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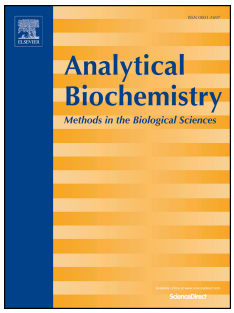

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Immunochromatographic strip biosensor for the rapid detection of N-glycolylneuraminic acid based on aptamer-conjugated nanoparticle

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ABSTRACT

N-glycolylneuraminic acid (Neu5Gc) is a type of sialic acid that is not typically produced in healthy humans but detective in some visceral cancer cells. As a new carcinoma biomarker, the level change in the serum and urine from the patient could potentially have the relation to the disease progression. So the measurement of the Neu5Gc will help to take a better response to therapeutic schedule for the sufferers. A sensitive and rapid aptamer-nanoparticle immunochromatographic strip for the visual detection of Neu5Gc was developed. The assay is based on the competitive reaction of binding the DNA aptamer targeting the candidate molecule selected by SELEX between Neu5Gc and complementary DNA. The sensing results indicated that the aptamer-based strip was sufficiently sensitive to detect Neu5Gc. The visual limit of detection (LOD) for semi-quantitative detection was 30 ng/mL under the optimal conditions and a quantitative detection limit of 5.38 ng/mL could be obtained using a scanning strip reader. The average recovery of the spiked cancer cell samples was 88.86%, with a coefficient of variation (CV) of 5.27%. The detection could be performed in less than 15 minutes using a simple procedure without any complicated equipment, demonstrating that this aptamer-nanoparticle biosensor strip has great potential for use to Neu5Gc-related cancer diagnosis.

Keywords: N-glycolylneuraminic acid (Neu5Gc); aptamer; gold nanoparticle; immunochromatographic strip

1. Introduction

Malignant tumors overexpress cell-surface glycans, particularly certain types of sialic acid[1-4]. There have been reports suggesting that sialylated tumor markers involve changes in the non-human sialic acid N-glycolylneuraminc acid (Neu5Gc)[1, 5]. Humans are unable to generate Neu5Gc as a result of a mutation in the gene encoding Cytidine monophosphate (CMP)-N-acetylneuraminic acid (Neu5Ac) Hydroxylase (CMAH)[6, 7]. Although there is no alternative pathway for the synthesis of Neu5Gc in the human body, some evidence has indicated that Neu5Gc accumulates in a number of cancers, including melanomas, colon carcinomas, breast cancers and retinoblastoma[5, 8-14]. The presence of this sialic acid may be the result of incorporation from animal foods, especially red meats and milk products[14-17]. Some epidemiological research has also linked an increased cancer risk to the consumption of certain foods[14, 17-20]. Meanwhile, Neu5Gc has also been detected in various bio-therapeutic products[4, 21]， Thus, the presence of Neu5Gc or its antibody could be used as a biomarker for carcinomas to monitor the progression or therapeutic response of various cancers[10, 22, 23].

Traditional methods for the detection of Neu5Gc are chemical analysis or mass spectrometry and immunological methods based on monoclonal or polyclonal anti-Neu5Gc antibodies[21, 24, 25] . However, although monoclonal antibodies are highly specific to some Neu5Gc-containing glycans, they cannot detect this molecule on other glycans. Polyclonal antibodies from chickens have low specificity and high background reactivity[8, 25]. Furthermore, while High-performance liquid chromatography (HPLC), Liquid chromatography-mass spectrometry (LC-MS), UPLC-FLD and other relevant methods can be more definitive[24, 26-28]; These methods have the merit of accuracy, efficient and good limit, But it is not compatible with routine laboratory utilization due

to the precise instrument and professional operator. Therefore, a simple, practical and sensitive method for the detection of Neu5Gc for routine batch use is necessary.

The immunological assay based on aptamers is developed in recent years. The aptamers are oligonucleotides, such as single-strand deoxyribonucleic acid (ssDNA) and ribonucleic acid (RNA), or peptide molecules that have high affinity and specificity for their targets as a result of their specific three-dimensional structures[29-31]. Compared with other probes for detection, aptamers have high specificity, low mass and versatile applications, and they are easy to produce and convenient to manipulate[32-35]. These features make aptamers useful in analysis, diagnosis and therapy[29, 34, 36-39]. We previously reported, for the first time, Neu5Gc-binding aptamers designed by SELEX (systematic evolution of ligands by exponential enrichment) selection[40]. More recently, several applications of aptamer-conjugated AuNPs have been reported for detection of cancer cells, foodborne pathogens and small molecules[35, 41-44].

In the present study, we developed an aptamer-based immunochromatographic test strip for the sensitive, cost-effective, and rapid visual detection of Neu5Gc from cancer cells. This method is based on the combination of a selective Neu5Gc aptamer with gold nanoparticles (AuNPs). The sulfhydrylated aptamer probe is conjugated to the Au-NPs, while biotinylated test and control probes are immobilized to the test line and control line, respectively. Neu5Gc first interacts with the selected aptamer probes on the AuNP-aptamer conjugates to produce an AuNP-aptamer-Neu5Gc complex and continues to migrate along the strip. Then, a representative red line can be created as a result of accumulating the AuNPs on the test line. The biotinylated control probes capture the excess AuNP-aptamer, causing a second red line. Herein, this aptamer-based chromatographic strip is used for the convenient qualitative and quantitative

analysis of Neu5Gc.

2. 2. Materials and Methods

2.1 Reagents and materials

N-glycolylneuraminc acid (Neu5Gc), N-acetylneuraminic acid (Neu5Ac), Ketodeoxynonulosonic acid (KDN), Chloroauric acid ($HAuCl_4$), streptavidin, dithiothreitol (DTT), Triton X-100, bovine serum albumin (BSA) and trisodium citrate were purchased from Sigma Aldrich (St. Louis, MO, USA) and used without further purification. The nitrocellulose membranes, polyvinyl chloride sheet, cellulose absorbent pad and glass fiber membranes were from Millipore (Billerica, MA, USA). Other reagents were of analytical grade purity and were obtained from Beijing Chemical Reagent Co. (Beijing, China). All other solutions were prepared using ultrapure (>18 MΩ) water from a Millipore Milli-Q water purification system (Billerica, MA, USA). All glassware was cleaned thoroughly with aqua regia [HCl/HNO_3 (3v/v)] for approximately 36 h and oven-dried for use.

The Neu5Gc-binding specific aptamer applied in this paper is a 70 nucleotide-long truncated and optimized version of the 82 nucleotide-long aptamer screened by SELEX in our previous experiment [40]. Additionally, it was modified with poly A and thiol at the 3′ end: 5′-TGGTCCCATGTACGCCGCGGTGTACCTCCGCGTGTACGCTACTTTCTGTGCGTGCTGA GCGTGAATTCGCAAAAAAAAAAAAAAAAAA-SH-3′.The complementary DNA to the aptamer was used as test line probe and modified with biotin at the 5′ end: 5′-Biotin-GCGAATTCACGCTCAGCACGCACAGAAAGTAGCGTACACGCGGAGGTACAC CGCGGCGTACATGGGACCA-3′. The biotinylated Oligo-dT 18 was used as control line

probe：5′-Biotin-TTTTTTTTTTTTTTTTTT-3′

They were synthesized by Shanghai Sangon Biological Engineering Technology & Services Company (Shanghai, China) and purified with PAGE.

2.2 Cancer cells culture

The cancer cells used in this study including human gastric cancer cell lines BGC-823, MGC-803 and SGC-7901, human colorectal cancer cell lines HCT-8 and SW116, and murine myeloma cells SP2/0 were all preserved in our laboratory**.**

The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco Life Technologies, Paisley,UK) supplemented with 10% fetal bovine serum (Gibco), 1% penicillin-streptomycin at 37 °C in an atmosphere of 5% CO_2. The cell suspension in logarithmic phase vaccinate in serum-free culture medium (SFM) at the 1 x 10^3/mL concentration to culture 48 h as the detected samples.

2.3 Preparation of gold nanoparticles (AuNPs)

Gold nanoparticles (AuNPs) were synthesized using the classic reported procedure[44, 45] with slight modifications. Briefly, 100 mL of 0.01% $HAuCl_4$ solution in deionized water was thoroughly boiled, and 2.2 mL of 1% (w/w) trisodium citrate solution was quickly added under vigorous stirring. The colloidal gold solution was boiled for another 6 min after the color of the mixture turned to wine-red within 3 min. Then, the reaction solution was cooled to room temperature (RT) under constant stirring and stored at 4 °C in a brown bottle. The dispersity and

diameters of the prepared AuNPs were characterized by transmission electron microscopy (TEM, H-7650) and UV/Vis spectroscopy (Shimadzu, Japan). The given diameters represent the average of at least 200 AuNPs.

2.4 Preparation of AuNP–aptamer conjugates

The AuNP–aptamer conjugates were synthesized according to the establishment of a sulfydryl–gold bond. In brief, 1 OD of thiolated aptamer was activated by adding 2 μL of triethylamine and 8.0 mg of DTT to incubate for 1 h at 37 °C. In order to remove the excess DTT, the solution was extracted three times using 100 μL of ethylacetate solution. The thiol modified aptamer solution was added to the 1 mL of 10 times concentrate AuNP suspension and shaken gently at 4 °C for 24 h. The mixture was then subjected to stabilized and "aging" process by adding 1 % sodium dodecyl sulfate (SDS) and 2 M NaCl solution respectively. The concentration of SDS and NaCl were finally brought to 0.05 M and 0.01% respectively. The mixture was incubated at 4 °C for another 24 h, and the solution was centrifuged for 15 minutes at 14000 rpm to remove the excess reagents. The red precipitate at the bottom was resuspended in the same volume of suspension buffer (20 mM Na_3PO_4, 5% BSA, 2.5% Tween-20 and 10% sucrose). The solution was confirmed by agarose gel electrophoresis and stored at 4 °C until use.

Successful conjugations of the aptamer to the AuNP were measured by dynamic light scattering (DLS) using a Zetasizer ZS (Malvern). The measurements performed for analysis were carried out three consecutive times to get reliable error bars, and the results were presented as mean values with a standard deviation.

148 *2.5 Preparation of streptavidin–biotin–DNA probe conjugates*

149 To prepare the streptavidin-biotin-DNA conjugates, a 5 µL aliquot of the streptavidin solution
150 (1mg/mL, 0.01 mM PBS, pH 7.4) and 35 µL of a 10 µM DNA probe solution were mixed and
151 incubated 2 h at 37 °C. To remove excess DNA probes, the solution was centrifuged at 6000 rpm
152 with a centrifugal filter (cutoff 30,000, Millipore) for 30 min. The streptavidin–biotin–DNA probe
153 conjugates were washed two times with the PBS solution in the same filter, and thereafter
154 dissolved in 40 µL of PBS solution for future use.

155

156 *2.6 Construction of the aptamer-based lateral flow strip*

157 A schematic description of the strip construction is shown in Figure 1. The sample application pad,
158 AuNP-aptamer conjugated pad, nitrocellulose (NC) membrane, and absorbent pad were laminated
159 2 mm with each other in sequence to ensure the migration of the solution through the strip and
160 mounted on a plastic back plate with a 4 mm width. The sample application pad was saturated
161 with buffer (0.05 M Tris-HCl, 0.25% [v/v] Triton X-100, 5% [v/v] Tween-20 and 0.15 M NaCl,
162 pH 8.0) and dried for future use. The streptavidin–biotin-DNA control probe and test probe
163 conjugates were sprayed onto the NC membrane with the BioDot XYZ 3060 dispenser as the
164 control zone and test zone, respectively. The DNA probe-loaded membrane were dried at RT for
165 10 min. The AuNP–aptamer probes (15 µL/strip) were dispensed on the glass fiber membrane and
166 vacuum-dried at RT for 1 h. The whole assembled strips were sealed in plastic bag and stored at
167 4 °C until use.

168

2.7 Immunochromatographic assay of Neu5Gc

The detection of Neu5Gc was carried out by applying 100 µL of the Neu5Gc standard substrate solution with different concentrations (0, 1, 5, 10, 15, 20, 25, 30 and 35 ng/mL) to the sample application pad. After 15 min, the results were observed by eye or measured by a BioDot TSR3000 Membrane Strip reader to determine the red intensity of the test line (Gene Company Limited, Shanghai, China).

2.8 Detection of Neu5Gc in cancer cells

The cancer cells were first hydrolyzed using the method described by other reports[21, 46]. With minor modifications. Briefly, samples were treated with 0.1 M NaOH to remove O-acetyl and hydrolyzed with 2.5 mL of H_2SO_4 0.05 M, heated for 1 h at 80 °C and then cooled to room temperature. To predicate the sample, the hydrolyzed sample was centrifuged and the supernatant was collected. This was followed by filtering the supernatant with a Millipore Ultra Filter. Analysis of the spiked cells samples were also carried out using the test strip. The standard Neu5Gc was dissolved in the cells sample solution at the final concentrations of 10, 20, 30 and 40 ng/mL, respectively.

3. Results and Discussion

3.1 Principle of the aptamer-based lateral flow strip

The 70 nucleotide-long truncated and optimized version aptamer was also showing the

corresponding high specificity and affinity to Neu5Gc with the 82 nucleotide-long aptamer that selected in our previous research using the SELEX method[40]. The protocol of the aptamer-nanoparticle lateral flow test device is schematically illustrated in Figure 1. The principle of the test strip designed in the present study is based upon a competitive hybridization reaction between the DNA test probe and the analyte Neu5Gc with the colloidal gold-labeled aptamers. The sulfhydrylated aptamer was immobilized on the AuNP surface via an Au-S bond, and the AuNP-aptamer conjugates were dispensed on the conjugate pad. In the presence of Neu5Gc in sample solution, the solution migrates along the strip by capillary action, allowing Neu5Gc to combine with the aptamers on the AuNP-aptamers conjugates, which decreases the number of available AuNP-aptamers that can hybridize to the DNA test probe, producing a characteristically weaker colorimetric signal in the test region. Thus, the less analyte Neu5Gc in the sample, the stronger the colorimetric signal in the test region. Regardless of the existence of analyte Neu5Gc in the sample, the AuNP-aptamers conjugates will be captured by hybridization with the DNA control probe immobilized in the control line by T-A complementary combination, showing that the lateral flow strip is working accurately. The DNA test probe and the DNA control probe are complementary to different fragments on the aptamer. Qualitative analysis is achieved by inspecting the colorimetric color change of the test region, while quantitative analysis is performed by measuring the optical intensity of the test region using a scanning reader.

Insert Figure 1

Figure 1. Schematic diagram of the detection of Neu5Gc using the aptamer-based lateral flow strip. The control line and test line are coated with a streptavidin-biotinylated control probe and a streptavidin-biotinylated test probe. The AuNP-aptamer conjugates are applied to the conjugate pad. The sample is loaded onto the sample pad for qualitative and quantitative analysis of Neu5Gc.

3.2 Characterization of AuNPs and AuNP-aptamer conjugates

In this study, we used gold nanoparticles as a probe for the lateral flow strip due to their size tailorability, range of applications and monodispersity in the aqueous phase. The AuNPs were characterized by TEM to determine their size and distribution. The average diameter of the AuNPs was found to be approximately 20 nm, with a desirably narrow size distribution (Figure 2A). The absorption spectrum of the AuNPs was also measured using UV-Vis spectroscopy from 350 to 800 nm. The maximum absorbance wavelength observed at 520 nm is characteristic of AuNPs (Figure 2B). The AuNP-aptamer conjugates were also scanned by UV-Vis spectroscopy, which revealed a red shift in the maximum absorbance wavelength compared to the AuNP solution.

We further determined the optimal aptamer concentrations during the conjugation process. As shown in Figure 3C, the hydrodynamic diameter (dh) of the AuNP-aptamers complexes increased as the concentration of aptamer increased and finally reached a plateau of approximately 90 nm. This is probably due to the accumulation of aptamer on the gold surface. When there had a low surface coverage, the thiol-modified aptamer would lay flat on the gold surface as a result of non-specific binding via the long nitrogen electron pairs of the nucleotides. With the increasing of the aptamer concentrations, these aptamers are gradually forming a perpendicularity modality on the gold surface because of electrostatic repulsion between the aptamers' negatively charged phosphate backbones, resulting in a dh increase (Figure 2C). Using a base-to-base distance for ssDNA of 0.43 nm, we calculated that the length of the aptamer was 42.57 nm and the diameter of the aptamer-AuNP conjugate was approximately 106 nm. This result is close to the tested plateau

234 in the dh. We assume the decrease of the diameter is due to that the aptamers form the secondary
235 structure. The sulfhydrylated aptamers show a preferable affinity toward the Au surface, which
236 then lead to stabilization of the nascent nanoparticles satisfactorily.

237

238 **Insert Figure 2**

239 **Figure 2.** Characterization of AuNPs and AuNP-aptamer conjugates. (A) TEM image of AuNPs.
240 (B) UV-Vis spectrum of the AuNP solution. (C) The hydrodynamic diameter (dh) change of the
241 AuNP-aptamers complexes.

242

243 *3.3 Optimization of the lateral flow strip construction and assay parameters*

244 During the process of developing the lateral flow strip method, many elements must be considered
245 for increasing the sensitivity of the strip. The flow of the sample solution along the NC membrane
246 affects the reaction time of the assay. Four types of nitrocellulose membranes, including Sartorius
247 CN140, Millipore 180, Whatman Immunopore FP and Purabind, were used to construct the strip.
248 The Sartorius CN140 nitrocellulose membrane had a relatively higher response intensity and
249 shorter migration time and thus was used to construct the strip.

250 The amount of AuNP-aptamers dispensed onto the conjugate pad can affect the captured
251 AuNP-aptamer conjugates on the test zone and control zone, thus influencing the final intensities
252 of the colorimetric signals. Therefore, we optimized the volume of AuNP-aptamer conjugates
253 loaded onto the pad in order to achieve a maximum signal with a minimal quantity of
254 AuNP-aptamers conjugates; 15 μL of AuNP-aptamer conjugates were used for the assay due to
255 the minor nonspecific adsorption and background signal observed at this volume.

Another vital element that influences the specificity and reliability of the assay is the various buffer solutions. The nonspecific adsorption of the AuNP-aptamers may result in high background and low sensitivity, which could be minimized by using appropriate buffers. Throughout the process of preparing the AuNP-aptamer conjugates, the AuNP-aptamer conjugate pellets were dispersed in a buffer containing 20 mM Na_3PO_4, 5 % BSA, 2.5 % Tween-20 and 10 % sucrose. The utilization of these ingredients (sucrose, BSA and Tween-20) could not only stabilize the AuNPs and facilitate the release of the AuNP-oligonucleotide conjugates from the conjugate pad, but also reduce the non-specific adsorption of the AuNP-oligonucleotide conjugates to the NC membrane during the assay. After the strips being dipped into the sample solution, the AuNP-aptamer conjugates on the conjugate pad are rehydrated. The ingredients (sucrose, BSA and Tween-20) dispersed in the sample solution could block the NC membrane without other blocking procedures when migrating along the strip.

3.4 Sensitivity and stability of the strip for Neu5Gc detection

Under the optimized experimental conditions described above, Neu5Gc standard solutions at different concentrations ranging from 0 ng/mL to 35 ng/mL were analyzed by the strip (Figure 3A). With an increase in target Neu5Gc amount, the color density of the test zone decreased. The visual detection limit of 10 ng/mL was estimated due to the red color density of the test zone was visually weaker than the control zone. Furthermore, quantitative detection was performed by a scanning reader. The relationship between the Neu5Gc standard substrate concentration and the related peak area of relative optical density (Peak-ROD), shown in Figure 3B, demonstrates that

277 the Peak-ROD of the test zone decreased with increasing analyte concentration. The Peak-ROD
278 reduced eminently while the concentration of Neu5Gc increased from 1 ng/mL to 30 ng/mL. Up to
279 a concentration of 30 ng/mL, the decrease in Peak-ROD was small. However, the Peak-ROD
280 versus the concentrations of Neu5Gc are linear over the 5 to 30 ng/mL rang and satisfactory for
281 quantitative study (Figure 3C).

282 The LOD of the quantitative assay was defined as the concentration of Neu5Gc that induced a
283 10 % decrease in the Peak-ROD compared with a blank solution. Based on the resulting
284 calibration plot in Figure 4B and Figure 4C, the calculated LOD of the quantitative detection was
285 5.38 ng/mL. And the LOD of semi-quantitative estimate of the test strip was 30 ng/mL without
286 instrumentation. Although the LOD of the fabricated lateral flow test strip is not better than that of
287 ELONA (Enzyme Linked Oligonucleotide Assay) (paper not published) and other spectrometric
288 analysis methods[26, 27, 46]. It is more convenient and rapid, making it suitable for routine
289 detection.

290 The repeatability of the strip was studied by measuring a series of Neu5Gc concentrations (0
291 ng/mL, 10 ng/mL, 30 ng/mL). Samples at the same concentration were measured 5 times with 5
292 different aptamer-based lateral flow strips from the same batch preparation. We assumed that
293 analogous results were achieved at the same concentrations. The coefficients of variation (CV) of
294 the signals at 0 ng/mL, 10 ng/mL and 30 ng/mL were 4.1%, 5.8% and 5.3%, respectively (n=5).
295 The overall relative standard deviation (RSD) was no more than 6%, demonstrating that the
296 developed strip was suitable for determining the concentration of Neu5Gc.

297 We also evaluated the stability of the assay by testing the same batch of strips with Neu5Gc at

298 concentration of 10 ng/mL. The strips were sealed and stored at 4°C for 1, 2, 4, 8 and 16 weeks.
299 The analysis results suggested nearly no disparity in visual detection of the test line for the 1-, 2-,
300 4- and 8-week strips. However, the intensity of the test line for the 16-week strip was slightly
301 weaker than the intensity of the 1-week strip. With a portable strip reader, we got a coefficient of
302 variation for the detection of less than 10 %, indicating that the aptamer-nanoparticle
303 immunochromatographic strips display good stability.

304

305 **Insert Figure 3**

306 **Figure 3**. (A) Photographic images of the aptamer-based lateral flow strip for a series of Neu5Gc
307 concentrations. The assay was carried out using 50 µL of standard Neu5Gc solution with equal
308 volumes of running buffer in each well. Neu5Gc at 0, 1, 5, 10, 15, 20, 25, 30 and 35 ng/mL was
309 used as a standard. (B) The detection curve of Neu5Gc using the aptamer-based lateral flow strip
310 by the scanning reader. Assay time, 10 min. (C) Change in absorption signal at Neu5Gc
311 concentrations ranging from 5 ng/mL to 30 ng/mL. The red line represents a linear fit to the data.
312 Each sample was analyzed three times, and the error bars represent the standard deviations.

313

314 *3.5 Specificity*

315 The obtained aptamer by our previous study showed relatively high specificity and affinity for
316 Neu5Gc. The developed aptamer-nanoparticle test strip in theory should have favorable specificity.
317 However, we substituted N-acetylneuraminic acid (Neu5Ac) and Ketodeoxynonulosonic acid
318 (KDN) for Neu5Gc in the samples to tested the selectivity. Three different concentrations, 5
319 ng/mL, 10 ng/mL and 30 ng/mL, of Neu5Ac and KDN were detected. We found that the strip
320 could differentiate between low and high Neu5Gc concentrations without any fake
321 positive/negative outcomes. Additionally, the assay did not provide false positives in the presence

of the non-Neu5Gc solutions, indicating its good specificity. From the above assay outcomes for the Neu5Gc and non-Neu5Gc solutions, we concluded that the fabricated test strips displayed low cross-reactions with other sialic acids.

3.6 Sample analysis

To evaluate the potential applicability and veracity of the lateral flow strip approach, Neu5Gc was detected from cancer cells samples. Simulated samples were prepared by spiking known amounts of Neu5Gc into cells samples that did not contain Neu5Gc according to our assay method. We employed the average recoveries of the measurements as the symbol for the assay. As shown in Table 1, the average recovery of the strip was 88.86%, with a coefficient of variation (CV) of 5.27%, showing good detection capability. The cancer cells surface normally show aberrant glycosylation and accumulation of this molecule might induce oncogenesis[47, 48]. So it is necessary to analyze the level of Neu5Gc in the cancer cells and this might provide a hint for the carcinogenesis.

Insert Table 1

Note:
Y represents ‘‘yes’’ when the test line developed a red color at the T region; N represents ‘‘no’’ when no test line was developed at the T region; minus sign (–) represents a negative result when the color density of the test line was the same as that of the control line; plus/minus sign (±) represents a weakly positive result when the color density of the test line was lighter than that of the control line; plus sign (+) represents a positive result when no test line was developed at the T region.

[a] Results are expressed as the mean±SD (n=4), SD=standard deviation.

[b] The coefficient of variation is defined as the ratio of the standard deviation to the mean.

[c] The result is out of the range for detection.

4. Conclusions

Here we successfully established an aptamer-nanoparticle immunochromatographic strip biosensor for both qualitative and quantitative Neu5Gc detection under the optimized conditions. The limit of the qualitative detection was as low as 5.38 ng/mL with a strip reader, meanwhile the LOD of semi-quantitative estimate of the test strip was 30 ng/mL without instrumentation, Although this aptamer-nanoparticle strip assay is not exactly accurate and also showed a higher LOD than traditional antibody-based ELISA and other methods, it does not require any instruments or skilled analysts and can be completed in a short time. To sum up, the developed aptamer-based immunochromatographic strip might provide an alternative technique for simple, rapid and sensitive screening of Neu5Gc in the batch detection and displays impressive promise for point-of-care relative carcinoma diagnosis and treatment.

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Conflict of interests

All the authors listed here declare that we have no conflict of interest.

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Table 1. Detection of spiked cells using the strip

Spiked cells	Spiked concentration (ng/mL)	Showing test line/visual results	Detected concentration (ng/mL)[a]	Recovery (%)	Coefficient of variation (CV, %)[b]
BGC-823	0	Y/-	---[c]	---[c]	---[c]
	10	Y/±	9.52±0.38	95.20	3.99
	20	Y/±	19.36±0.59	96.8	3.05
	30	N/+	26.40±0.91	88.00	3..45
	40	N/+	---[c]	---[c]	---[c]
SGC-7901	0	Y/-	---[c]	---[c]	---[c]
	10	Y/±	8.95±0.56	89.53	6.26
	20	Y/±	18.99±1.84	94.93	9.69
	30	N/+	26.00±1.27	86.66	4.88
	40	N/+	---[c]	---[c]	---[c]
HCT-8	0	Y/-	---[c]	---[c]	---[c]
	10	Y/±	8.81±0.28	88.08	3.18
	20	Y/±	18.39±0.74	91.96	4.02
	30	N/+	26.90±1.96	89.68	7.29
	40	N/+	---[c]	---[c]	---[c]
MGC-803	0	Y/-	---[c]	---[c]	---[c]
	10	Y/±	8.29±0.35	82.90	4.22
	20	Y/±	17.51±0.86	87.54	4.91
	30	N/+	25.31±1.10	84.38	4.35

	40	N/+	---[c]	---[c]	---[c]
	0	Y/-	---[c]	---[c]	---[c]
SW116	10	Y/±	8.29±0.62	82.90	7.48
	20	Y/±	18.00±0.53	90.00	2.94
	30	N/+	25.32±1.91	84.39	7.54
	40	N/+	---[c]	---[c]	---[c]

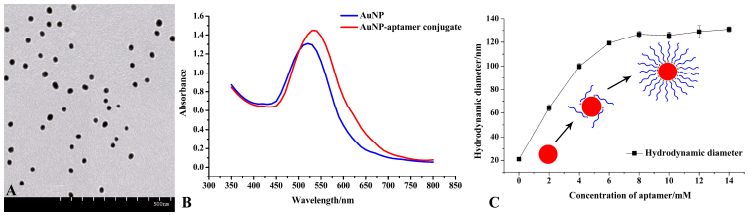
Note:

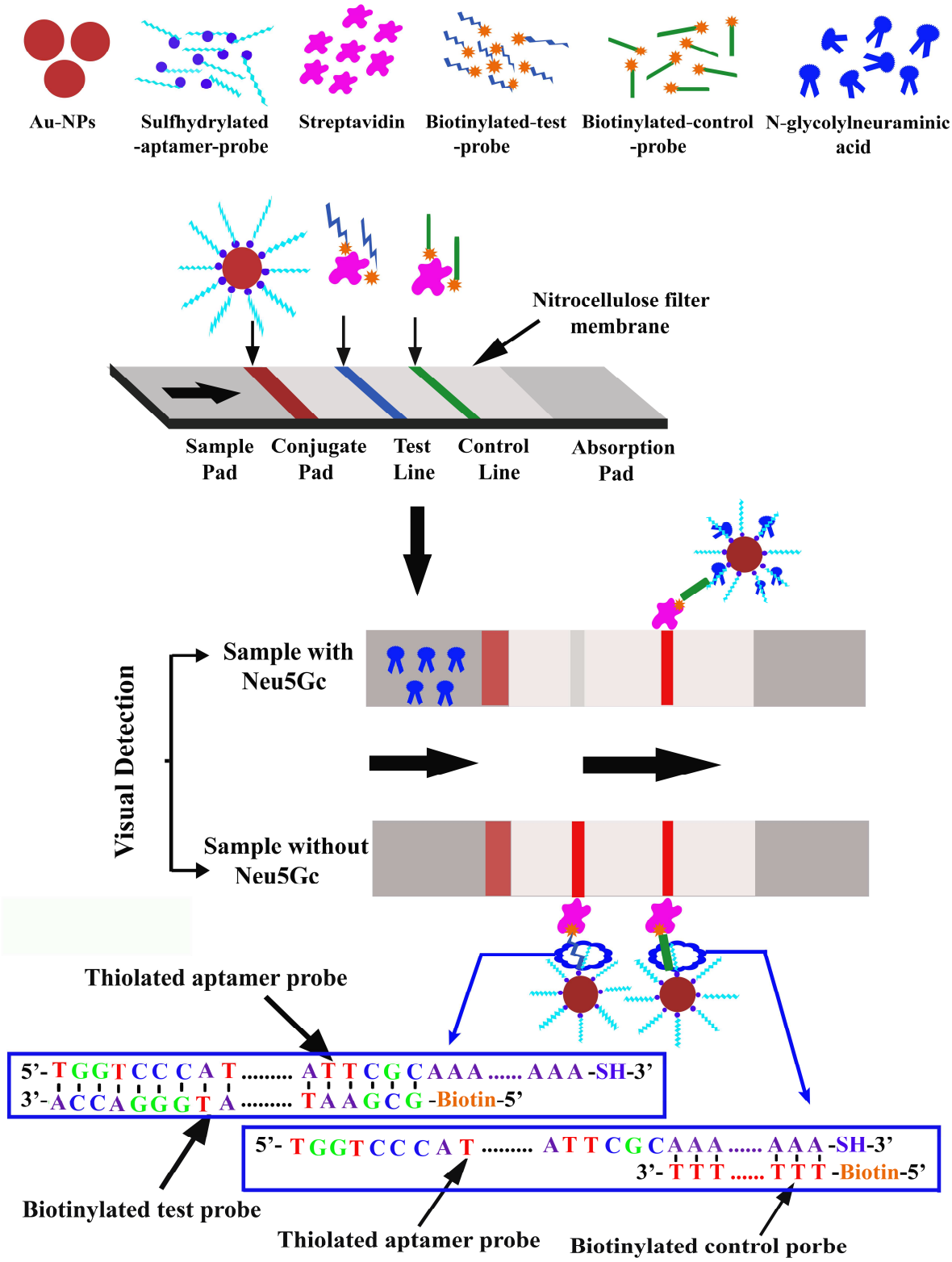
Y represents ‘‘yes’’ when the test line developed a red color at the T region; N represents ‘‘no’’ when no test line was developed at the T region; minus sign (–) represents a negative result when the color density of the test line was the same as that of the control line; plus/minus sign (±) represents a weakly positive result when the color density of the test line was lighter than that of the control line; plus sign (+) represents a positive result when no test line was developed at the T region.

a Results are expressed as the mean±SD (n=4), SD=standard deviation.

bThe coefficient of variation is defined as the ratio of the standard deviation to the mean.

c The result is out of the range for detection.




Au-NPs
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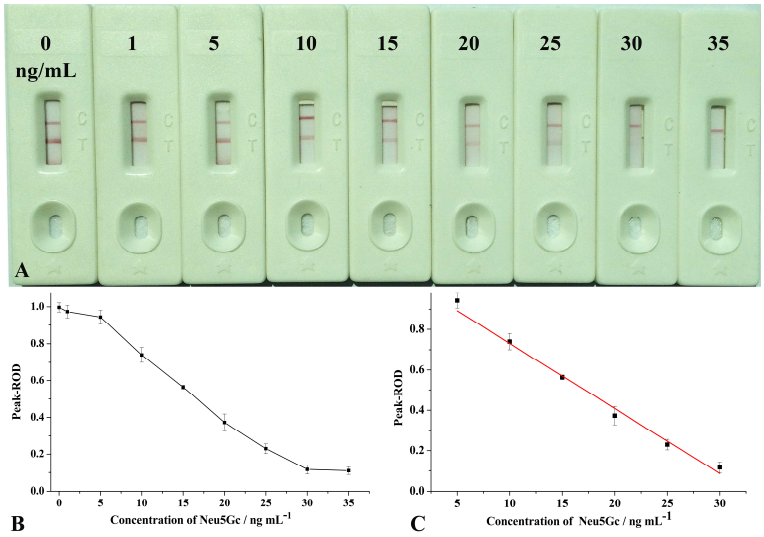
Streptavidin
Biotinylated-test -probe
Biotinylated-control -probe
N-glycolylneuraminic acid
Nitrocellulose filter membrane
Sample Pad
Conjugate Pad
Test Line
Control Line
Absorption Pad
Visual Detection
Sample with Neu5Gc
Sample without Neu5Gc
Thiolated aptamer probe
5'-TGGTCCCAT ..... ATTCGCAAA ..... AAA-SH-3'
3'-ACCAGGGTA ..... TAAGCG-Biotin-5'
5'- TGGTCCCAT ..... ATTCGCAAA ..... AAA-SH-3'
3'- TTT ..... TTT-Biotin-5'
Biotinylated test probe
Thiolated aptamer probe
Biotinylated control porbe



Research Highlights

The simple, rapid and sensitive aptamer-based immunochromatographic strip was developed to analyze the new cancer biomarker of Neu5Gc.

Both qualitative and quantitative Neu5Gc detection are achieved.

The visual limit of detection of the strip is 30 ng/mL and the quantitative limit of detection is 5.38 ng/mL.

Some relative cancer cell samples spiked different levels of Neu5Gc were studied using the developed strip.

The method displays impressive promise for point-of-care relative carcinoma diagnosis in order to further better response to therapeutic schedule.