analytical chemistry

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Real-Time Wireless Monitoring of Cell Proliferation and Detachment Based on pH-Responsive Conductive Polymer Dots

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Cite This: *Anal. Chem.* 2021, 93, 8638−8646 Read Online

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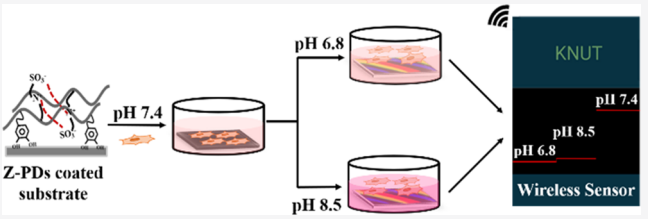
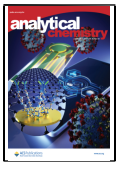
ABSTRACT: *In situ* wireless monitoring for cell proliferation and detachment kinetics was conducted using pH-responsive zwitterionic polymer dots (Z-PDs), based on changes in electrochemical signals derived from Z-PD-coated substrates *via* the interaction of charges transferred between Z-PDs and cells. Z-PD-coated substrates were found to be a potent means to monitor and manipulate cell adhesion and detachment because of their high sensitivity over a wide range of pH conditions, and modification of the coated substrates was confirmed using a wireless system. At neutral pH, Z-PD-coated wireless sensors exhibited $\pi-\pi$ stacking involving aromatic rings with hydrophobic interactions, thereby promoting cell proliferation; consequently, an increase in the measured resistance was observed. In contrast, Z-PD-coated substrates triggered by acidic and basic conditions promoted cell detachment, which induced an increase in the resistance compared with Z-PD substrates at pH 6.8, as a result of charges transferred to support Z-PD internalization through cell membranes after detachment. Therefore, as a wireless biosensor with excellent pH responsiveness that facilitates cell proliferation and detachment and whose electrochemical signals could be additionally acquired *via* a smartphone, Z-PD biosensors demonstrated a more favorable approach for monitoring cell−surface interactions than conventional optically based methods.



Interactions between cells and substrates have been a fundamental premise that play important roles in medical research as they contribute to the vast majority of biomedical applications, especially in cell delivery and tissue engineering with further investigation of anoikis process-induced cell detachment from the extracellular matrix (ECM).[1−6] Controlling and monitoring the number of cells attached to substrates facilitate the examination of cell characteristics for further applications. Traditionally, cell monitoring processes have been conducted using microscopic methods *via* conventional cell detachment regulated by trypsin, a cleaving reagent that might enable mutations in cells to occur over a long period. Current studies have proposed favorable detachment methods to promote cell detachment; however, with many reagents being accompanied by complicated synthesis steps and harmful effects on cells, it is necessary to fabricate an inventive material that promotes cell harvesting along with cell monitoring with rapidity, accuracy, and simplicity.[7−11] To support cell growth and detachment, research groups have reported several stimuli-responsive biomaterials featuring intrinsic characteristics that respond to exogenous environments such as pH and temperature, mostly based on hydrophobic−hydrophilic transitions.[12−18] pH-responsive zwitterionic polymers, which have been applied in cell delivery applications, represent promising materials for cell growth as well as cell harvesting owing to their inherent properties that enable them to exist in both hydrophobic and hydrophilic forms over a wide pH range.[19−23] However, methods for monitoring cell growth and cell detachment have been dependent on microscopic methods such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM), which are both costly, time-consuming, and have poor sensing ability. Therefore, an innovative approach for cell monitoring should be adopted.

Functional nanomaterials such as graphene and quantum dots have attracted attention in biosensing fields with utilization of electrochemical properties.[24] However, the limitation of functionalization ability of graphene and metallic core-induced toxicity of quantum dots, accompanied by low solubility, made these materials less biocompatible and showed low response to biological stimuli.[25] Recently, polymer dots (PDs) with sp^2 bond chains carbonized from synthesized polymers have been widely utilized in many biological applications because of their excellent photoluminescence, biocompatibility, and low toxicity as well as competent electronic properties.[26−33] Park et al. successfully exploited electroconductivity of PDs for electrochemical biosensors combined with a wireless sensing system connected to a smartphone for real-time monitoring based on changes

Received: April 26, 2021
Accepted: June 2, 2021
Published: June 10, 2021



https://doi.org/10.1021/acs.analchem.1c01778
Anal. Chem. 2021, 93, 8638−8646

detected in electrical resistance signals displayed on the phone screen.[34−37] Carbonized zwitterionic PDs (Z-PDs) are considered a favorable material for biosensor fabrication to monitor cell growth and cell detachment, as PDs can be tethered firmly to solid substrates using adhesive moieties. Moreover, at neutral pH, the biosensors exhibit high binding affinity for cells, proving their ability to promote cell proliferation and possibly leading to an increase in the resistance after cell proliferation. Additionally, the switchable hydrophobic−hydrophilic nature of zwitterionic surfaces triggered by acidic and basic pH conditions allows the biosensors to become cell-resistant, gradually enabling the removal of cells from the surface, accompanied by charge transfer. This transfer facilitates cellular uptake in an acidic environment, thus altering the electrochemical signals of the biosensor.[38−40] Therefore, zwitterionic PDs are ideal candidates for cell delivery as well as cell harvesting, which could be utilized for electrochemical monitoring.

In this study, we fabricated a pH-switchable wireless electrochemical biosensor to monitor cell proliferation and detachment by utilizing surface-coated zwitterionic PDs composed of carbonized sultone-conjugated poly(vinylpyrrolidone) (PVP) and catechol-derivative polymer-coated substrates. Owing to the hydrophobic−hydrophilic switchability associated with charge transfer under various pH conditions, the wireless biosensor exhibits great potential for manipulating cell growth and detachment, which was supported by the effective internalization of PDs at positively and negatively charged surfaces. Therefore, the electrochemical signals of the biosensors obtained using a wireless device were altered, demonstrating rapidity and sensitivity in monitoring cell−surface interactions that overcome the disadvantages of conventional microscopic methods.

EXPERIMENTAL SECTION

Synthesis of Zwitterionic Polymer Dots (Z-PDs) Based on Carbonized CCDP/Sultone-Conjugated PVP (PVP-CCDP-Sultone). To prepare PVP-CCDP-sultone, PVP (5 g) and CCDP (0.61 g) were dissolved in ethanol (125 mL) and tetrahydrofuran (THF) (30 mL) at 70−80 °C for 24 h. The obtained product was purified by the precipitation method in vacuum oven. To obtain Z-PDs, PVP-CCDP-SO_3^- (1.0015 g) was dissolved in double distilled water (DDW) (50 mL) and carbonized by the hydrothermal process at 180 °C for 8 h. The product was subsequently freeze-dried to obtain the final product.

UV−Vis and Fluorescence (FL) Spectroscopic Studies for Z-PDs. For the UV−vis spectroscopic study, Z-PDs were dispersed in phosphate-buffered saline (PBS), pH 7.4, with a concentration of 0.1 mg/mL. The absorbance was then recorded at a wavelength range of 200−900 nm. For the fluorescence spectroscopic study, 1 mg/mL Z-PDs was dispersed in PBS, pH 7.4. The pH treatment was adjusted using 1 M HCL and 1 M NaOH solutions. The FL intensity was recorded at an excitation wavelength of 460 nm.

Water Contact-Angle Measurements for Z-PDs Coated on Various Substrates as a Biosensor. Z-PDs were coated on different surfaces (poly(ethylene terephthalate) (PET) and Si wafer) to evaluate the material-independent surface coating. The substrates were first washed with acetone and ethanol. Z-PDs were dissolved in pH 8.5 Tris-buffer solution at a concentration of 10 mg/mL using the dip-coating method for substrate coating. After 24 h, at room temperature, Z-PD-coated substrates were washed with DDW several times. The pH of the coated substrates was adjusted to 6.8 and 8.5 using 1 M HCl and 1 M NaOH solutions, respectively. The water contact angle of Z-PD-coated substrates varied pH was measured.

Electrochemical Studies for Cell Proliferation on Z-PD-Coated Biosensors. The change in electrochemical signals was recorded by electroimpedance spectroscopy. The resistances of Z-PD-coated Si wafer substrates were measured at different pH treatments (pH 6.8, 7.4, and 8.5) before cell seeding experiments. Then, the HeLa cells (density 10^3 cells) in cell suspension were cultured on various pH 7.4-treated Z-PD biosensors and incubated for 6, 12, 24, and 48 h at 37 °C in a humidified atmosphere with 5% CO_2. After incubation, the cell-cultured biosensors were washed by PBS to remove nonattached cells.

Electrochemical Studies for Cell Detachments on Z-PD-Coated Biosensors by pH Treatment Depending on Cell Proliferation. HeLa cells (200 μL, density 10^3 cells) in cell suspension were cultured on distinct pH 7.4-treated Z-PD biosensors and incubated for 6, 12, 24, and 48 h at 37 °C in a humidified atmosphere with 5% CO_2. After incubation, the cell-cultured biosensors were washed by PBS to remove nonattached cells. Subsequently, 200 μL of pH 6.8 buffer was added onto the cell-cultured substrates and washed after 1.5 h of incubation.

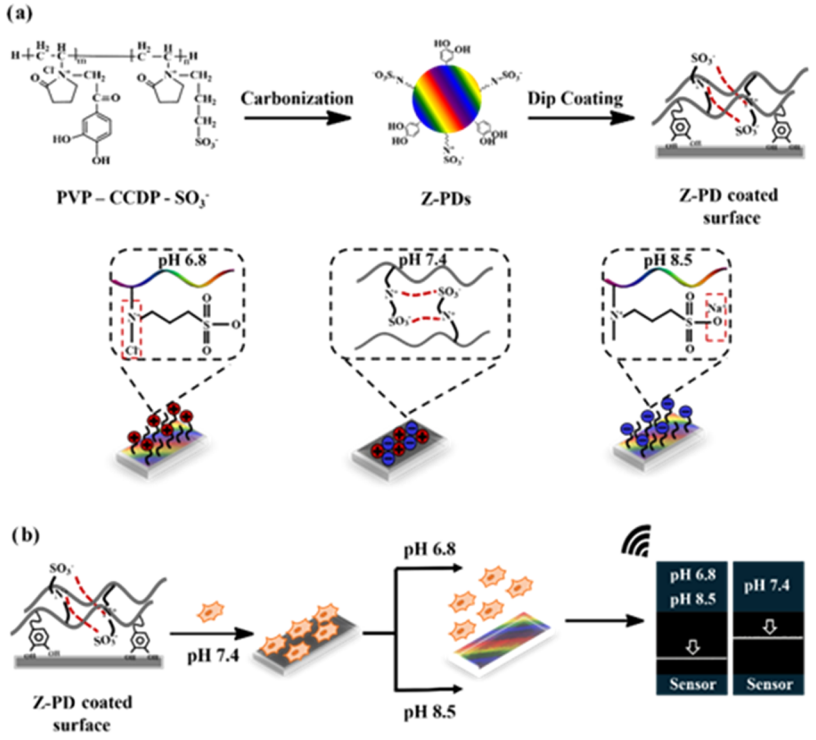
Electrochemical Studies for Cell Detachment. The resistance of the Z-PD-coated Si wafer substrate was recorded in advance. HeLa cell suspension with a density of 10^3 cells was cultured on pH 7.4-treated Z-PD-coated biosensors for 48 h and incubated at a condition of humidified atmosphere with 5% CO_2 and 37 °C. After 48 h, the cell solution was removed and the biosensors were washed with PBS several times to remove nonattached cells. Subsequently, the cell-cultured biosensors were treated with PBS solution adjusted pH 6.8 and 8.5 for cell detachment. After pH treatment for 1.5 h, the biosensors were rinsed with PBS several times to remove the detached cells. Electroimpedance spectroscopy was used to measure resistance changes in each step.

Confocal Imaging for Cell Proliferation and Detachment of Z-PD-Coated Substrates after pH Treatment. To observe cell proliferation on Z-PD-coated substrates, 200 μL of HeLa cells with a density of 10^3 cells were cultured individually on pH 7.4-treated Z-PD-coated substrates, following by 6, 12, 24, and 48 h incubation with the conditions of the humidified atmosphere with 5% CO_2 and 37 °C. Subsequently, the cell suspension was removed and the substrates were washed with PBS, pH 7.4, several times for observing under a confocal laser scanning microscope (CLSM). The cell detachment by pH 6.8 treatment was conducted after each interval of incubation time. Briefly, after incubation, the cell-cultured coated substrates were treated with 200 μL of PBS, pH 6.8, adjusted using 1.0 M HCL and then incubated for 1.5 h. Finally, the substrates were washed several times with PBS, pH 7.4, to remove detached cells. The cell-detached substrates were monitored to investigate fluorescence recovery by pH treatment by CLSM.

Fluorescence-Activated Cell Sorting (FACS) Analysis for Cellular Uptake after Cell Detachment by pH Treatment. Z-PDs were coated on a six-well plate for 24 h and then washed with DDW and PBS thoroughly. After being dried, 2 mL of HeLa cells (10^5 cells) were seeded onto individual wells and incubated for 6, 12, 24, and 48 h in a

https://doi.org/10.1021/acs.analchem.1c01778
Anal. Chem. 2021, 93, 8638−8646

Scheme 1. (a) Preparation of pH-Responsive Z-PD-Coated Electrodes and (b) Illustration of Z-PD-Coated Wireless Biosensor for Detecting Cell Proliferation and Detachment under Different pH Conditions



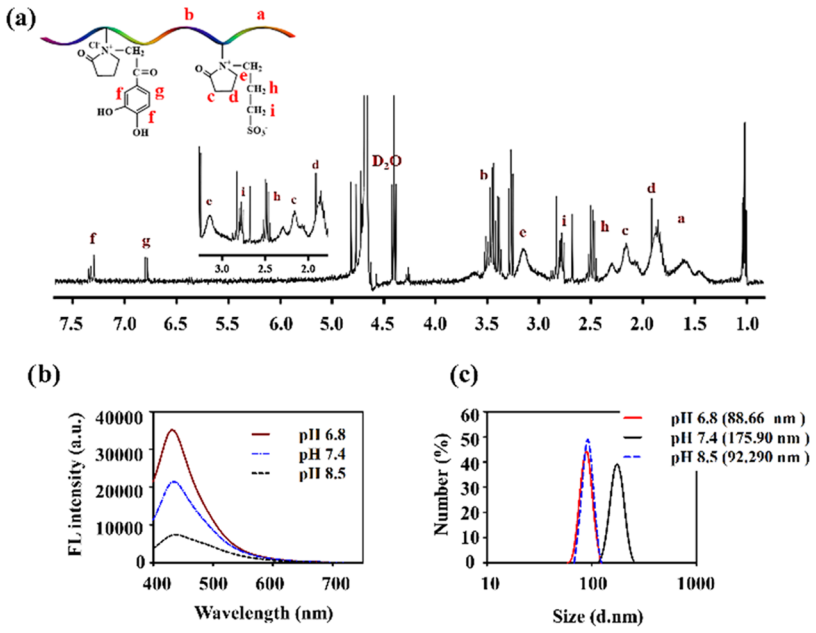


Figure 1. Characterization of Z-PDs. (a) ^{1}H NMR spectra, (b) change in the FL intensity of Z-PDs with pH treatment, and (c) particle size distribution of Z-PDs with pH treatment.

humidified atmosphere with 5% CO_2 and 37 °C. The cells at different incubation times were collected by pH 6.8 treatment for 1.5 h and resuspended in 1% fetal bovine serum (FBS) in PBS, pH 7.4. The cellular uptake was then analyzed by Attune NxT Acoustic Focusing Cytometer at an excitation wavelength of 405 nm and an emission wavelength of 450 nm. For comparison, HeLa with the same amount were seeded under above conditions followed by the treatment of pH 7.4 on Z-PD-coated surfaces. The cells were collected by trypsin treatment after different hours of incubation and resuspended in 1% FBS in PBS, pH 7.4. The cellular uptake was then analyzed by Attune NxT Acoustic Focusing Cytometer at an excitation wavelength of 405 nm and an emission wavelength of 450 nm.

Live and Dead Assays. For CLSM assay, 200 μL of cell solution containing 1×10^3 cells per mL was cultured on Z-PD-coated substrates and then incubated at 37 °C and 5% CO_2 atmosphere for 48 h. The cell-cultured substrates were

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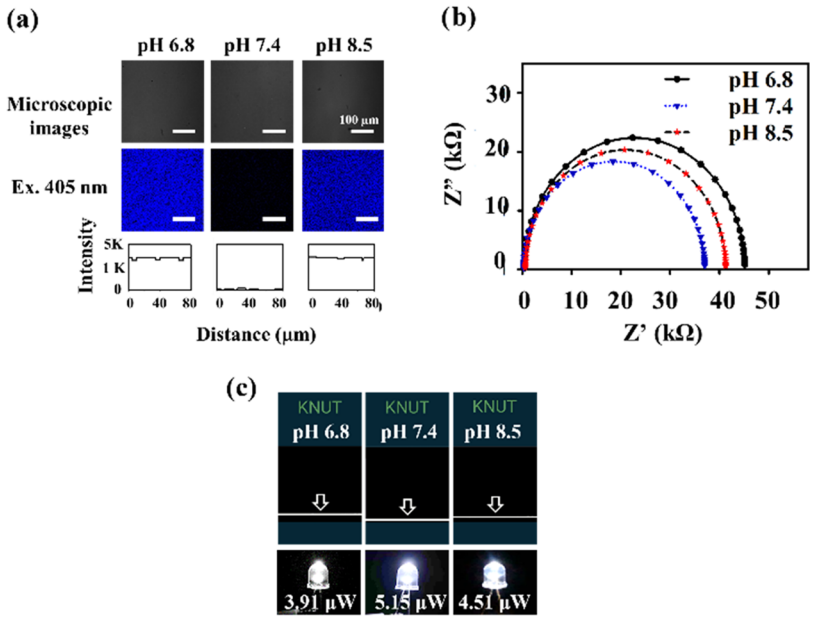

Figure 2. Effects of Z-PDs on coated substrates. (a) CLSM images of Z-PD-coated surfaces at pH 6.8, 7.4, and 8.5, (b) EIS studies, and (c) wireless and LED Z-PD-coated surfaces at pH 6.8, 7.4, and 8.5.

then detached by treatment at pH 6.8 and 8.5. Subsequently, the detached cells were centrifuged at 1500 rpm for 3 min. The supernatant was removed and the cells were mixed with 100 μL of calcein AM for 30 min followed by the addition of propidium iodide (PI) for 5 min. The cells were then observed under a confocal laser scanning microscope.

RESULTS AND DISCUSSION

Design of pH-Responsive Z-PD-coated Biosensor for Cell Attachment and Detachment. A wireless electrochemical biosensor was fabricated using pH-responsive zwitterionic PDs (Z-PDs) coated onto surfaces by catechol moieties modified by treatment over a wide pH range. At neutral pH, the fluorescence of Z-PD biosensors was quenched due to stacking of cationic ammonium groups and anionic sultone groups on the coated surface. Nevertheless, at pH 6.8 and 8.5, the Z-PDs on the coated biosensor reacted with abundant exogenous anions and cations from pH buffers to interrupt the inherent ionic interactions causing the charge transfer phenomenon, thus dissociating the charge-dense structure and recovering the fluorescence of Z-PD biosensors. The Z-PD-coated surfaces were alternatively utilized as electrochemical sensors to monitor cell proliferation and detachment owing to their remarkable responses to varied pH conditions that effectively changed the electroconductivity. The Z-PD-coated surfaces displayed a high affinity for cells at pH 7.4, owing to the hydrophobic aggregation of inherent anions and cations that promote cell proliferation. However, the coated surfaces demonstrated a highly potent cell detachment method *via* acidic and basic treatment that enhanced hydrophilic transitions on the coated surfaces for efficient cell removal. At the same time, the resistance of the cell-detached surfaces was higher than that of the cells without pH treatment, which implied the high sensitivity and detection accuracy of Z-PD biosensors. Moreover, electrochemical signals could be obtained by a wireless device connected to a smartphone, which allows simple and rapid observations compared to those using optically based methods (Scheme 1).

Characterization of Z-PD Nanoparticles. ^{1}H NMR analysis was conducted to verify the structure of the Z-PD nanoparticles (Figure 1a). Generally, the peaks indicated successful synthesis of Z-PDs with 7.0−7.5 ppm for catechol moieties and 2.2−3.0 ppm for sultone groups.[41,42] To investigate the absorption of the Z-PD nanoparticles, UV−vis analysis was performed (Figure S1). As shown in the figure, catechol groups were confirmed corresponding to the absorption peaks at 280 and 260 nm due to $\pi-\pi^*$ interactions involving aromatic rings and the n−π^* transition in catechol groups.[43,44] The fluorescent ON/OFF behavior in response to pH treatment of Z-PD nanoparticles was analyzed by the FL intensity (Figure 1b). At pH 6.8, the Z-PDs exhibited a strong fluorescence intensity due to their small particle size (Figure 1c). Nevertheless, at pH 8.5, the fluorescence of Z-PDs was quenched, which was related to the aggregation of polydopamine by catechol residues in solution. At pH 7.4, the size of Z-PDs was significantly increased, which was a result of electrostatic interactions generating the dense stacking of $\pi-\pi$ aggregated on the polymer chain of Z-PDs (Figure 1d).[45] Under acidic and basic conditions, the addition of cations and anions disturbed such electrostatic interactions and released ion complexation, leading to a decrease in the size of the Z-PD nanoparticles.

pH-Responsive Electrochemical Performance of Z-PD Biosensors. To investigate the properties of Z-PD biosensors with varying pH, Z-PD nanoparticles were coated onto poly(ethylene terephthalate) (PET) and silicon wafer substrates by the dip-coating method. Contact-angle measurements were performed to examine the wettability of the Z-PD-coated substrates under various pH conditions. As shown in Figure S2, there was a decline in contact angles after dip coating and acidic−basic treatment, implying that the increase in hydrophilicity originated from stretched structures of Z-PDs under acidic and basic conditions by existing ammonium and sultone moieties on the coated surfaces, which enhanced the hydrophilicity of the biosensors.[46,47] However, at neutral pH, the hydrophilicity of the coated substrates decreased, which

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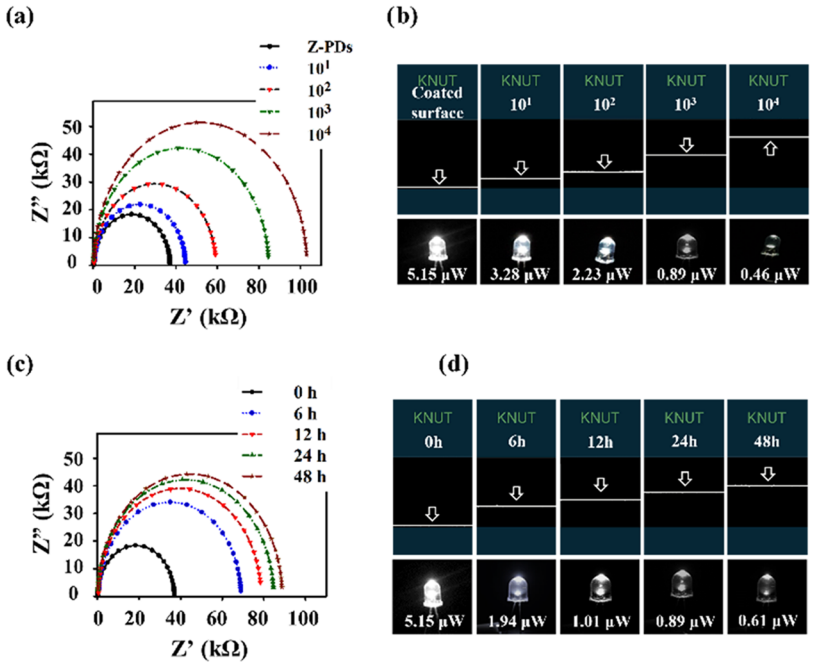

Figure 3. Electrochemical studies of Z-PD-coated surfaces cultured with HeLa cells: (a) EIS studies, (b) wireless and LED results with varied HeLa cell numbers of 10^1, 10^2, 10^3, and 10^4, (c) EIS study, and (d) wireless and LED results with 10^3 HeLa cells and varied proliferation times of 6, 12, 24, and 48 h at pH 7.4.

could be explained by the highly packed ionic complexation of inherent anions and cations on the surface preventing wettability. Confocal laser scanning microscopy was used to characterize the fluorescence ON−OFF behavior of Z-PD biosensors in response to pH, as shown in Figure 2a. At pH 7.4, the fluorescence of the Z-PD-coated surfaces was quenched due to the aggregation of Z-PDs resulting from electrostatic stacking interactions between the positively and negatively charged ions existing on the polymer chain coated on the surfaces, which consequently demonstrated good conductivity of the Z-PD biosensor at neutral pH. Changes in conductivity were measured by electrochemical impedance spectroscopy (EIS), as shown in Figure 2b. However, at pH 6.8 and 8.5, the fluorescence was recovered due to the disaggregation of Z-PDs caused by the protonation of sultone and ammonium groups on the coated surfaces. Accordingly, with free sodium ions remaining on the Z-PD-coated surfaces at pH 8.5, the conductivity of the surface increased by more than that of the Z-PD-coated surfaces at pH 6.8, leading to a decrease in its resistance. These changes in electrochemical signals were confirmed using a wireless light-emitting diode (LED) system, as shown in Figure 2c. As shown in the figure, the resistance line of pH 7.4 treatment was lower than that of pH 6.8 and 8.5 and verified the high conductivity of Z-PDs at neutral pH owing to the dense stacking of inherent anions and cations with strong electrostatic interactions, which at the same time led to an increase in the power of the LED output. To verify the biocompatibility and low cytotoxicity of Z-PDs suitable for cell−surface modification, an MTT assay was performed (Figure S3). Based on the results, the viability of HeLa cells incubated with various concentrations of Z-PDs from 0.01 to 1 mg/mL for 24 h remained almost 100%, that proved the biocompatibility of the probe. Subsequently, cell experiments were conducted to evaluate interactions involving cells and Z-PD-coated surfaces. Briefly, HeLa cells at different cell densities ranging from 10^1 to 10^4 cells were cultured on Z-PD-coated surfaces for 24 h under the indicated conditions. Cell growth was observed by CLSM, as shown in Figure S4. Electrochemical methods, including EIS measurements and wireless sensors, which have been reported to be effective methods for surface monitoring as the resistance is altered by cell attachment and detachment, were employed to verify the cell growth with increasing cell density (Figure 3a,b). As can be observed in the figure, the resistance of the biosensors significantly increased as the cell density increased, followed by a decline in LED power, which confirmed the high adhesion of cells to Z-PD-coated surfaces under neutral pH conditions. Correspondingly, to investigate cell growth without pH treatment, cell proliferation on Z-PD biosensors with 10^3 cells was arranged. The Z-PD-coated surfaces were first incubated at different times to serve as a control (Figure S6). The cell-seeded biosensors were individually incubated for 6, 12, 24, and 48 h at pH 7.4, to monitor cell proliferation, followed by the reported conditions. The cell-seeded biosensors were individually incubated for 6, 12, 24, and 48 h at pH 7.4, to monitor cell proliferation, followed by the reported conditions. The number of cells that proliferated after different incubation periods was tracked *via* CLSM imaging (Figure S5), followed by confirmation of cell generation using an electrochemical instrument (Figure 3c,d). The electrochemical signals noticeably changed after each proliferation time, demonstrating the ability of cells to self-generate on Z-PD-coated biosensors without any pH influence, thus increasing the resistance of the Z-PD-coated biosensors as the number of cells increased. Accordingly, FACS for cell counting was performed to confirm the number of cells grown during proliferation. Briefly, 10^3 of HeLa cells were seeded on individual pH 7.4-treated Z-PD-coated petri-dishes, followed by reported incubation conditions with various proliferation times. After each proliferation time, pH 6.8 treatment was performed to remove the attached cells. The number of cells detached were counted by FACS as shown in Figure S7.

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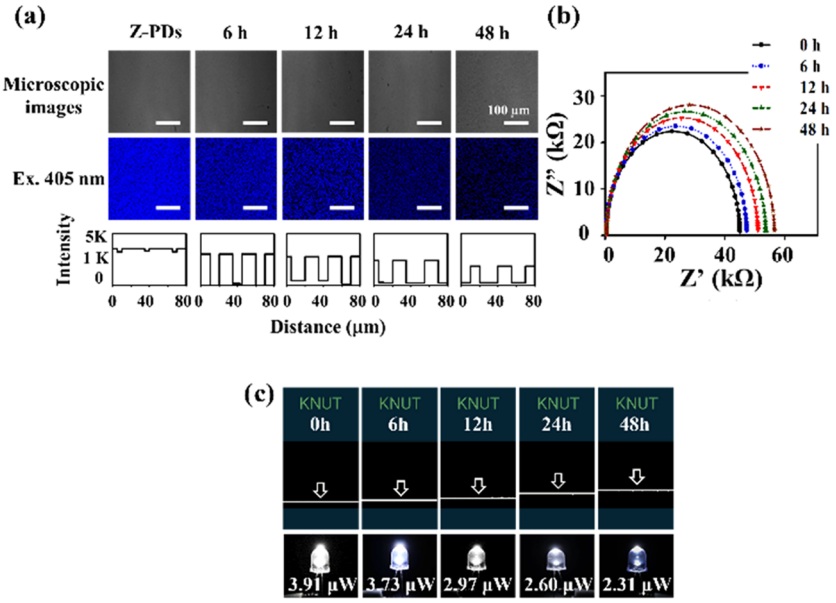

Figure 4. (a) CLSM images of cell-detached Z-PD-coated surfaces by pH 6.8 treatment after 0, 6, 12, 24, and 48 h proliferation on pH 7.4-treated Z-PD-coated substrates. Electrochemical analysis for cell detachment on Z-PD-coated surfaces varied with cell proliferation time. (b) EIS studies and (c) wireless and LED results for Z-PD-coated surfaces cultured with 10^3 HeLa cells detached by pH 6.8 treatment after 0, 6, 12, 24, and 48 h proliferation on pH 7.4-treated Z-PD-coated substrate.

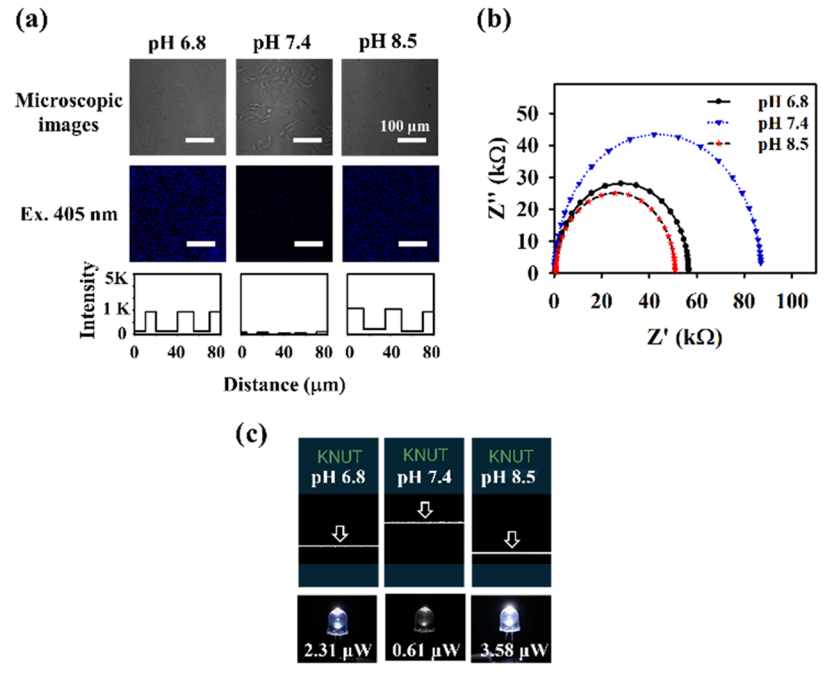

Figure 5. (a) CLSM images of Z-PD-coated surfaces cultured with 10^3 HeLa cells over 48 h at pH 7.4 and subsequently treated with pH 6.8 and 8.5, (b) electrochemical analysis for cell detachment on Z-PD-coated surfaces with EIS studies, and (c) wireless and LED for Z-PD-coated surfaces cultured with 10^3 HeLa cells over 48 h at pH 7.4 and then treated with pH 6.8 and 8.5.

Electrochemical Performance of pH-Responsive Z-PD Biosensors for Cell Proliferation and Detachment. The transition of Z-PD-coated surfaces from hydrophobic to hydrophilic in response to an acidic environment was applied for cell detachment. In brief, cell proliferation involving 10^3 HeLa cells on Z-PD-coated surfaces treated at pH 7.4 was carried out similar to the above conditions. After incubation, the cell-cultured Z-PD-coated surfaces were treated with PBS buffer at pH 6.8 to achieve cellular detachment; complete detachment of cells was observed on the Z-PD-coated surfaces after 1.5 h of acidic treatment (Figure 4a, first row). The impact of pH on the coated surfaces after cell detachment was investigated by CLSM imaging to determine the fluorescent responses of Z-PDs to cell detachment with different numbers of cells based on proliferation time, as shown in Figure 4a, second row. As displayed in the figure, the fluorescence intensity exhibited a gradual decline as the number of detached cells increased; at the same time, Z-PDs existed in an unevenly scattered state on coated surfaces, which was attributed to

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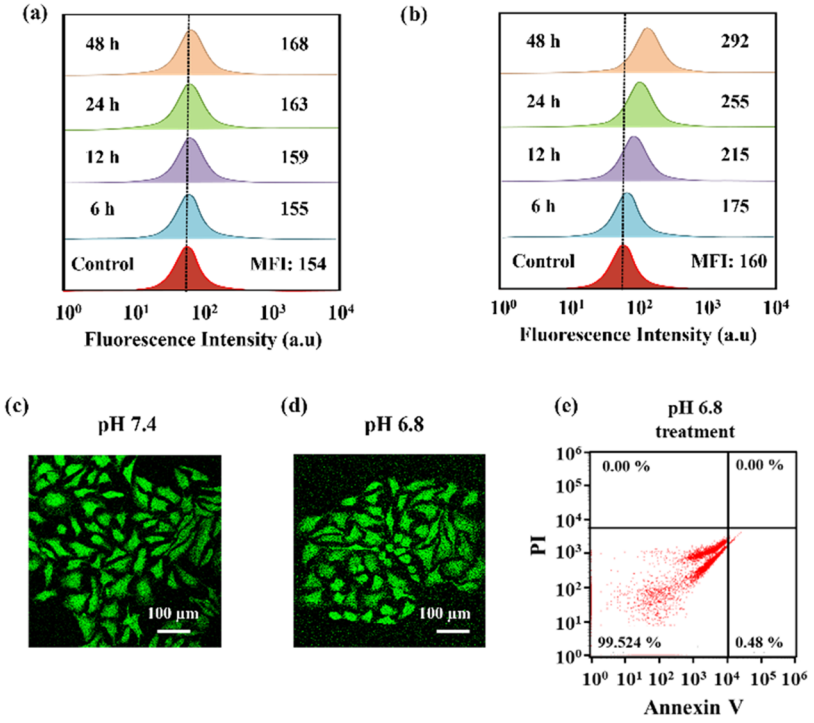

Figure 6. Flow cytometric analysis of HeLa cells cultured on Z-PD-coated substrates at (a) pH 7.4 detached by trypsin and (b) detached by pH 6.8 treatment. Live and dead cell assay using CLSM imaging of HeLa cells cultured on Z-PD-coated substrates for (c) pH 7.4 and (d) pH 6.8. (e) Live and dead cell assay using FACS for detached HeLa cells after pH 6.8 treatment.

positively charged transfer that induced Z-PD internalization into cells occurring simultaneously with cell detachment.[48,49]

As shown in Figure 4b, the resistance of the cell-detached biosensors increased compared to that of the preliminary pH 6.8-treated biosensors, which supported the hypothesis that cellular uptake occurred during the cell detachment process and increased the resistance of the biosensor as the number of detached cells increased due to more amount of Z-PD nanoparticles taken up as the proliferation time increased. The resistance signals obtained *via* the wireless system corresponded to the EIS signals, and the LEDs decreased in the intensity power, which was effectively sensed by the electrochemical instrument (Figure 4c).

Wireless Sensing of pH-Switchable Behavior of Z-PD Biosensors for Cell Detachment. Z-PD biosensors were used to compare the cell adhesion ability and cell detachment of Z-PD biosensors under various pH conditions. Acidic and basic treatment was performed after incubation of 10^3 HeLa cells on Z-PD-coated surfaces over 48 h at pH 7.4. As shown in Figure 5a, first row, at neutral pH, cells exhibited a strong affinity for the Z-PD-coated surface, which retained the quenching state of the Z-PD-coated surface. Meanwhile, cells cultured on Z-PD-coated surfaces treated with pH 6.8 and 8.5 were progressively removed from the surface due to hydrophilic transitions that partially recovered the fluorescence of the Z-PD-coated surfaces. The pH-switchable behavior that influenced cell−surface interactions was further confirmed by EIS analysis of pH-treated cell-cultured Z-PD-coated surfaces. The pH-switchable behavior that influenced cell−surface interactions was further confirmed by EIS analysis of pH-treated cell-cultured Z-PD-coated surfaces. As shown in Figure 5b, after treatment with pH 6.8 and 8.5, the resistance of cells detached from coated substrates decreased remarkably, indicating the efficient detachment of cells under acidic and basic conditions. Additionally, the wireless signals at pH 6.8 and 8.5 represented much lower resistance lines, which corresponded to the EIS signals, constantly intensifying the power output of the LED lamps.

Flow Cytometric Analysis of Cells Detached from Z-PD-Coated Surfaces by pH Treatment. At pH 7.4, Z-PD-coated substrates displayed a potential for cell proliferation because of the stacking state, which exhibited high affinity in terms of cell adhesion. However, at pH 6.8 and 8.5, antifouling effects occurred that enabled cells to be removed from the coated surfaces due to the hydrophobic−hydrophilic transition, simultaneously causing a recovery of the fluorescence intensity on Z-PD-coated surfaces, accompanied by charge transfer. Flow cytometry analysis was conducted to determine the fluorescence intensity recovery of Z-PDs through cellular uptake on Z-PD-coated surfaces in response to acidic treatment that resulted in cell detachment. HeLa cells (10^5 cells/well) were separately incubated for 6, 12, 24, and 48 h, followed by the indicated conditions. PBS buffer (pH 6.8) was used 1.5 h after each incubation time to completely detach cells from Z-PD-coated surfaces; the cells were then collected and analyzed by FACS. HeLa cells cultured on coated surfaces at pH 7.4 were employed as controls and detached by trypsin treatment. As shown in Figure 6a, the group treated at pH 7.4 did not show any significant change in terms of the mean fluorescence intensity, and the highest value after the cells were incubated for 48 h and had detached was 168 (with the control value of 155) corresponding to 8.3%, as the Z-PD nanoparticles existed in the quenched state at neutral pH. However, the mean fluorescence intensity value gradually increased and reached a value of 292 (with the control value of 160) after the cells were detached at pH 6.8, representing approximately 81.2%, which was due to the fluorescent recovery of Z-PD nanoparticles in cationic form in an acidic environment that

https://doi.org/10.1021/acs.analchem.1c01778
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exhibited a strong fluorescence intensity, leading to an effective increase in the mean fluorescence intensity value. The enhanced cellular uptake of Z-PDs at pH 6.8 demonstrated the distinction of the Z-PD biosensor in accordance with various pH treatments, which proved the utility of the pH-responsive probe in manipulating and monitoring cell−surface adhesion and detachment. To observe viability of cells after pH detachment, live and dead assays were performed in the presence of calcein AM and PI (Figures 6c,d and S8a). No red signal appeared in the cells after detachment, which proved the viability of the detached cells after treatment. In addition, viable and dead cells were detected by FACS to confirm the living cells, which showed that almost all cells were viable after detachment by acidic and basic treatments (Figures 6e and S8b).

CONCLUSIONS

Herein, we developed a wireless electrochemical biosensor using Z-PD-coated substrates to monitor cell proliferation and detachment, owing to the hydrophobic−hydrophilic transition of the zwitterionic PD-coated substrates in response to different pH levels. At neutral pH (pH 7.4), the Z-PD biosensors demonstrated a high binding affinity for cell adhesion due to the hydrophobic interactions that occurred on the coated surfaces featuring electrostatic effects, which gradually led to an increase in the resistance signals of the biosensors as the cells proliferated continuously. In contrast, under acidic and basic conditions, Z-PD biosensors exhibited relatively positive/negative charge transfer, accompanied by hydrophilic transitions on the coated substrates to induce cell detachment which, in addition, altered the electrochemical signals of the biosensors. The electrical resistance of the cell-cultured biosensors after pH 6.8 buffer treatment progressively increased as the cells initiated detachment with regard to the internalization of Z-PDs into the cell membrane simultaneously induced *via* positively charged surfaces. Therefore, Z-PD biosensors exhibited a high potential for manipulating cell adhesion as well as monitoring cell detachment, which could be identified by changes in electrochemical signals promoted by pH treatment, whose signals could be easily obtained *via* a wireless system without any interference from conventional optical methods.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.1c01778.

Material and characterization; UV−vis spectroscopy of PVP-CCDP-SO_3^-; water contact angle of the Z-PD-coated substrate under pH 6.8, 7.4, and 8.5 treatment; cell viability of Z-PDs at different concentrations; CLSM imaging of cell attachment depending on cell density on Z-PD-coated substrates at pH 7.4; CLSM imaging of cell proliferation at pH 7.4 depending on incubation time with a cell density of 10^3 cells/substrate; EIS study for the stability of Z-PD-coated substrate; FACS analysis for cell counting after cell proliferation at pH 7.4 depending on incubation time with cell density 10^3 cells/well; live−dead assays after cell detachment by pH 8.5 treatment after 48 h proliferation at pH 7.4 with cell density 10^3 cells (PDF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Basic Science Research Program (2020R1A2B5B02001500 and 2018R1A6A1A03023788), the Regional Innovation Strategy (2020 RIS) through the National Research Foundation of Korea (NRF), and the Korea Medical Device Development Fund grant funded by the Korea government (the Ministry of Science and ICT, the Ministry of Trade, Industry and Energy, the Ministry of Health & Welfare, Republic of Korea, and the Ministry of Food and Drug Safety) (202011D08)

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https://doi.org/10.1021/acs.analchem.1c01778
Anal. Chem. 2021, 93, 8638−8646