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Efficient Cancer Cell Capturing SiNWAs Prepared via Surface-Initiated SET-LRP and Click Chemistry

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Circulating tumor cells (CTCs) are existing in the blood of patients with an extraordinarily low number, thus discovering a more effective, economical and specific way to capture tumor cells is essential and still remains a tremendous challenge. In this work, glycopolymers poly (N-acryloyl glucosamine) (PAGA) and TD05 aptamers were combined together on silicon nanowire arrays (SiNWAs) to capture Ramos cells through SET-LRP and click chemistry for the first time. The polymerizations showed controllable living features using 2-hydroxyethyl α-bromoisobutyrate (HEBiB) as sacrifice initiator. In serum-containing environment, PAGA-modified surfaces could catch small amount of Ramos cells. Furthermore, the number of captured specific Ramos cells increased extensively compared with control after introducing the aptamer molecule TD05 onto the PAGA-modified surface. Few of non-specific Baf3 cells were captured to the surfaces prepared. The results revealed synergistic effect generated by combining glycopolymer and aptamer that could achieve a multivalency-enhanced effective and specific cancer cells capturing, suggesting to be a promising approach for cancer detection.

Introduction

Cancer involves abnormal cell growth with the potential to invade or spread to other parts of the body. Circulating tumor cells (CTCs) are existing in the blood of patients with solid tumors, which shed from the primary tumor into the bloodstream, travelled to different tissues, and their detection could be vital in prognosis, diagnosis and treatment of cancer. CTCs have an extraordinarily low number, approximately as few as one in 10⁹ blood cells, thus discovering a more effective, economical and specific way to capture tumor cells is essential and still remains a tremendous challenge. Many functional molecules, such as antibodies, peptides, DNA, have been shown to specifically recognize cancer cells. Among them, aptamers, a form of single-stranded oligonucleotides (ssDNA), can recognize their targets, specifically live cancer cells, and bind to them with high affinity in complicated biological environments. Aptamers are stable in the blood stream with low toxicity, and have been applied in exploiting as exceptional targeting elements for cancer cells. More specifically, silicon nanowire arrays (SiNWAs) with unique micro/nanostructures and excellent biocompatibility have shown to be effective in cell-capturing, especially after modified with aptamers. Though with so many advantages, aptamers are relatively expensive and not easy to synthesize in large scale, which limit their applications to fabricate devices functionalized with only aptamers.

Carbohydrates play a vital role in biological processes such as cell signalling and cell-cell recognition. Synthetic polymers with pendant sugar moieties, typically termed “glycopolymers”, are easy to synthesize in large scale and able to interact with specific proteins as multivalent ligands. Researches have shown that the membrane protein glucose transporters (GLUTs), especially GLUT1 or GLUT3, are over-expressed in many cancer cells, and possess high affinity toward glucose and glucosamine. It was found that glycopolymers with pendant glucosamine moieties presented high binding ability toward GLUT-over expressing cancer cells. Bearing these in mind, we envisaged to develop an approach that can capture cancer cells in a more effective, economical and specific fashion by using glycopolymers with the assistance of small amount of aptamers.

To fabricate the device, SiNWAs were chosen as our base material, due to the high surface areas, good biocompatibility and unique micro/nanostructures. Single electron transfer-living radical polymerization (SET-LRP) has been demonstrated to be an excellent method for the synthesis of functional polymers due to its distinct advantages such as low reaction temperatures, low concentration of catalyst and fast polymerization rates. We have recently reported the surface-initiated SET-LRP in aqueous media on flat silicon surface. Herein for the first time, poly(N-acryloyl glucosamine) (PAGA) was introduced into the SiNWAs through SET-LRP and the TD05 aptamers were further attached via click chemistry. Therefore, by using the robust synthetic method of SET-LRP and click chemistry, we fabricated multivalency-enhanced specific cancer cells capturing SiNWAs with the synergistic effect generated by combining the
glycopolymers and aptamers together.

**Experimental Section**

**Materials**

Silicon wafers [p-doped, (100)-oriented, 0.45 mm thick and 100 mm in diameter] were purchased from Guangzhou Semiconductor Materials Laboratory (Guangzhou, China), cut into square chips of 0.5 cm × 0.5 cm. D- (+)-glucosamine hydrochloride (TCI), acryloyl chloride (96%, aladdin), 3-aminopropyltriethoxysilane (APTES) (98%, Sigma-Aldrich), α-bromoisobutyl bromide (BIBB) (98%, Sigma-Aldrich), tris(2-dimethylamino) ethyl) amine (Me3TREN) (Sigma-Aldrich), 2-hydroxyethyl α-bromoisobutyrate (HEBiB) (Sigma-Aldrich) and L-(+)-sodium ascorbate (J&K) were used as received. Copper (II) sulfate (CuSO4·5H2O) was purchased from Shanghai Chemical Reagent Co., LTD. (Shanghai, China) and used without further treatment. Copper (I) bromide (CuBr) (98%, Fluka). Fluaka was purified by stirring in acetic acid, washed with methanol and dried in vacuum. Toluene, triethylamine and all the other solvents were purchased from Shanghai Chemical Reagent Co., LTD. and purified according to standard procedures before being used. Deionized water used in all experiments with a minimum resistivity of 18.2 MΩ cm was purified by a Millipore water purification system. Nitrogen gas was of high-purity grade. N-acryloyl glucosamine (AGA) was prepared as previously reported.35-36. TD05 aptamer (APT) with highly specific affinity to Ramos cells, purchased from Shanghai Sangon Biotechnology Co., LTD. (Shanghai, China). Its sequence is: 5'-CAC CGG GAG GAT AGT TCG GTG GTT CAG GGT CTC CCG GTG TTT TT(3')=5'-CAC CGG GAG GAT AGT TCG GTG GTT CAG GGT CTC CCG GTG TTT TT-C3'. Ramos cells (CL1012, B-cell, human Burkitt’s lymphoma) were from Abgent Biotechnology Co., Ltd.

**Preparation of aptamer-modified SiNWAs via SET-LRP and click reactions**

Silicon nanowire arrays (SiNWAs) were fabricated as described in our previous work by the chemical etching method.31 Cleaned silicon wafers were immersed in AgNO3 (0.015 M)/HF (5 M) aqueous solution at 50 °C for 10 min. Then, the chips were immersed in 20 % nitric acid, and rinsed with excessive deionized water subsequently. The synthesis of initiator-immobilized SiNWAs was performed using a similar way to the preparation of bromide-modified silicon wafers reported previously.40 Surface-initiated SET-LRP of AGA was proceeded in a glove box at room temperature. First, AGA monomers (2.01 mmol) was dissolved in 8 mL mixture of N,N-dimethyl formamide/water (3/1). Second, 10 μL of Me3TREN (0.0037 mmol) was added and was stirred under nitrogen for 30 min. Third, the mixture was transferred into a round-bottom flask containing CuBr (5.3 mg, 0.0037 mmol) in a glove box. Finally, initiator-functionalized SiNWAs were immersed in the fresh prepared solution mentioned above to form PAGA grafted SiNWAs (SN-PAGA). Polymerization was conducted at room temperature in nitrogen atmosphere.

Afterward, sodium azide (0.065g, 1 mmol) was dissolved in 6 mL of wet DMF and initiator-functionalized SiNWAs or PAGA grafted SiNWAs were added, standing at 70 °C overnight, fabricated the azide end-functional SiNWAs. Aptamer was introduced onto the surface by copper-catalysed azide-alkyne cycloaddition (CuAAC) to prepare SN-PAGA-APT surface. Briefly, aptamer was dissolved in phosphate buffer solution (PBS, pH = 7.4), followed by the addition of azide end-functional SiNWAs into the solution with aptamer at a concentration of 5 μM, CuSO4·5H2O and sodium ascorbate. This click reaction was processed at room temperature. The general process for SN-PAGA-APT is illustrated in Scheme 1.

To investigate the kinetic and living properties of the surface-initiated SET-LRP of AGA, HEBiB (0.016 mmol) was added into the solutions as sacrifice initiator.

**Polymer characterization**

1H NMR spectrum of glycopolymers was obtained with Agilent 400 MHz NMR equipment using DMSO-d6 as solvent. Molecular weights and molecular weight distributions of polymers were acquired by a Waters 1515 gel permeation chromatography (GPC) system equipped with a PL aquagel-OH MIXED-M column using PEG as the standard samples. Millipore water containing 70 % 0.2 M NaNO3, 0.1 M NaH2PO4 with a pH of 7 and 30 % methanol has been used as the mobile phase and delivered at a flow rate of 1 mL/min.

**Surface characterization**

X-ray photoelectron spectroscopy (XPS, VG Scientific Ltd.) was used for determining the chemical composition of the modified SiNWAs. The surface topography of the unmodified SiNWAs was characterized by scanning electron microscopy (SEM, Hitachi S-4700). Single silicon nanowire (SN and SN-PAGA) was studied by transmission electron microscopy (TEM, JEOL JEM-2100) running at 200 kV. Static water contact angle tests were performed with the sessile drop method by a SL200C optical contact angle meter (USA Kino Industry Co., Ltd.) at room temperature. Fourier transform infrared (FT-IR) spectra of SiNWAs at different modification steps were recorded with a Nicolet 6700 FTIR spectrometer fitted with attenuated total reflection (ATR) with wavelengths ranging from 500-4000 cm⁻¹ for 128 scans. The background spectra were carried out in air. The copper content on the SiNWAs was measured on a Varian 220FS Atomic Absorption Spectrometer (AAS) System.

**Cell culture and capture assay**

Ramos and BaF3 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified atmosphere with 5% CO2 at 37 °C. The media for BaF3 cells contains IL-3 as well. Samples were treated with 75% ethanol for 20 min and rinsed with sterile ultrapure water three times. Cells were centrifuged, washed with sterile PBS (pH = 7.4) and collected by centrifugation. The samples were placed in 48-well plate (Costar) and cells with a density of 5 × 10⁶ cells/well were injected to each well containing 10% FBS, followed by incubation at 4°C for 1.5 h. For cell capture assay, all the samples were rinsed with PBS three times to remove unattached cells, fixed with 4% paraformaldehyde for 10 min and stained with 0.5 μg/mL DAPI. Cells captured by samples were determined by fluorescence microscopy (BX51, Olympus). The cell density was analysed using Image-Pro Plus 6.0 software.
Results and Discussion

**SiNWAs surface-initiated polymerization in aqueous media**

Single electron transfer-living radical polymerization (SET-LRP) is a robust technique for polymer synthesis. In our previous work, we had proved that polymer brushes could be prepared quickly by surface-initiated SET-LRP. In the present research, the glycomonomer, poly (N-acryloyl glucosamine) (PAGA), was introduced onto the SiNWAs surface by using SET-LRP as shown in Scheme 1. Firstly, a homogeneous, dense monolayer of initiator was immobilized on SiNWAs surface. Then, surface-initiated SET-LRP of AGA was carried out by immersing the initiator-modified SiNWAs in the reaction medium for required time at ambient temperature. In order to get more information about the polymerization kinetics on silicon surface, silicon wafer was first used to investigate the surface initiated SET-LRP. As shown in Figure S2, the thickness of polymer brush on the surface increased almost linearly with the polymerization time, demonstrating the fine controllability of the surface-initiated polymerization. Meanwhile, kinetic properties of polymerization on SiNWAs had been investigated by the introduction of the sacrifice initiator-HEBiB during the reactions. As shown in Figure S3, with the increase of polymerization time, the integration of vinyl groups in monomer-AGA gradually decreased. Using 1, 3, 5-trioxane (ppm = 7.81) as the standard sample, the conversion of the polymerization can be calculated and it reached about 47% at 5 h with a molecular weight of 13000 and PDI of 1.26 as illustrated in Figure 1a and 1b. During the reaction, glycopolymers with controlled molecular weight and narrow polydispersity (PDI < 1.3) were obtained (Figure 1a and 1b), confirming the controllable features using sacrifice initiator in this surface-initiated polymerizations. We then chose the samples at a polymerization time of 4 h for further investigation.

Post-modification is one of the common methods to obtain...
functional terminal polymers. Click chemistry has been proved as a convenient and convicive tool to introduce new functional groups to the end of polymer chains. Herein as demonstrated in Scheme 1, TD05 aptamer which has a strong affinity to Ramos cells was further immobilized to the chain-end of glycopolymer brush on the SiNWAs segments by Cu-catalyzed alkyne-azide cycloaddition (CuAAC).

![Graph](image1.png)

Figure 1. (a) Kinetic plots of LRP using HEBiB during the surface-initiated polymerizations. (b) Molecular weights and PDIs at different times.

**Preparation and modification of SiNWAs**

The SiNWAs were prepared by chemical etching method, and scanning electron microscopy (SEM) was applied to characterize the topography of SiNWAs. Figure 2a indicated that SiNWAs with a length of ~10 μm and diameter of 55 nm were obtained. After the surface-initiated LRP of AGA, single silicon nanowires became thicker with the observation of a modified layer with ~7 nm (Shown in Figure 2c measured by high-resolution TEM). Furthermore, a spectroscopic ellipsometer (M-88, J.A. Woollam Co., Inc.) was employed to detect the variation of thickness on flat silicon wafers after grafting PAGA. The data revealed that after modification by PAGA, the thickness increased by 14 nm. From these results, it can be inferred that the covered layer on single silicon nanowires measured by TEM after surface-initiated LRP of AGA should be a layer of PAGA. After end-group modification with aptamer, there was no obvious change of single silicon nanowires (data not show), possibly due to the “grafting to” method and the low amount of aptamer used. For TEM measurement, the small amount of aptamer grafting was in a collapsed state on the surface, which could not make significant variations in SiNWAs thickness.

![Graph](image2.png)

Figure 2. SEM (a) and TEM (b) (c) characterization of SiNWAs. (a) Top and side (inset) view image of SiNWAs. (b) Single silicon nanowire after surface-initiated PAGA. (c) High-resolution TEM image of PAGA-modified single silicon nanowire.

**XPS Spectrum**

In order to prove whether PAGA and aptamer have been immobilized on SiNWAs surface successfully, XPS, WCA and FTIR were further performed. As shown in Figure 3a, XPS revealed the changes in chemical composition of each modification step of SiNWAs. For SN-OH surface, silicon, carbon, oxygen, and nitrogen can be detected. Because the substrate is consisting of silicon and the surface was covered by a large number of hydroxyl group, the contents of silicon and oxygen were at a higher level. Small amount of nitrogen element was detected due to adsorption from the environment. After grafting the initiator onto the surface through amidation reaction, the appearance of bromide indicated that the immobilization of Br-initiator was successful. Figure S4 shows the high-resolution spectra of the Br 3d regions on SN-Br surface, the peak at 70.2 eV represents the Br 3d region (3d refers to the electron on the 3d orbit of Br). After the surface-initiated LRP of AGA, the content of bromide became less in contrast with that of SN-Br surface (Figure 3a SN-PAGA), due to polymers grafted onto the surface.
Meanwhile, the content of silicon had a sharp decrease, indicating the surface was covered by polymers. It should be noted that C/N/O atomic ratio determined by XPS (47.7/6.3/43.4) was very close to the theoretical value of PAGA, suggesting that the surface was completely covered by PAGA layer (SN-PAGA). When the end group of PAGA modified SiNWAs (SN-PAGA) was converted to azide group, the increased content of nitrogen and the disappearance of bromide demonstrated the success of azide modification, with almost full conversion. As a single-stranded DNA, aptamer has high content of phosphate and hydroxyl, when the end of PAGA chains were modified with aptamer through click reaction, the appearance of phosphorus and the increased content of oxygen confirmed the successful immobilization. Figure 3b shows the high-resolution XPS spectra of the SN-PAGA-APT surface, with the peak at 133.1 eV representing the P 2p region (2p refers to the electron on the 2p orbit of P).

Water contact angle and ATR-FTIR spectrum

The wettability of modified SiNWAs were further characterized by static water contact angle and ATR-FTIR (Figure 4). From Figure 4a, the SiNWAs surface became super-hydrophilic and its water contact angle was less than 2° after being treated with Piranha. When the surface was silanized and amidated by -Br, the surface became hydrophobic, with a water contact angle of about 115°. And the ATR-FTIR results of the SN-Br surface (Figure 4b) showed that there was an obvious peak at 1535 cm⁻¹ and 1640 cm⁻¹, representing the N-H bending vibration and C=O stretching vibration, respectively. The tremendous changes of water contact angle and the appeared characteristic peaks in ATR-FTIR fully explained that -Br was successfully grafted onto SiNWAs surface by amidation. Then, we grafted AGA onto the surface through the surface-initiated polymerization by SET-LRP, and the surface became hydrophilic (WCA ≈ 2°) again. The peaks at 1535 cm⁻¹ and at 1640 cm⁻¹ became significantly larger, and a clear peak appeared at 3203 cm⁻¹ indicating the existence of -OH from glycopolymers. These results demonstrated that SiNWAs surface was covered with PAGA. For modifications with azide and aptamer, the FTIR result (Figure 4b) proved the success of azide modification by the appearance of peak at 2108 cm⁻¹ from -N₃.

![Figure 3](image-url) Atomic concentrations of each functionalization process on SiNWAs. (a) XPS atomic concentration. (b) High-resolution spectra of the P 2p regions on SN-PAGA-APT surface.

![Figure 4](image-url) (a) Water contact angles and (b) ATR-FTIR spectra of SiNWAs after each modification step.
After aptamer was introduced into the surface by click chemistry, the surface became super-hydrophilic and its water contact angle is about 0°, which indicated that phosphate, hydroxyl, and other hydrophilic groups of aptamer made the surface super-hydrophilic after the modification. FTIR (Figure 4b) showed that the peak at 3203 cm⁻¹ became significantly larger, and the new peak at 1100 cm⁻¹ indicated the existence of -PO₃, which is consistent with the structure of aptamer. The azide peak decreased obviously, however didn’t disappear, because the amount of aptamer used is less than the N₃ functional groups on the surface. The same phenomenon has been observed for the conversion from SN-N₃ to SN-APT, as shown in Figure S6b. Furthermore, there is no detectable remaining aptamer left in the reaction solution after click reaction via UV-Vis, indicating an almost full conversion of aptamer. The results proved that aptamer was grafted at the chain-end of glycopolymer. All the results mentioned above strongly confirmed that we have successfully prepared an aptamer-terminated-PAGA modified SiNWAs through surface-initiated LRP and the post-modification by CuAAC click chemistry. To remove copper from the click reaction, the chips were immersed into water for 3 days with changing the water several times before further applications. Atomic absorption spectrometer (ASS) was used to measure the remaining copper content. A value of ~1 μg copper in 25 mg substrate was measured, indicating that the attached copper on the surface is negligible.

![Figure 5](image-url)  
**Figure 5.** Capture of Ramos cells and Baf3 cells on modified SiNWAs surfaces in serum-containing environment. (a) Fluorescence images of cell experiments for both Ramos cells and Baf3 cells. Scale bar: 200 μm. (b) The density of Ramos and Baf3 cells captured by SN-OH, SN-APT, SN-PAGA and SN-PAGA-APT surface, respectively. Data are the mean ± SD (n = 5).

**Cell capture on modified SiNWAs surfaces**  
On the purpose of preparing a surface that can capture cancer cells specifically and efficiently, Ramos cells was cultured to prove the specific cell adhesion of the surface, while taking Baf3 cells as control. The ability to capture Ramos cells and Baf3 cells on the different surfaces were measured in complex environments containing 10% serum. Previous researches have reported that...
TD05 aptamer has a higher affinity for Ramos cells at 4 °C. 46-48 Therefore, the capture assays of aptamer modified surfaces were performed at 4 °C in the present work.

The fluorescence images showed that only a very small amount of Ramos cells adhered on the pristine SN-OH surface (Figure 5a) with a cell density of only 640 cells/cm² (Figure 5b). After modified with PAGA, the captured cell significantly increased (Figure 5a) and the cell density increased to 6500 cells/cm² (Figure 5b). These results proved our initial vision that the PAGA modified surface can indeed capture Ramos cell membranes by binding with the overexpressed glucose transporter protein on the membrane of cancer cells. The captured cell number increased nearly 10 folds compared with the SN-OH surface. Before testing the cell-capturing ability of surfaces modified with both PAGA and TD05, we first test the ability of TD05 alone modified surface. It is found that the number of captured Ramos cells increased extensively after modified with aptamer TD05 (Figure 5a) with a cell density of about 18300 cells/cm² (Figure 5b), higher than SN-PAGA. The result clearly shows that TD05 can bind with Ramos cells specifically. Therefore, aptamer TD05 was further introduced to the PAGA modified surface to investigate if the efficiency of cell capture can be further improved. In our research, the amount of aptamer added is very small, much less than the amount of N₃ groups available. After the click reaction, we checked the content of aptamer by UV-Vis and there is no detectable signals of aptamer remaining, indicating that the click reaction is highly efficient with almost full conversion. Therefore the amount of clicked aptamer can be assumed to be the same as the amount added. In order to ensure the comparability between two samples, the amount of aptamer was the same as that on SN-APT surface. The number of captured Ramos cells further increased (Figure 5a), with a cell density of 39200 cells/cm² (Figure 5b), the number of cells increased 60-folds compared with the SN-OH surface. In order to prove that AGA and aptamer modified surface can capture Ramos cell specifically, Baf3 cell was used as a control to study the capture efficiency of normal cell under the same conditions. From Figure 5a, it can be seen that the tested surfaces showed little ability to capture Baf3 cells. Therefore, the materials we prepared have specific binding ability to Ramos cancer cells with high efficiency, with a good anti-fouling performance to normal cells. Furthermore, SiNWAs chips with different amount of aptamers were also prepared to investigate their ability to capture Ramos cells. As shown in Figure S7, the more aptamer, the better capturing. However, aptamer is more expensive than glycopolymer, therefore a good cancer cell capturing with reduced usage of aptamer will be demanded. The chip modified with 5 μM aptamer has a cancer cell capturing ability close to that of 10 μM, which might be an optimum condition for the cancer cell capturing applications.

Conclusions

In summary, three types of SiNWAs surfaces modified with glycopolymer or/and aptamer (SN-PAGA, SN-APT and SN-PAGA-APT) were successfully prepared through SET-LRP and click reactions. Ramos cells and Baf3 cells were used to investigate the specific and non-specific interactions between the bio-interfaces and cells in detail, respectively. We can conclude that: (a) SiNWAs hardly catch any circulating cells such as Ramos and Baf3, due to the excellent hydrophilicity. Similarly, other hydrophilic surfaces tested here catch little Baf3 cells, indicating if there is no specific interaction, hydrophilic surfaces are not favorable to catch circulating cells. (b) The glycopolymer-coated surface (SN-PAGA) can catch Ramos cells, possibly through the multivalent interactions between PAGA and GLUTs. However glycopolymer alone is not good enough for efficient capturing. (c) Integrating glycopolymers PAGA with aptamers TD05 on SiNWAs produced the most efficient and specific Ramos capturing surface, a multivalency-enhanced consequence was achieved with more than 60-folds over the SN-OH surface. Here we provide a facile way of using SI-SET-LRP and click chemistry to fabricate surfaces that can capture tumor cells in a more effective, economical and specific fashion through the synergistic effect generated by combining the glycopolymers and aptamers together.

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Notes and references

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c Electronic Supplementary Information (ESI) available: [H NMR of glycopolymers and XPS, contact angles, ATR-FTIR of surfaces. Dry thickness of polymer brush and captured Ramos cell density of substrates.]. See DOI: 10.1039/b000000xd


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Enhanced specific cancer cells capturing effect generated by combining glycopolymer and aptamer together through SI-SET-LRP and click chemistry.