermore, a new P 2p peak appeared on the spectrum of the G7-aptamer modified surfaces when compared to those of other surfaces, providing support that aptamer immobilization was successful.

Moreover, the success of surface modification was further investigated using high-resolution XPS spectra, as shown in Figure S3B. Specifically, in comparison with PS surfaces, the surfaces treated with KOH and plasma showed more oxygen-related deconvoluted peaks (C 1s scan), including C-CO_2 (285.3 eV), C-O (286.1 eV), C=O (287.3 eV) and O-C=O (289.2 eV) [1-3], confirming the success of surface activation. Moreover, after the APTES treatment, the spectrum of the resulting surfaces showed new amino-related peaks at 285.8 eV (C-N, C 1s), 401.4 eV (NH_3^+, N 1s), and 399.6 eV (-NH_2, N 1s) [1, 4, 5] while all the oxygen-related C 1s peaks from KOH and plasma treated surface disappeared, indicating the success of surface amination. In addition, after G7-COOH immobilization, three new constituent peaks related to chemical structures of G7-COOH molecules emerged at 289.0 eV (O-C=O, C 1s), 288.1 eV (N-C=O, C 1s), and 400.1 eV (N-C=O, N 1s) [1, 4, 5], supporting the notion that G7-COOH surface immobilizations were successfully carried out. Furthermore, after conjugating aptamers, two new constituent peaks related to nucleic acids emerged at 289.3 eV (N-C(=O)-N, C 1s) and 398.3 eV (=NH, N 1s) [6, 7], indicating that the aptamer conjugation was successful.



Sample	Surface composition of major elements (in atomic %)							
	C 1s	N 1s	O 1s	Si 2p	P 2p	O/C	N/C	Si/C
Polystyrene	99.28	/	0.72	/	/	0.01	/	/
KOH and plasma treatment	89.90	/	10.10	/	/	0.11	/	/
APTES treatment	80.58	3.12	11.19	5.11	/	0.14	0.04	0.06
G7 modification	72.45	9.92	14.62	3.01	/	0.20	0.14	0.04
G7-aptamer modification	69.70	10.34	15.89	2.97	1.11	0.23	0.15	0.04

Figure S3. XPS analysis of surface modification. A) Survey scan of each modified surface and the surface composition of the major elements (in atomic %). B) High-resolution XPS spectra of C 1s and N 1s of each modified surface.

S4 Surface blocking test

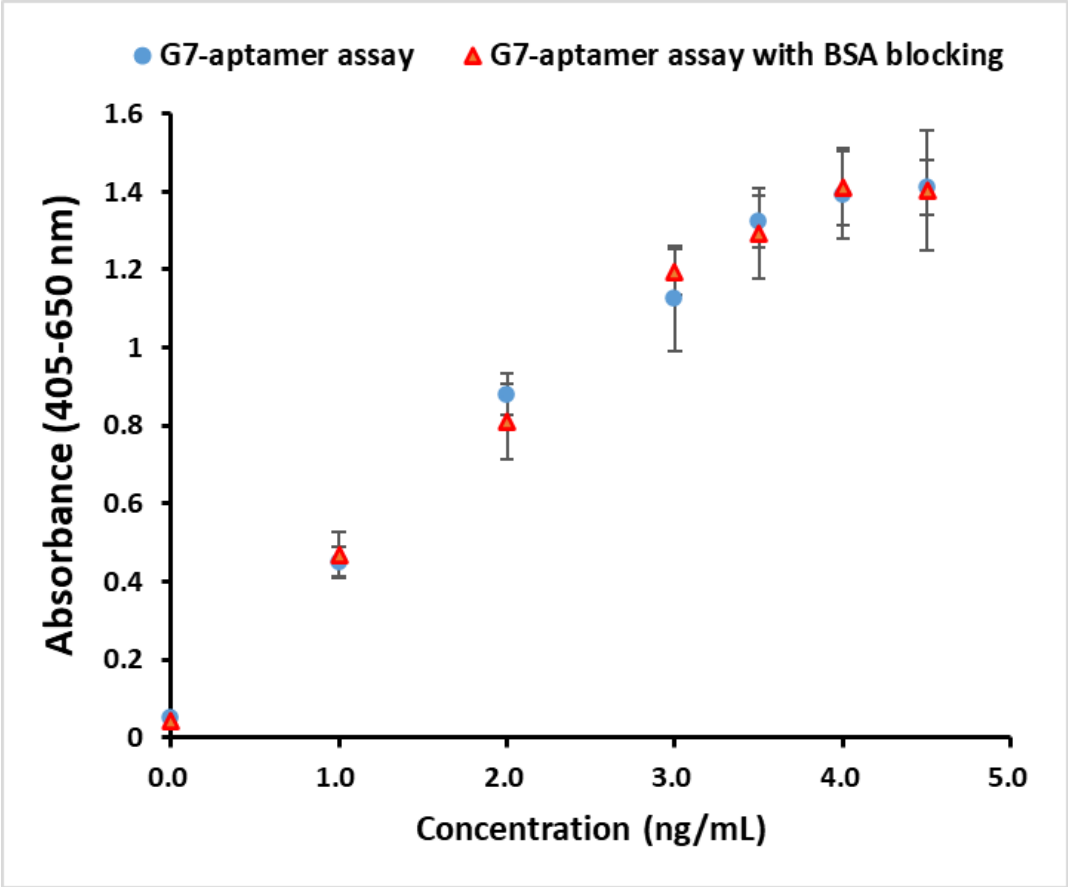


Figure S4. Comparison of G7-aptamer assay detection performances with and without BSA blocking steps.

S5. Materials

3-Aminopropyl-triethoxysilane, 99% (APTES), N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) liquid substrate solution, 2-(N-morpholino) ethanesulfonic acid (MES), and hydrazide-biotin were purchased from Thermo Fisher Scientific (Ottawa, ON). Anhydrous alcohol, Tween-20, phosphate-buffered saline (PBS) (0.01 M, pH 7.4), bovine serum albumin (BSA), Tris(hydroxymethyl) aminomethane (Tris), and NHS-biotin were purchased from Sigma-Aldrich (Oakville, ON). Poly(amidoamine) dendrimer generation 7 with carboxyl functional groups (G7-COOH) was purchased from Dendritech (Midland, MI). Human EGF, human FGF, sonic hedgehog, BMP-4, and Human PDGF-BB Standard ABTS ELISA Development Kit were purchased from PeproTech (Rocky Hill, NJ). Poly(styrene) flat bottom 96-microwell plates (not treated, product number 3370) were purchased from Corning (Kennebunk, ME). Aptamers were custom synthesized by Integrated DNA Technologies (Coralville, IA).

S6 Conjugation efficiency of the aptamer to the G7-COOH

To estimate the conjugation efficiency of the aptamer to the G7-COOH, amine capped aptamers with fluorescent tags (*i.e.,* NH_2-aptamer-cy3) were employed to conjugate with G7-COOH molecules in solution. Specifically, a reaction mixture containing 1 μM NH_2-aptamer-cy3, 1 nM G7-COOH, 5 mM NHS and 2 mM EDC in 0.1 M MES solution (pH 6.0) was reacted at room temperature for 2 h. Subsequently, the resulting mixture was filtered using an Amicon Ultra centrifugal filter (cutoff = 50 KDa) to

remove the unreacted aptamers (Mw = 14.5 KDa) and to purify the resulting G7-aptamer conjugates (Mw > 100 KDa). Subsequently, the purified G7-aptamer conjugates were used to estimate the concentration of fluorescent aptamers conjugated to the G7 dendrimers against a calibration curve established using known concentrations of NH_2-aptamer-cy3. The conjugation efficiency was calculated using the equation below:

$$\text{Conjugation efficiency} = \frac{\text{Concentration of the quantified aptamers}}{\text{Concentration of G7 molecules} \times 512 \text{ branches}} .$$

References

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