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Highly Fluorescent Conjugated Polyelectrolyte for Protein Sensing and Cell-Compatible Chemosensing Applications

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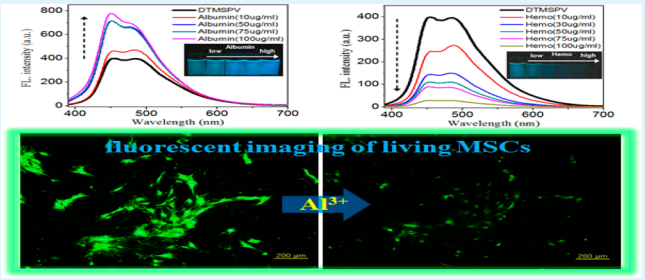
Supporting Information

ABSTRACT: Using a highly fluorescent, water-soluble polymer derived from a triazine-bridged copolymer (DTMSPV), we explored the tunable fluorescence properties of the water-soluble DTMSPV by solvent polarity to function as a fluorescence sensory probe for protein sensing. The green-blue fluorescence from DTMSPV was significantly enhanced in the presence of bovine serum albumin through hydrophobic interactions. Meanwhile, complete quenching of the fluorescence from DTMSPV occurred in the presence of hemoglobin through iron complexation with the polyelectrolyte. In addition, the DTMSPVs were highly fluorescent and permeated into living mesenchymal stem cells (MSCs), enabling effective imaging of the MSCs. This permeation into stem cells is crucial to the detection of Al^{3+} in living MSCs. The interaction between the triazine units in DTMSPV with the Al^{3+} ions allows for the detection of Al^{3+} in living cells. Thus, a strong fluorescence from living MSCs pretreated with DTMSPV was quenched as a function of the Al^{3+} concentration, confirming that DTMSPV is a cell-permeable fluorescent polymer that can function as a versatile probe to detect Al^{3+} in living cells.



KEYWORDS: *water-soluble polymer, conjugated polymer, protein sensor, cell imaging, chemosensing*

1. INTRODUCTION

Fluorescent conjugated polymers that interact with external stimuli have received considerable attention as potential fluorescence sensors and chemosensors because of their distinct electronic and optical properties, which arise from their highly delocalized backbone structures.[1−7] In particular, water-soluble fluorescent conjugated polyelectrolytes (CPEs) with a protein-sensing capability and cell permeability are of great importance in applications for proteomics, pathogen detection, and medical diagnostics; this is because a specific change in protein expression in living cells is strongly related to the generation of diseases.[8,9] CPEs consist of electron-delocalized conjugated polymer backbones with tunable charged (anionic or cationic) side chains. These electron-delocalized polymer backbones lead to signal amplification and high sensitivity based on rapid intrachain and interchain energy transfer. In one study, the fluorescence of conjugated polymers was quenched 60 times more effectively than that of the corresponding monomeric molecules in response to target molecules.[10] In addition, their numerous charges offer opportunities for multivalent interactions with biomolecular targets other than just water solubility.[11] Their unique electrical and optical properties are heavily impacted by molecular confirmation and supramolecular assembly. Therefore, understanding the structural changes in CPEs in response to external targets is necessary to design new materials with improved performance and to study conformational changes in biomolecules at a fundamental level.

Recently, we reported novel fluorescent polymers showing well-organized organic nanostructures, establishing the relationship between the polymer properties and molecular interaction-induced nanostructural changes.[12−14] Specifically, the highly fluorescent, water-soluble triazine-bridged copolymer poly-[(diphenylamino-*s*-triazine)-*co*-(2-methoxy-5-propyloxysulfonate-1,4-phenylenevinylene)] (DTMSPV) is highly sensitive and selective toward Al^{3+} ions (Figure 1).[14] Thus, it is a challenge to explore the specific interactions of this highly fluorescent polyelectrolyte with its biomolecular target analytes, given that the low fluorescence quantum yields of previously known polyelectrolytes have limited their use in biological assays. The absolute fluorescence quantum yield of DTMSPV in water is 31%, which is much higher than that of the homopolymer poly(2-methoxy-5-propyloxysulfonate-1,4-phenylenevinylene) (MPS-PPV, <1%; Figure 1).[15] Under the same analysis conditions, the aqueous solution of DTMSPV exhibited ∼60 times higher intensity than that of MPS-PPV. This high quantum yield for DTMSPV is probably due to the alternatively connected rigid units of triazine and phenylene,

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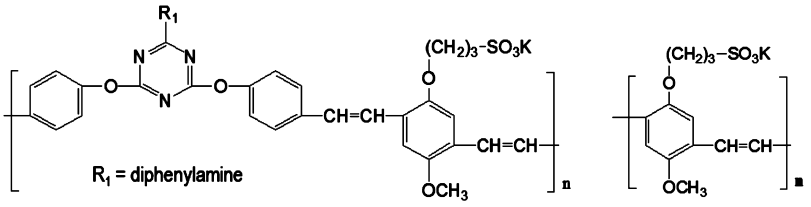

Figure 1. Chemical structures of the anionic water-soluble copolymer DTMSPV and homopolymer MPS-PPV.

which likely maintain the planarity along the polymer backbone, reducing self-quenching.[16] The high fluorescence quantum yield and intensity of DTMSPV probes are exceptionally desirable for detecting the presence of target analytes and deciphering sensor signals.

In this study, we report the tunable fluorescence characteristics of the water-soluble polyelectrolyte (DTMSPV) as a fluorescence sensory probe for protein sensing as well as a fluorescence imaging probe for living cells. We first examined the effect of the solvent polarity on the fluorescence of DTMSPV and then explored protein sensing and selective detection of Al^{3+} in cells with DTMSPV. The identification and quantification of aluminum ions in living cells are significant issues in medical and clinical research, given that aluminum ions have severe toxic effects and have been implicated in neurodegeneration, dementia, Alzheimer's disease, anemia, and bone and joint disease.[17−19]

2. EXPERIMENTAL SECTION

2.1. Materials. DTMSPV and MPS-PPV were synthesized with 4,6-bis(4-formylphenoxy)-2-diphenylamino-*s*-triazine and 2-methoxy-5-(3-sulfonatopropoxy)-1,4-xylenebis(triphenylphosphonium bromide) through the Wittig condensation and Gilch reactions, respectively, according to previously reported procedures.[14,20,21] Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), antibiotics (penicillin/streptomycin), phosphate-buffered saline (PBS; pH 7.4), trypsin/ethylenediaminetetraacetic acid (0.05%), and trypan blue (0.4%) were purchased from Gibco (Invitrogen, Carlsbad, CA). Gelatin from porcine skin type A, albumin from bovine serum (BSA), and hemoglobin (Hb; human) were purchased from Sigma-Aldrich. α-Amylase (α-Am, from *Bacillus licheniformis*), human serum albumin (HSA), lipase (Lip, from *Candida rugosa*, type VII), gelatin, and poly(L-lysine) were also purchased from Sigma. Collagen was purchased from BD Biosciences. Potassium *tert*-butoxide was purchased from TCI Co. (Tokyo, Japan) and used without any further purification. Other chemicals and solvents were purchased from Aldrich and used as received. Bone-marrow-derived mesenchymal stem cells (MSCs), obtained with patient approval (Severance Hospital), were used in this study. A frozen stock of MSCs at passage 3 was provided by the Cell Therapy Center, Severance Hospital (Yonsei University, Seoul, Korea).

2.2. Instruments and Sample Preparation. ^{1}H NMR spectra were obtained using a Varian Unity/Inova NMR 500 MHz spectrometer. Fluorescence spectra were obtained with a luminescence spectrometer (model LS55, Perkin-Elmer, Waltham, MA) under excitation at 370 nm. The absolute quantum yield of the water-soluble conjugated copolymer was determined using an absolute photoluminescence quantum yield measurement system (C9920-02; Hamamatsu Photonics, Shizuoka, Japan) at an excitation wavelength of 370 nm produced by a 150 W xenon lamp. The weights of DTMSPV, aluminum ions, and proteins were measured using a microbalance with an accuracy of 0.001 mg (CPA2P; Sartorius, Göttingen, Germany). Distilled water was used in all experiments. The polymer concentration was calculated with the monomer repeat unit.

2.3. Imaging of MSCs Incubated with DTMSPV and Al^{3+}. MSCs were thawed and plated at a density of about 10000 cells/cm^2 in 15 mL of a medium (DMEM supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin) in a 75 cm flask (Nunc, Roskilde, Denmark), at 37 °C in 5% humidified CO_2. Two days before imaging, MSCs were passaged and cultured on 12-mm glass coverslips coated with gelatin (0.1%). Immediately before imaging, MSCs were washed with a PBS buffer (without calcium and magnesium) to remove any residual serum in the cell medium and incubated with DTMSPV (6.6×10^{-5} M) in a PBS solution for 1 h at 37 °C. After MSC staining was confirmed, MSCs were washed twice with PBS to remove any remaining DTMSPV solution. Then, MSCs were further treated with Al^{3+} (3.6×10^{-5} and 3.6×10^{-7} M) in a PBS solution. The treated MSCs were imaged using an Olympus BX51 fluorescence microscope with WB dichroic mirrors DM500 and DM570, excitation filters BP450-480 and BP510-550, barrier filters BA515 and BA590, and an Olympus inverted research microscope (model IX71). To confirm fluorescence quenching caused by Al^{3+}, control samples of MSCs, which were not treated with Al^{3+}, were prepared and examined to determine the quantitative degree of the fluorescence change.

3. RESULTS AND DISCUSSION

3.1. Effect of the Solvent Polarity on the Fluorescence of DTMSPV. As shown in Figure 1, the water-soluble polyelectrolytes (DTMSPV) are alternatively connected with rigid and hydrophobic units of triazine and phenylene in a polymer backbone; this is a structure different from that of the homopolymer, MPS-PPV, which has *p*-phenylene units only. As mentioned previously, this unique chemical structure of DTMSPV leads to a high quantum yield, making it suitable for use as an effective fluorescence probe.

In our previous study,[14] we showed that the amphiphilic polymer (DTMSPV) self-assembled into a micelle-like structure (DTMSPV-MS) in a dilute aqueous solution but formed a π-stacking structure (DTMSPV-πS) in a concentrated solution (DTMSPV > 6.8×10^{-5} M). The nanostructures of DTMSPV at different concentrations were easily detected by the fluorescence of the solution; thus, any change in the concentration, the nanostructure of DTMSPV, or the metal-ion concentration could be estimated simply by monitoring the intensity and spectral shift of the fluorescence of the DTMSPV solution. Here, to clearly understand the interaction of DTMSPV with proteins based on hydrophobic interactions, the fluorescence of DTMSPV in different solvent polarities was examined by changing the tetrahydrofuran (THF) content in an aqueous solution.

The aqueous solution of DTMSPV (4.6×10^{-4} M) showed a fluorescence band maximized at ∼500 nm, indicating formation of the π-stacking structure (DTMSPV-πS), as previously reported.[14] The fluorescence intensity of DTMSPV increased upon the addition of THF (Figure 2). Interestingly, the relative intensity, as represented by the intensity ratio (I/I_0) of the THF solution (I) to water (I_0), increased to 3.77 when the THF content reached 70% [water/THF ratio of 3:7 (v/v)].

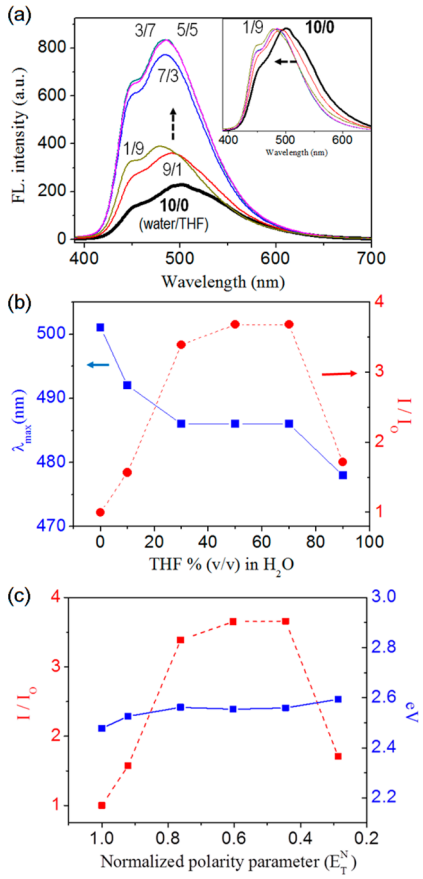

Figure 2. (a) Fluorescence spectra of DTMSPV in a mixed solution (4.6×10^{-4} M) of pure water and THF (excited at 370 nm). (b) Relative fluorescence intensity (I/I_0) and maxima of DTMSPV as a function of the THF content in water (the DTMSPV concentration is 4.6×10^{-4} M). (c) Relative fluorescence intensity (I/I_0) and maxima of DTMSPV as a function of the polarity parameter of the mixed solvent.

The fluorescence intensity was maximized in solutions containing 50% and 70% THF [water/THF ratio of 5:5 and 3:7 (v/v)] and then decreased under a high THF content [water/THF ratio of 10:90 (v/v)]. The maximum fluorescence wavelength was blue-shifted when the THF content was increased (Figure 2b).

The solubility parameters of distilled water (δ_t = 23.4 MPa$^{1/2}$) and THF (δ_t = 9.5 MPa$^{1/2}$) were obtained, and that of DTMSPV (δ_t = 35.9 MPa$^{1/2}$) was determined according to the Hansen solubility parameters to understand the fluorescence of DTMSPV in different solvents by changing the THF content in an aqueous solution.[22] Figure 2c shows the relative intensity and spectral shift in electronvolts (eV) versus the difference in the solubility parameters between DTMSPV and the solvent. It is well established that the fluorescence of conjugated polymers mainly comes from intrachain excitons and is very sensitive to the polymer structure.[23] In an aqueous solution, both electrostatic and hydrophobic interactions between the polymer chains are dominant factors influencing the fluorescence, and these interactions are critical in the πS structure. When THF was gradually added to the water solution, a strong interaction between the hydrophobic polymer backbone and THF evolved. This interaction could reduce the interchain interaction in the πS structure, which would cause an increase in the fluorescence intensity arising from decreased self-quenching.[24]

Although the mechanism for the enhanced fluorescence by the addition of THF requires further research, it is noteworthy that the enhancement is directly related to the polarity parameters of the solvents shown in Figure 2c. The normalized polarity parameters of the mixed solvents with different ratios are summarized in Table S1 in the Supporting Information (SI). These results indicate that the fluorescence of DTMSPV could be a measure of the polarity change around the polyelectrolytes, making it possible to analyze the polyelectrolyte interaction with proteins.

3.2. Detection of Proteins Using DTMSPV. The interaction between the anionic CPE DTMSPV and proteins such as BSA and Hb was investigated by fluorescence spectroscopy. In general, the detection of proteins using a fluorescent polymer can be explained in terms of three major processes: (1) Föster resonance energy or electron transfer between the polymer and analyte, (2) analyte-induced conformational change of the polymer; (3) analyte-induced aggregation of the polymer.[25,26]

As shown in Figure 3a, the fluorescence intensity of DTMSPV gradually increased as the concentration of BSA increased from 10 to 100 μg/mL. This fluorescence increase is similar to the solution having THF (Figure 2). Thus, such a fluorescence increase by the addition of BSA could be ascribed to the hydrophobic interaction between BSA and DTMSPV; this weakens the self-aggregation of DTMSPV, resulting in decreased self-quenching.[26] Although BSA has a negative charge in an aqueous solution (isoelectric point, PI = 5.4),[27] BSA has a hydrophobic unit.[28,29] Therefore, the hydrophobic interaction seems to be much stronger than the electrostatic force.

On the other hand, the fluorescence intensity of DTMSPV gradually decreased as the concentration of Hb increased from 10 to 100 μg/mL (Figure 3b). This fluorescence quenching of DTMSPV by Hb is thought to be attributable to the presence of Fe^{3+} in the heme group of the protein,[30] which strongly quenches the fluorescence of the conjugated polymer. This Hb-induced fluorescence quenching is supported by our previous study, in which DTMSPV exhibited a high quenching efficiency (Stern–Volmer quenching constant, K_{SV}) of 182700 M^{-1} in response to Fe^{3+} ions. Figure 3c shows the relative fluorescence intensity of the DTMSPV solution containing two different proteins. The fluorescence intensity increased as the BSA content increased, while it decreased as the Hb content increased.

In addition, the behavior of DTMSPV toward six different kinds of proteins was examined by fluorescence spectroscopy. These proteins possess diverse structural characteristics including metal/nonmetal-containing, molecular weight (M_w), PI, etc., as described in the literature.[31] The fluorescence response patterns were subjected to the kinds of proteins shown in Figure 3d. The relative fluorescence intensity of DTMSPV was gradually increased as the concentration of HSA increased from 10 to 100 μg/mL. This fluorescence increase is similar to the solution having BSA. Both BSA and HSA do not contain any metal ions. The hydrophobic interaction between HSA and DTMSPV increased the fluorescence intensity because of the decreased self-quenching in DTMSPV. The metal ion (Ca^{2+}), in α-Am, does not show strong interaction with DTMSPV, which matched to the poor sensitivity of DTMSPV on Ca^{2+} in a previous paper.[14] Therefore, the fluorescence intensity of DTMSPV did not change much with increasing Am concentration unlike Hb, of which Fe^{3+} ions

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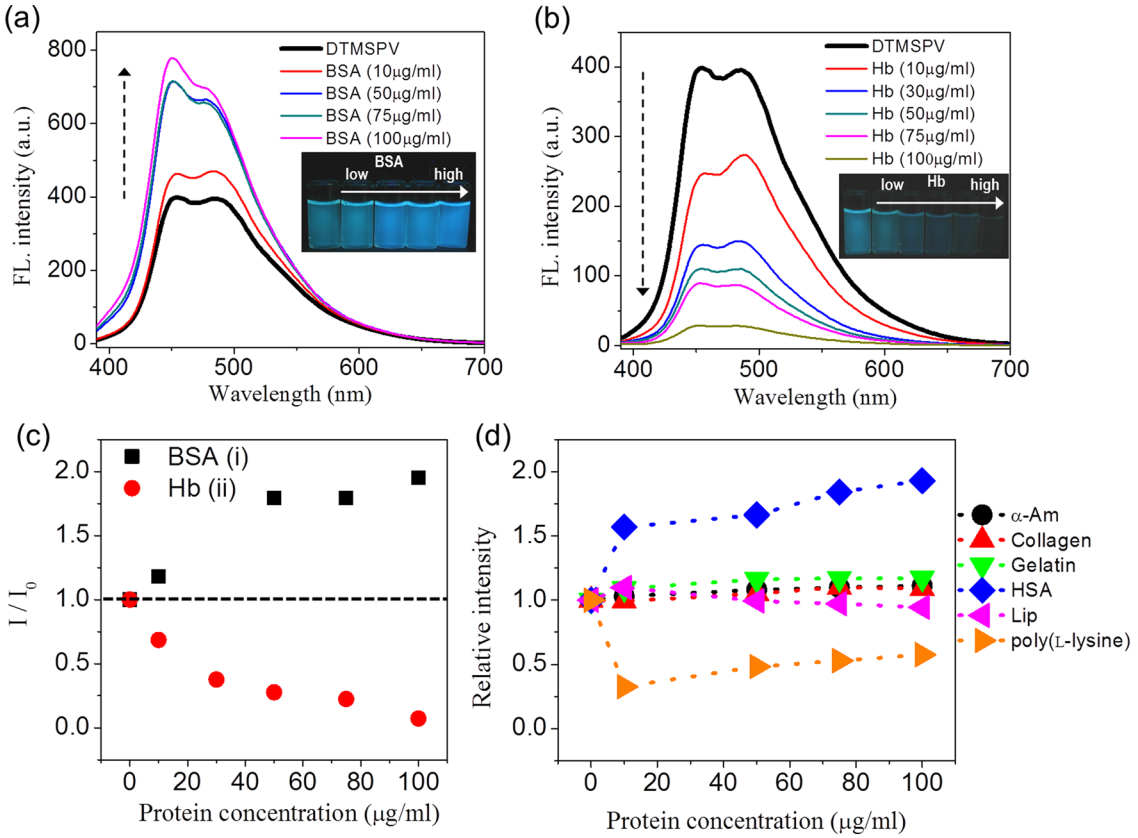

Figure 3. (a) Emission spectra of the DTMSPV solution (6.8 × 10−6 M) in the absence (black) and presence of (a) albumin and (b) Hb in aqueous solution. λ_{exc} = 370 nm The inset shows photographic images of DTMSPV in an aqueous solution with different protein concentrations excited at 365 nm using a hand-held UV lamp. (c) Plot of the relative fluorescence intensity (I/I_0) for DTMSPV in the presence of BSA (i) and Hb (ii) at different concentrations. (d) Relative fluorescence intensity for DTMSPV in the presence of α-Am, collagen, gelatin, HSA, Lip, and poly(L-lysine).

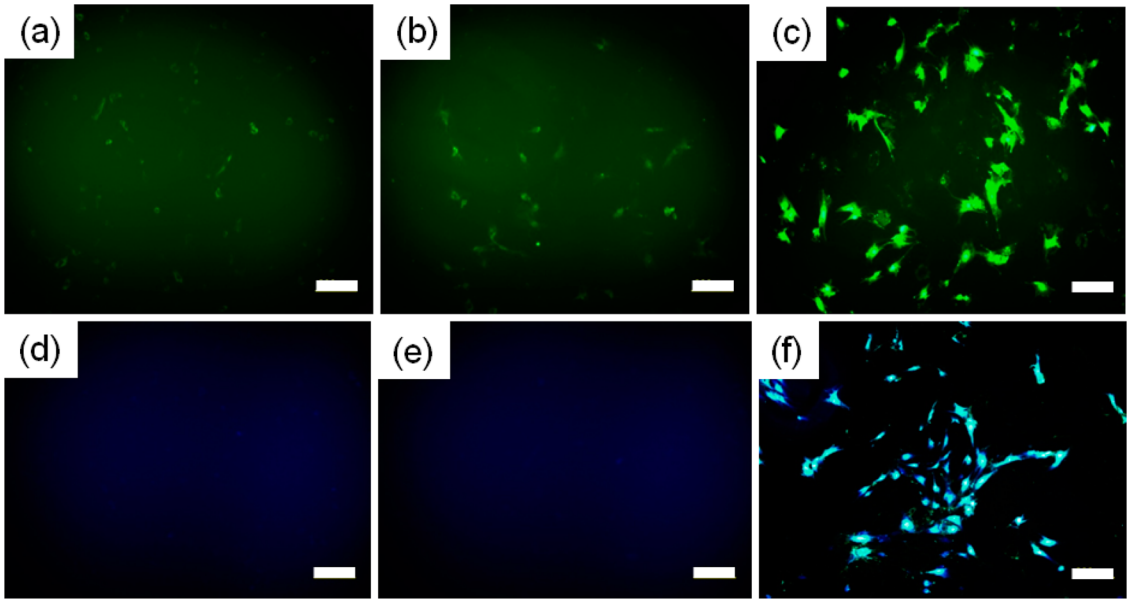

Figure 4. Fluorescence microscope images of MSCs with (a) and without (d) the fluorescent polymer, (b and e) with MPS-PPV and (c and f) with DTMSPV in a PBS solution (6.6 × 10^{-5} M). (a–c) Images obtained with a blue probe beam (450–480 nm). (d–f) Images obtained with a green probe beam (510–550 nm). Scale bar = 200 μm.

interact strongly with DTMSPV. Poly(L-lysine) is positively charged in water because of its high PI (9). Because of the positive charge with less hydrophobic structure, poly(L-lysine) showed a significant decrease in the fluorescence intensity of DTMSPV because it interacts strongly with DTMSPV. Lip has properties similar to those of BSA. However, the weak hydrophobic property of Lip resulted in no influence on the fluorescence intensity. Finally, the interactions of DTMSPV

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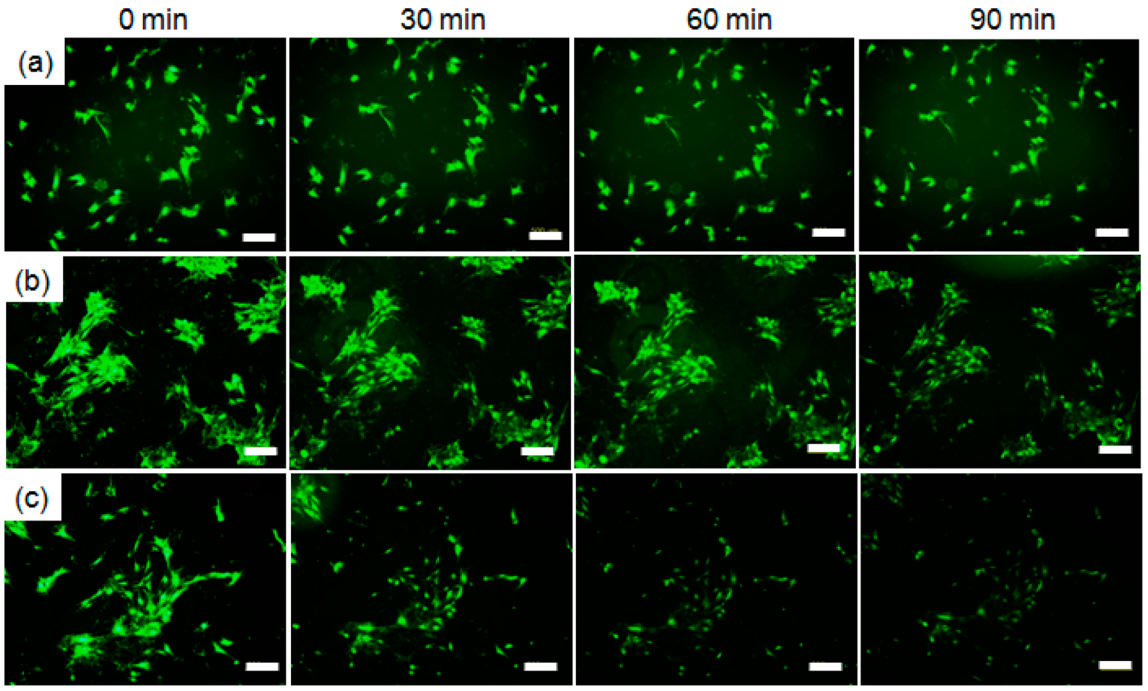

Figure 5. Fluorescence microscope images of DTMSPV-treated MSCs (a) in the absence of Al^{3+} and in the presence of (b) 0.36 and (c) 36 μM Al^{3+} for 0, 30, 60, and 90 min. Scale bar = 200 μm. See also the magnified images in Figure S1 in the SI.

with two similar ECM proteins, collagen and gelatin, were also analyzed. They did not show any significant fluorescent changes. Although more proteins should be further characterized, it seems that the metal ions, hydrophobicity, and surface charges of proteins are important to the sensitivity of DTMSPV. These results indicate that DTMSPV has potential applications in protein assays.

3.3. Imaging of MSCs and Detection of Al^{3+} in Living Cells. Taking advantage of the high fluorescence and water solubility of DTMSPV, MSCs were treated with the polyelectrolyte to image the cells through a fluorescence microscope. MSCs were incubated with DTMSPV in a PBS solution (6.6×10^{-5} M) for 1 h at 37 °C. Both MPS-PPV- and DTMSPV-untreated MSCs and only MPS-PPV-treated MSCs showed negligible intracellular fluorescence under a green probe beam, as well as no intracellular fluorescence under a blue probe beam, while DTMSPV-incubated MSCs showed a strong green-blue fluorescence and normal cell morphology (Figure 4). These results indicate that DTMSPV is a cell-permeable fluorescent polymer that can be applied as an imaging material for MSCs.

In our previous study, DTMSPV was shown to be a highly sensitive and selective sensing polyelectrolyte for Al^{3+} in the presence of competitive metals.[5] This distinctive concentration-dependent interaction of DTMSPV with Al^{3+} indicated that it would be possible to use DTMSPV to detect Al^{3+} in living cells. To detect and trace the Al^{3+} level in living cells, MSCs were treated with PBS solutions containing Al^{3+}. When the DTMSPV-pretreated MSCs were further treated with PBS solutions containing different Al^{3+} concentrations (0.36 and 36 μM), fluorescence quenching of the DTMSPV-treated MSCs varied depending on the Al^{3+} concentration (Figure 5b,c). When MSCs were treated with a solution containing a high Al^{3+} concentration (36 μM) for 90 min, MSCs exhibited a very weak fluorescence compared to those treated with a low Al^{3+} concentration (0.36 μM). MSCs untreated with Al^{3+} (Figure 5a) still showed a strong fluorescence signal even after 90 min, indicating that fluorescence quenching was caused by Al^{3+} (see also magnified images in Figure S1 in the SI). As shown in Figure 6, about 90% of the fluorescence was extinguished when

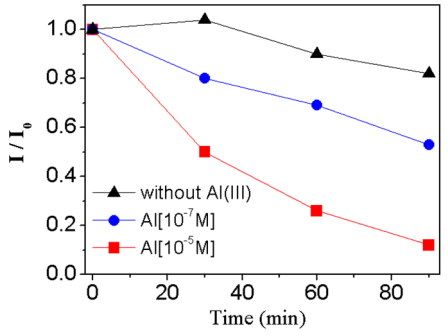

Figure 6. Fluorescence decay (I/I_0) of DTMSPV-treated MSCs over time under different Al^{3+} concentrations. I = fluorescence intensity after Al^{3+} treatment. I_0 = fluorescence intensity before Al^{3+} treatment.

MSCs were treated with a solution containing a high Al^{3+} concentration (36 μM) for 90 min. Importantly, ~50% of the fluorescence was extinguished when MSCs were treated with a solution containing a low Al^{3+} concentration (0.36 μM), being 10 times lower than the critical Al^{3+} concentration (3.7×10^{-6} M) in drinking water. Despite the low Al^{3+} concentration, it is known that dementia and Alzheimer's disease may be associated with Al^{3+} even at a level as low as 0.36 μM. In the absence of Al^{3+}, fluorescence quenching of MSCs after 90 min was less than 15% (Figure 6). These results indicate that DTMSPV can be used as a probe for efficient monitoring of intracellular Al^{3+} levels within living cells.

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4. CONCLUSION

The fluorescence of DTMSPV was enhanced when THF was added because of interaction of the hydrophobic unit of the polyelectrolyte with THF. Similarly, in the presence of BSA, DTMSPV showed a fluorescence increase through the hydrophobic interaction between DTMSPV and BSA. On the other hand, the fluorescence of the DTMSPV solution decreased in the presence of the metalloprotein Hb because iron ions in the heme units quench the fluorescence of DTMSPV. The DTMSPV polyelectrolyte, being cell-permeable, was used in the fluorescent imaging of living MSCs. The strong fluorescence from DTMSPV-treated MSCs was quenched as a function of the Al^{3+} concentration, confirming that DTMSPV is a suitable probe for efficient monitoring of intracellular Al^{3+} levels within living cells.

■ ASSOCIATED CONTENT

Supporting Information

Normalized polarity parameter of mixed solvents and magnified fluorescent microscope images of MSCs with Al^{3+}. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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