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Dual-Responsive Surfaces Modified with Phenylboronic Acid-Containing Polymer Brush To Reversibly Capture and Release Cancer Cells

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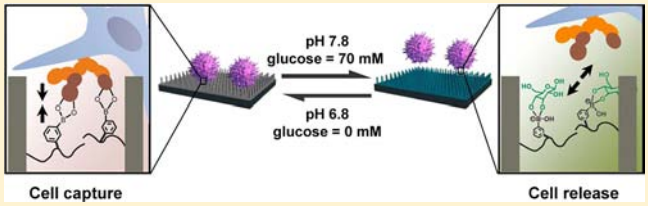
Supporting Information

ABSTRACT: Artificial stimuli-responsive surfaces that can mimic the dynamic function of living systems have attracted much attention. However, there exist few artificial systems capable of responding to dual- or multistimulation as the natural system does. Herein, we synthesize a pH and glucose dual-responsive surface by grafting poly(acrylamidophenylboronic acid) (polyAAPBA) brush from aligned silicon nanowire (SiNW) array. The as-prepared surface can reversibly capture and release targeted cancer cells by precisely controlling pH and glucose concentration, exhibiting dual-responsive AND logic. In the presence of 70 mM glucose, the surface is pH responsive, which can vary from a cell-adhesive state to a cell-repulsive state by changing the pH from 6.8 to 7.8. While keeping the pH at 7.8, the surface becomes glucose responsive—capturing cells in the absence of glucose and releasing cells by adding 70 mM glucose. Through simultaneously changing the pH and glucose concentration from pH 6.8/0 mM glucose to pH 7.8/70 mM glucose, the surface is dual responsive with the capability to switch between cell capture and release for at least 5 cycles. The cell capture and release process on this dual-responsive surface is noninvasive with cell viability higher than 95%. Moreover, topographical interaction between the aligned SiNW array and cell protrusions greatly amplifies the responsiveness and accelerates the response rate of the dual-responsive surface between cell capture and release. The responsive mechanism of the dual-responsive surface is systematically studied using a quartz crystal microbalance, which shows that the competitive binding between polyAAPBA/sialic acid and polyAAPBA/glucose contributes to the dual response. Such dual-responsive surface can significantly impact biomedical and biological applications including cell-based diagnostics, in vivo drug delivery, etc.



INTRODUCTION

Stimuli-responsive materials[1−7] have been used in various biomedical and biological applications, because their physicochemical properties, such as wettability, stiffness, adhesion, and optical property, can undergo dynamic changes in accord with changes in living systems. For example, thermo-responsive poly(*N*-isopropylacrylamide) has been successfully used for tissue engineering, including cell sheet engineering[8] and tissue building blocks.[9−11] Recently, stimuli-responsive biointerfaces which are functionally similar to natural surfaces in a dynamic manner have also been designed for bioseparation,[12−14] drug delivery systems,[15−19] cell-based diagnostics,[20] and so on. In our previous study, we designed thermo-responsive surfaces for reversible capture and release of targeted cancer cells without damage.[21] However, most of these smart surfaces are focused on single response, such as light,[22,23] voltage,[24] enzyme,[25] temperature,[26,27] or sugar.[28,29] These single-responsive surfaces cannot satisfy the needs of double or multiple stimulation in complex environments. It is therefore desirable to design dual- or multiresponsive surfaces that can efficiently regulate the adhesion behavior of targeted cells.

Polymer brushes known as dense layers of chains that are grafted chemically from/to a surface or an interface[30−32] have been utilized extensively to fabricate a diverse range of stimuli-responsive surfaces, such as poly(*N*-isopropylacrylamide) or poly(ethylene glycol) brush-based thermo-responsive surfaces,[8,21,27] polyelectrolyte brush-based pH-responsive surfaces,[33] and copolymer brush-based solvent-responsive surface.[34] In recent years, phenylboronic acid (PBA)-containing polymer brushes on different surfaces have been fabricated for sensor chips of glycoproteins,[35] glucose detection,[36] and controllable cell adhesion,[37−40] depending on the fast and stable formation of boronate esters between PBA and diols.[41−43] However, almost all of the studies are performed under specific alkaline conditions (usually close to or higher than the pK_a of PBA), because only tetrahedral anionic PBA can form a stable complex with diols-containing targets.[44] As an exception, recent works have shown that PBA can form a stable complex with sialic acid, a principal carbohydrate component generally occupying the terminal positions of the

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carbohydrate chains of glycoproteins and glycolipids in cell membrane, in acidic medium below the pK_a of PBA.[45,46] Although the underlying mechanism for specific binding between PBA and sialic acid in relatively low pH still remains unclear,[41,46−48] the unique binding property between PBA and sialic acid is acknowledged. Due to this property, PBA-modified gold electrode is able to quantify sialic acid at the cell surface under physiological conditions[49] and further monitor tumor metastasis by determination of cell-membrane sialic acid expression.[50] Therefore, the question arises can we design new-generation dual-reponsive surfaces to regulate cell adhesion by utilizing the pH-dependent capability of PBA complexation with small biological molecules?

In this paper, we demonstrated a pH and glucose dual-responsive surface that can efficiently and rapidly switch between capture and release of targeted cancer cells by grafting a poly(acrylamidophenylboronic acid) (polyAAPBA) brush from aligned silicon nanowire (SiNW) array (Figure 1a). We selected

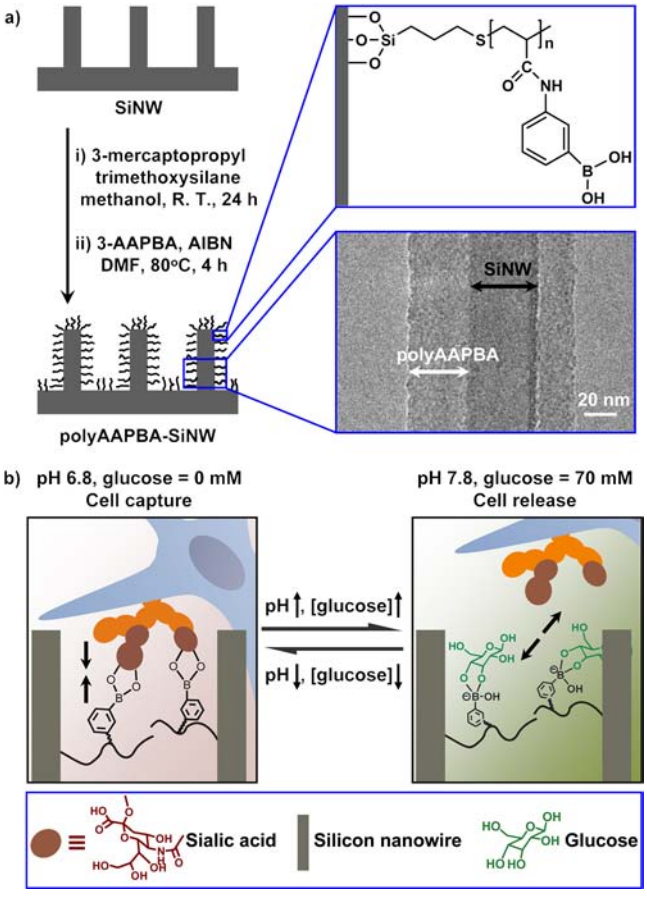

Figure 1. Schematic of the pH and glucose dual-responsive surface for cell capture and release. (a) Synthesis of poly(acrylamidophenylboronic acid) (polyAAPBA) brush on aligned silicon nanowire (SiNW) array. TEM image shows that the thickness of polyAAPBA is 30−40 nm (bottom-right). (b) Cell capture and release induced by pH and glucose. At pH 6.8 in the absence of glucose, the as-prepared surface captures targeted cells due to the specific binding between polyAAPBA brush on the surface and sialic acid existing in the membrane of the cells. By elevating the pH to 7.8 and increasing glucose concentration to 70 mM, competitive binding between tetrahedral anionic polyAAPBA and glucose leads to release of targeted cells.

breast cancer cells (i.e., MCF-7 cells) as model cells because (1) the adhesion of cancer cells is closely related to cancer metastasis and diagnostics and (2) MCF-7 cells overexpress sialic acid in the membrane.[51,52] At pH 6.8, polyAAPBA brush on the SiNW array can form specific binding with sialic acid expressed in the membrane of MCF-7 cells (left, Figure 1b). Because the pK_a of 3-AAPBA-containing polymers is ~9.0,[53] when elevating the pH to 7.8, about 6% of 3-AAPBA units in polyAAPBA are in tetrahedral anionic form.[54] With further addition of glucose, a stable complex between tetrahedral anionic polyAAPBA and glucose[37,55,56] replaces the polyAAPBA/sialic acid complex, thus releasing the MCF-7 cells (right, Figure 1b). When decreasing pH to 6.8 without glucose, the tetrahedral anionic polyAAPBA is dehydroxylated and can again form specific binding with sialic acid, thereby reversibly capturing targeted MCF-7 cells. In addition, amplified responsiveness and accelerated cell capture/release were observed on polyAAPBA-SiNW, owing to a three-dimensional (3D) contact mode[57] between the SiNW array and the cell protrusions. In this way, we obtained a dual-responsive surface that can switch between cell capture and release efficiently and rapidly.

RESULTS AND DISCUSSION

In our experiment, we chose phenylboronic acid-containing polyAAPBA brush as the cell-capture ligand because flexible end-grafted polymer chains can improve reactivity of the ligand.[40] We fabricated a polyAAPBA brush-based dual-responsive SiNW array (polyAAPBA-SiNW) by combining wet chemical etching[58] with chain-transfer radical polymerization.[59] We used a wet-etching chemical method to obtain SiNW with lengths of about 8 μm and diameters of 40−160 nm (Figure S2, Supporting Information). Then, chain-transfer radical polymerization of 3-acrylamidophenylboronic acid (3-AAPBA) was performed on a thiol-terminated SiNW array with the use of 2,2′-azobis(2-methylpropionitrile) (AIBN) as the initiator (Figure 1a). X-ray photoelectron spectroscopy (XPS) shows four peaks with binding energies at about 191.6, 284.8, 400.1, and 532.4 eV (Figure S1, Supporting Information), which correspond to B1s, C1s, N1s, and O1s, respectively. The TEM image of a single SiNW indicates that the thickness of the polyAAPBA brush was 30−40 nm (bottom-right, Figure 1a). These results suggest that polyAAPBA brush is modified on the surface successfully.

As a proof of concept, we demonstrated the capability of polyAAPBA-SiNW to switch between cell capture and release. Figure 2a shows that polyAAPBA-SiNW is pH responsive in the presence of 70 mM glucose. At pH 6.8, the cell capture efficiency was about 60% (left, Figure 2a). After changing the pH to 7.8, few cells remained on the surface (right, Figure 2a). Figure 2b exhibits that polyAAPBA-SiNW is glucose responsive at pH 7.8, and it can reversibly capture (glucose = 0 mM) and release cells (glucose = 70 mM) through changing the glucose concentration. By simultaneously altering the pH and glucose concentration (Figure 2c), polyAAPBA-SiNW was pH and glucose dual responsive with the capability to reversibly capture and release cells. Figure 2d shows that polyAAPBA-SiNW can switch between cell capture and release cleanly and repeatly by variation between pH 6.8 without glucose and pH 7.8 with 70 mM glucose, exhibiting as a pH and glucose dual-responsive AND logic system.[60−62] Moreover, viability of cells captured on or released from polyAAPBA-SiNW is important for practical applications, so we further investigated cell viability using the Live/Dead staining method (see section 4 in the Supporting Information for details). Both the captured and the released cells exhibited good viability, and the percentage of live cells was higher than 95% (Figure 3). This noninvasive cell capture and release would facilitate subsequent cell culture and

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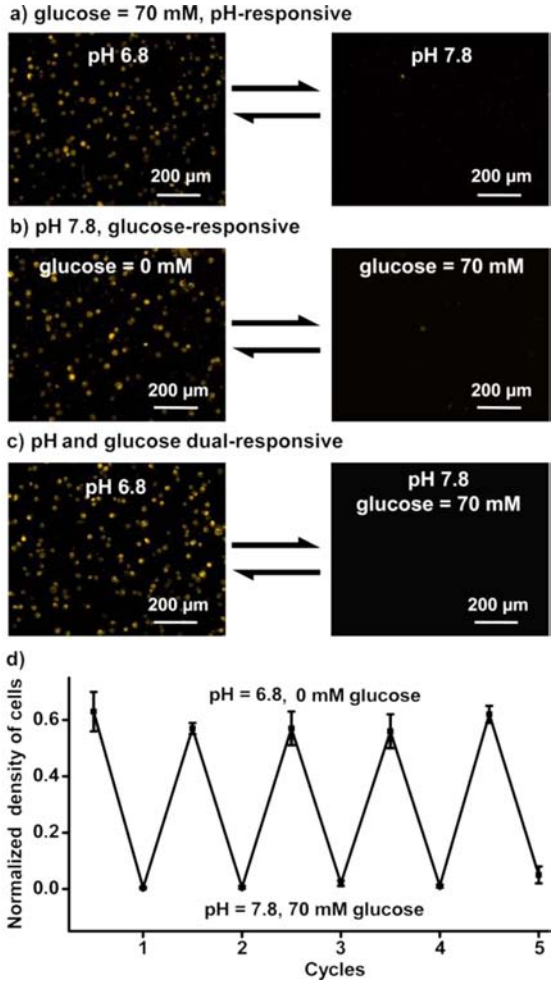

Figure 2. Cell capture and release on the as-prepared polyAAPBA-SiNW. (a) In the presence of 70 mM glucose, polyAAPBA-SiNW is pH responsive. (b) At pH 7.8, polyAAPBA-SiNW is glucose responsive. (c) PolyAAPBA-SiNW is pH and glucose dual responsive. (d) Normalized density of cells on polyAAPBA-SiNW at two different pH values and glucose concentrations. Normalized density of cells means the ratio of the actual density of captured cells and theoretical density of captured cells. Error bar represents the standard error of mean from three repeats.

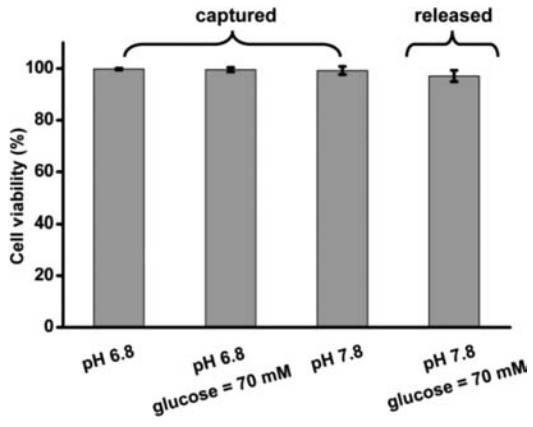

Figure 3. Quantitative evaluation of the viability of MCF-7 cells captured on and released from the as-prepared polyAAPBA-SiNW. Error bar represents the standard error of mean from three repeats.

molecular analysis. Thus, polyAAPBA-SiNW is able to reversibly capture and release cells with high viability, responding to both pH and glucose.

Surface topography has been reviewed to have a significant influence on cell adhesion.[63−70] To make clear the role of the SiNW array in the process of cell capture and release, we compared the cell capture and release performance on polyAAPBA-SiNW and polyAAPBA-modified flat silicon wafer (polyAAPBA-Si). The SEM image shows that polyAAPBA-SiNW exhibits aligned 3D nanostructure (Figure 4a). In contrast, polyAAPBA-Si is relatively

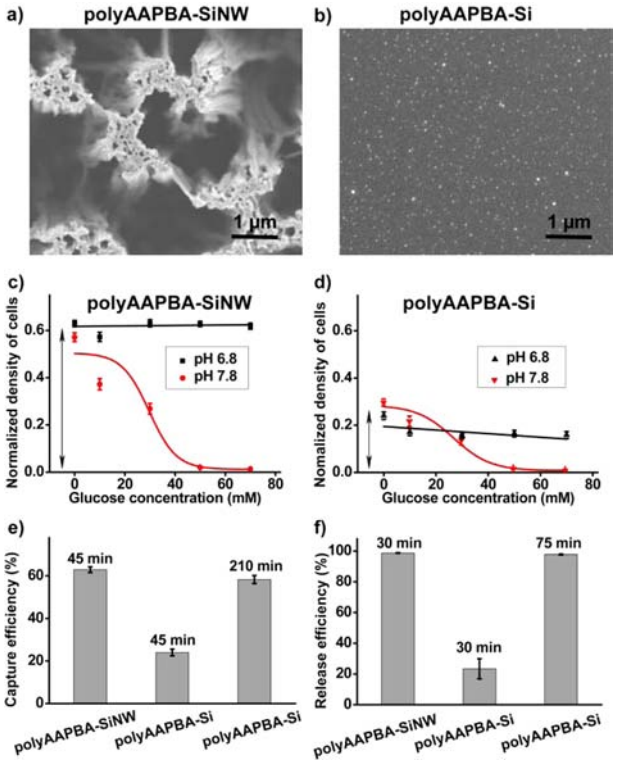

Figure 4. SEM images indicate that polyAAPBA-SiNW possesses aligned nanostructure (a), while polyAAPBA-Si is relatively smooth (b). (c and d) Glucose concentration and pH dependence of the normalized density of cells on polyAAPBA-SiNW and polyAAPBA-Si. Compared with polyAAPBA-Si (d), polyAAPBA-SiNW can greatly amplify the responsiveness between cell capture and release (c). Moreover, polyAAPBA-SiNW shows more rapid cell capture and release than polyAAPBA-Si (e and f). Normalized density of cells means the ratio of the actual density of captured cells and theoretical density of captured cells. Error bar represents the standard error of mean from three repeats.

smooth (Figure 4b). Figure 4c and 4d illustrates the cell capture performance of polyAAPBA-SiNW and polyAAPBA-Si at two pH values (pH 6.8 and 7.8) with different glucose concentrations. At pH 6.8, the normalized density of cells on polyAAPBA-SiNW was about 0.6 after incubation for 45 min (at 37 °C with 5% CO_2). Cell density had no obvious changes by increasing glucose concentration up to 70 mM. However, at pH 7.8, cell density was dramatically decreased when glucose concentration was higher than 20 mM, and only few cells were captured on polyAAPBA-SiNW when the glucose concentration was higher than 50 mM (Figure 4c). Similar pH and glucose dependence on cell capture behavior was also observed on polyAAPBA-Si (Figure 4d). However, the responsiveness of polyAAPBA-SiNW was greatly amplified compared to polyAAPBA-Si, which was mainly due to the higher cell density

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on polyAAPBA-SiNW than polyAAPBA-Si at pH 6.8 or pH 7.8 without glucose. SEM images show that cells captured on polyAAPBA-SiNW have extruded more protrusions (Figure 5a)

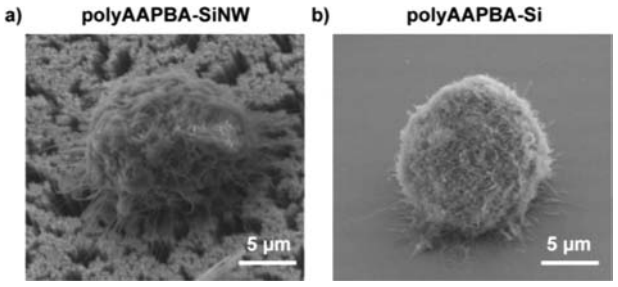

Figure 5. SEM images of cells captured on different substrates at pH 6.8. Cells captured on polyAAPBA-SiNW (a) extruded more protrusions than on polyAAPBA-Si (b).

than on polyAAPBA-Si (Figure 5b),[65] providing a 3D contact mode between cells and nanostructured surface, thereby enhancing the capture efficiency.[21,57] Moreover, we found that the nanostructured SiNW array was conductive to rapid cell capture and release (Figure 4e and 4f). On the one hand, at pH 6.8 the capture efficiency of the cells on polyAAPBA-SiNW was more than 60% after 45 min of incubation time. While the capture efficiency on polyAAPBA-Si was as low as 24% within the same period, it needed 210 min of incubation time to achieve a capture efficiency of 58% (Figure 4e). On the other hand, through elevating the pH to 7.8 in the presence of 70 mM glucose, the release efficiency of the cells on polyAAPBA-SiNW was 98% in 30 min. However, the release efficiency for polyAAPBA-Si was only 23% in 30 min and required 75 min to achieve a release efficiency of 97% (Figure 4f). We hypothesize that the rapid cell capture and release on polyAAPBA-SiNW may be attributed to the 3D contact mode between cells and the SiNW array, which benifits efficient diffusion of chemicals (i.e., proton and glucose) to the binding sites.[57] These results indicate that the 3D contact mode may not only amplify the responsiveness of the polyAAPBA-modified surface but also facilitate rapid cell capture and release.

To understand the fundamental responsive mechanism of the polyAAPBA brush-modified surface between cell capture and release, we used a quartz crystal microbalance (QCM)[71−74] to investigate the molecular interactions of polyAAPBA/sialic acid and polyAAPBA/glucose. SiO_2-coated QCM sensors were modified with polyAAPBA brush using the aforementioned protocol (Figure 1a). Figure 6 depicts typical sensorgrams upon binding of various concentrations of glucose and sialic acid conjugate to the polyAAPBA-modified QCM sensors at pH 6.8 and 7.8. We determined the binding constant (K_a) for polyAAPBA/glucose and polyAAPBA/sialic acid systems according to eqs 1 and 2[75]

$$\frac{[\text{glucose}]_0}{\Delta m} = \frac{[\text{glucos e}]_0}{\Delta m_{max}} + \frac{1}{\Delta m_{max} K_a} \quad (1)$$

$$\frac{[\text{sialic acid}]_0}{\Delta m} = \frac{[\text{sialic acid}]_0}{\Delta m_{max}} + \frac{1}{\Delta m_{max} K_a} \quad (2)$$

where Δm_{max} is the maximum binding amount, Δm is the measured binding amount, and $[\text{glucose}]_0$ and $[\text{sialic acid}]_0$ are the original concentration of glucose and sialic acid. From the data in Figure 6, the linear relation between $[\text{glucose}]/\Delta m$ and [glucose] and $[\text{sialic acid}]/\Delta m$ and [sialic acid] at pH 6.8 and

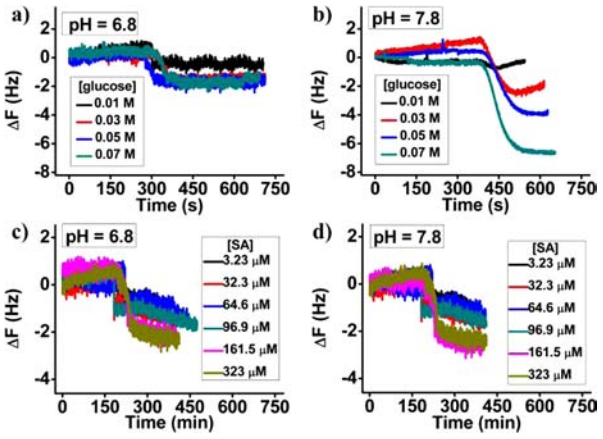

Figure 6. Typical curves of frequency changes following injection of different concentrations of glucose (a and b) and sialic acid (c and d) into the QCM chamber with polyAAPBA-modified QCM sensors.

7.8 can be obtained (Figure S3, Supporting Information). Thus, K_a can be obtained from the ratio of the slope to the intercept in Figure S3, Supporting Information. Calculated K_a values for polyAAPBA/sialic acid and polyAAPBA/glucose at pH 6.8 and 7.8 are summarized in Table 1. After injection of 161.5 μM

Table 1. Binding Constant K_a (M^{-1}) for PolyAAPBA/sialic acid and PolyAAPBA/glucose Systems at Different pH Values[a]

	polyAAPBA/sialic acid	polyAAPBA/glucose
pH 6.8	37	8
pH 7.8	17	141

[a]Determined by QCM measurements.

sialic acid solution at pH 6.8, frequency changes decreased immediately (Figure 7a). This suggests that polyAAPBA can form a stable complex with sialic acid under such slightly acidic condition. The binding constant (K_a) was about 37 M^{-1} (see Table 1). Frequency changes were not obvious upon further pH variation ranging from 6.8 to 8.8 (Figure 7a), suggesting that pH changes in the range from 6.8 to 8.8 hardly disrupted the complex between polyAAPBA and sialic acid. Figure 7b shows that the influence of glucose concentration on frequency changes is more obvious with increasing pH. At pH 6.8, K_a for polyAAPBA/glucose was about 8 M^{-1} (see Table 1). However at pH 7.8, K_a for polyAAPBA/glucose was as high as about 141 M^{-1} (see Table 1). These findings indicate that glucose can hardly replace sialic acid to form a complex with polyAAPBA at pH 6.8, while glucose can replace sialic acid to form a stable complex with polyAAPBA at pH 7.8. We repeatedly cycled the injection of 161.5 μM sialic acid solution at pH 6.8, 70 mM glucose solution at pH 7.8 and 6.8 buffer solution, and recorded the variation of the frequency changes (Figure 7c). After adding sialic acid solution at pH 6.8, the frequency changes decreased immediately due to the polyAAPBA/sialic acid complex (left, Figure 7d). Changes further decreased sharply when injecting 70 mM glucose solution at pH 7.8, owing to formation of a stable complex between tetrahedral anionic polyAAPBA and glucose (middle, Figure 7d). When subsequently changing the pH to 6.8, frequency changes increased rapidly because of disassociation of the polyAAPBA/glucose complex (right, Figure 7d). By changing pH values and glucose concentrations, frequency changes showed excellent reversibility for at least five

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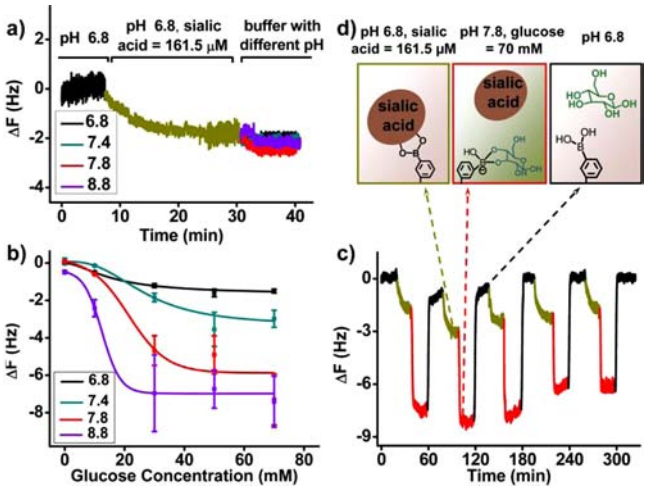

Figure 7. (a) Frequency changes of the polyAAPBA brush-modified QCM sensors decrease due to the stable complex between polyAAPBA and sialic acid. Changing pH in the range from 6.8 to 8.8 can hardly disrupt the complex between polyAAPBA and sialic acid. (b) Frequency changes of polyAAPBA brush-modified QCM sensors as a function of glucose concentration under different pH values. Influence of glucose concentration on frequency changes is more obvious with increasing pH. (c) Frequency variation during the cycle of injection of 161.5 μM sialic acid solution at pH 6.8, 70 mM glucose solution at pH 7.8 and pH 6.8 buffer solution. (d) Formation of polyAAPBA/sialic acid complex at pH 6.8 (left), competitive binding of glucose and sialic acid to tetrahedral anionic polyAAPBA at pH 7.8 (middle), and disassociation of polyAAPBA/glucose complex at pH 6.8 (right). Error bar represents the standard error of mean from three repeats.

cycles (Figure 7c). Therefore, the molecular mechanism of the dual-responsive polyAAPBA-modified surfaces may rely on competitive binding between polyAAPBA/sialic acid and polyAAPBA/glucose at different pH values.

To explore the specificity of our designed platform, we set up a parallel cell-capture experiment at pH 6.8 by choosing three kinds of cells: adherent COS-7 cells[76] (silic acid less-expressive cell line derived from the kidney of the African Green Monkey), MCF-7 cells (sialic acid overexpressive cell line), and sialidase treated MCF-7 cells (sialic acid residues were removed from glycoproteins on cell surfaces). In comparison with sialic acid overexpressive MCF-7 cells, the normalized density of COS-7 cells on the polyAAPBA-modified surfaces was much lower (Figure 8a). In particular, after treating sialic acid overexpressive MCF-7 cells with neuraminidase form *Clostridium perfringens*, a kind of sialidase which is usually used to remove sialic acid residues from glycoproteins on cell surfaces,[77,78] almost no cells were captured on the polyAAPBA-modified surfaces (Figure 8a). These results verify that our designed platform exhibits specificity to capture sialic acid overexpressive cells. In addition, the cell capture behavior on polyAAPBA-modified surfaces at pH 6.8 is indeed due to the specific recognition between sialic acid in the cell membrane and polyAAPBA immobilized on the surface. Moreover, the ability of polyAAPBA-SiNW to capture and release targeted MCF-7 cells was further demonstrated in a serum-containing buffer (pH 6.8). Although the normalized density of cells was lower in the serum-containing buffer than in pH 6.8 buffer without serum, the cell density was still as high as about 0.48 on polyAAPBA-SiNW and the captured MCF-7 cells could be easily released by simultaneously changing the pH to 7.8 and

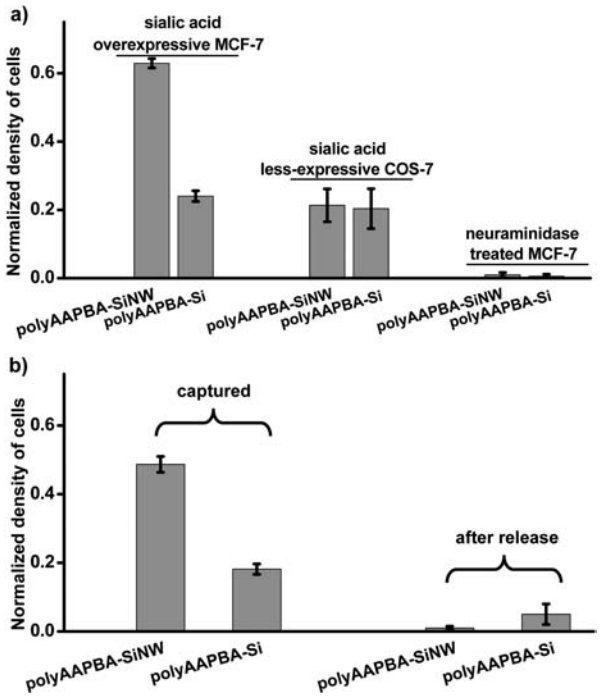

Figure 8. (a) As-prepared polyAAPBA-modified surfaces show good specificity for sialic acid overexpressive cells. Comparing with MCF-7 cells, the normalized density of COS-7 cells captured on polyAAPBA-modified surfaces is much lower. In particular, after treating MCF-7 cells with sialic acid-specific neuraminidase, few cells are captured on the polyAAPBA-modified surfaces. (b) PolyAAPBA-modified surfaces can also capture and release targeted cancer cells in serum-containing buffer. Normalized density of cells means the ratio of the actual density of captured cells and theoretical density of captured cells. Error bar represents the standard error of mean from three repeats.

adding 70 mM glucose (Figure 8b). Overall, the pH and glucose dual-responsive polyAAPBA-SiNW can specifically capture and release targeted cancer cells, even in serum-containing buffer.

■ CONCLUSION

We successfully fabricated a pH and glucose dual-responsive surface that can rapidly switch between cell capture and release with high efficiency. Switchable cell capture and release is based on the synergistic effect of molecular recognition and topographical interaction. Molecular recognition between polyAAPBA brush on polyAAPBA-SiNW and sialic acid moieties in the cell membrane provides dual-responsive property. Topographical interaction between nanostructured SiNW array and cell protrusions amplifies the responsiveness of the dual-responsive surface and accelarates cell capture and release. We illustrated the fundamental responsive mechanism of the polyAAPBA brush-modified surface at the molecular level using QCM. This work has extended the field of cell-related smart surfaces, from single to dual response, and would promote application of smart surfaces in complex environments for diagnostics, tissue engineering, drug delivery, and so on.

■ EXPERIMENTAL SECTION

Synthesis of polyAAPBA Brush-Based Surfaces. We first fabricated SiNW array using a wet-etching chemical method. Briefly, silicon substrates (1 cm × 1 cm) were ultrasonicated in acetone and ethanol at room temperature for 10 and 5 min, respectively, to remove contamination from organic grease. Then the degreased silicon was heated in boiling Piranha solution (3:1 (v/v) H_2SO_4/H_2O_2) for 1 h.

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Subsequently, silicon substrates were thoroughly rinsed with deionized water and dried with a flow of nitrogen. Then, the clean silicon substrates were treated with oxygen plasma (200 W, 90 s) to generate enough hydroxyl on the surface. Afterward, substrates were placed in a Teflon vessel containing the etching solution ([HF] = 7.0 mol L^{-1} and [$AgNO_3$] = 0.04 mol L^{-1}]) at ambient temperature for 1 h. After etching, substrates were immersed in 20% HNO_3 for 2 min to remove the silver film. Finally, substrates were rinsed with deionized water and dried with nitrogen gas.

Then we used chain-transfer radical polymerization to synthesize polyAAPBA brush-based SiNW array (polyAAPBA-SiNW). Briefly, freshly prepared SiNW array was immersed in anhydrous methanol containing 5 wt % 3-mercaptopropyl trimethoxysilane at room temperature for 24 h, generating chemically bonded −SH groups as the chain-transfer agent. After rinsing with methanol and DMF, polymerization of 3-AAPBA was performed by immersing the −SH-bonded SiNW array into a degassed solution of 3-AAPBA (0.3 g) in DMF (15 mL) containing AIBN (3 mg) at 80 °C for 4 h. After washing with deionized water, substrates were dried with a flow of nitrogen. For polyAAPBA-modified flat silicon wafer (polyAAPBA-Si) and QCM sensor (coated with SiO_2), the procedure was similar to the protocol for polyAAPBA-SiNW.

Cell Capture and Release Experiments. For cell capture, MCF-7 cells were stained with Dil for 30 min and suspended at pH 6.8 or 7.8 Tris-HCl buffers (0.1 M) with/without glucose to generate cell density of 1×10^5 cells/mL. Then the polyAAPBA brush-based substrates were placed into a 6-well cell culture plate, and 3 mL as-prepared cell suspension was loaded. The plate was protected from light with tinfoil and incubated in an incubator (37 °C, 5% CO_2) for 45 min. Substrates were gently washed with pH 6.8 or 7.8 Tris-HCl buffers for three times, and then we imaged the cells using a fluorescence microscope (Nikon, Ti-E). Color, brightness, and morphometric characteristics such as cell size and shape were considered in identifying MCF-7 cells and excluding cell debris. Cells that showed Dil+ (fluoresce yellow when exposed to green light) and met the phenotypic morphological characteristics were MCF-7 cells.

For cell release, after incubation in pH 6.8 Tris-HCl buffer (0.1 M) for 45 min (37 °C, 5% CO_2), polyAAPBA brush-based substrates were immediately transferred to pH 7.8 Tris-HCl buffer (0.1 M) with 70 mM glucose and incubated for further 30 min (37 °C, 5% CO_2), rinsing with pH 7.8 Tris-HCl buffer (0.1 M) three times. Then, we imaged and counted the cells using the fluorescence microscope.

The number of MCF-7 cells was obtained from three independent repeats, and for each at least 10 different areas were observed.

Quartz Crystal Microbalance (QCM) Measurements. All QCM measurements were performed at 37 °C using Q-Sense E1 system (Sweden). Prior to binding assays between polyAAPBA and glucose or sialic acid, the QCM channel was washed with deionized water and then pH 6.8 Tris-HCl buffer (0.1 M). Glucose and sialic acid were diluted at a series of concentrations using Tris-HCl buffer (0.1M) with different pH values. Then the solution was injected into the channel at a flow rate of 150 μL min^{-1}. After obtaining the binding curves, the polyAAPBA brush-modified sensor chip was regenerated using 70 mM glucose solution at pH 7.8 and then pH 6.8 Tris-HCl buffer for further measurements. All binding curves were recorded by Q-Sense software and analyzed by QTools.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Materials, detailed experimental procedures, XPS spectrum, SEM images, linear reciprocal plots of [glucose]/Δm against [glucose], [sialic acid]/Δm against [sialic acid], and fluorescence-activated flow cytometry and analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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