analytical chemistry

Article

pubs.acs.org/ac

Chromatic Biosensor for Detection of Phosphinothricin Acetyltransferase by Use of Polydiacetylene Vesicles Encapsulated within Automatically Generated Immunohydrogel Beads

Sung-Ho Jung,[†,‡,▽] Huisoo Jang,[†,‡,▽] Min-Cheol Lim,[§] Jae-Hwan Kim,[§] Kong-Sik Shin,[∥] Sun Min Kim,[‡,⊥] Hae-Yeong Kim,[§] Young-Rok Kim,[§] and Tae-Joon Jeon*[,†,‡]

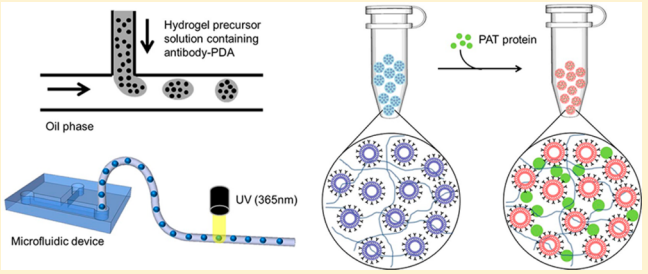
[†]Department of Biological Engineering, [‡]Biohybrid Systems Research Center, and [⊥]Department of Mechanical Engineering, Inha University, Incheon 402-751, Korea

[§]Institute of Life Science and Resources and Department of Food Science and Biotechnology, Kyung Hee University, Yongin 446-701, Korea

[∥]Biosafety Division, National Academy of Agricultural Science, Rural Development Administration, Jeonju 560-500, Korea

Supporting Information

ABSTRACT: We developed a simple and sensitive colorimetric biosensor in the form of microparticles by using polydiacetylene (PDA) vesicles encapsulated within a hydrogel matrix for the detection of phosphinothricin acetyltransferase (PAT) protein, which is one of the most important marker proteins in genetically modified (GM) crops. Although PDA is commonly used as a sensing material due to its unique colorimetric properties, existing PDA biosensors are ineffective due to their low sensitivity as well as their lack of robustness. To overcome these disadvantages, we devised immunohydrogel beads made of anti-PAT-conjugated PDA vesicles embedded at high density within a poly(ethylene glycol) diacrylate (PEG-DA) hydrogel matrix. In addition, the construction of immunohydrogel beads was automated by use of a microfluidic device. In the immunoreaction, the sensitivity of antibody-conjugated PDA vesicles was significantly amplified, as monitored by the unaided eye. The limit of detection for target molecules reached as low as 20 nM, which is sufficiently low enough to detect target materials in GM organisms. Collectively, the results show that immunohydrogel beads constitute a promising colorimetric sensing platform for onsite testing in a number of fields, such as the food and medical industries, as well as warfare situations.



Genetically modified organisms (GMOs) have been developed and cultivated in many countries to enhance crop yields.[1] To effectively manage labeling and safety evaluation systems along GMO production and distribution chains, there is an increased demand for monitoring of GMOs, food ingredients, and feed products derived from GMOs.[2,3] Among several GMO detection methods that have been developed to date, polymerase chain reaction (PCR)-based DNA detection methods are mainly used.[4,5] However, PCR-based methods are time- and labor-consuming, precluding routine and rapid analyses such as point-of-care diagnosis. Protein-based detection methods such as enzyme-linked immunosorbent assay (ELISA) are also commonly used for GMO detection.[6,7] One immunoassay method is the lateral flow test (LFT), which has advantages in terms of detection time and ease of use.[8] However, despite its many advantages, the LFT method is limited in terms of quantitative detection and sensitivity.[9]

Polydiacetylenes (PDAs) are often used as smart sensing materials in label-free biosensor systems due to their unique chromism properties, which include blue−red color transition and red fluorescence emission.[10] Polymerizable PDA vesicles consisting of diacetylene monomers readily self-assemble in aqueous solution.[11] Polymerized PDA vesicles then undergo a dramatic blue−red chromic transition, exhibiting red fluorescence emission upon exposure to external stimuli such as heat, pH changes, organic solvents, ionic liquids, and ligand−receptor interactions.[12−16] As a result, PDAs are widely used in qualitative and quantitative chemo/biosensor applications.[17,18]

In most PDA-based biosensors, PDAs are suspended in liquid phase or immobilized on a solid substrate.[19] PDAs suspended in liquid phase are often used to monitor the presence of target molecules after modification of the surface of PDA vesicles with target-recognizable ligands due to their ease of preparation.[20] The unique characteristics of PDAs enable the straightforward detection of target molecules by the blue−red color transition of PDA vesicles. In contrast, liquid-phase PDA sensors have some undesirable limitations, including aggregation of PDA vesicles, low detection sensitivity, and a large amount of analytes. In addition, the color intensity of the PDA

Received: May 14, 2014
Accepted: January 23, 2015
Published: January 23, 2015

DOI: 10.1021/ac501795x
Anal. Chem. 2015, 87, 2072−2078

vesicle solution may deteriorate upon addition of the sample solution, which makes the removal of PDA vesicles from the reaction solution unfeasible.[21] These problems can be alleviated by immobilization of PDA vesicles onto a solid surface.[22,23] However, immobilized PDA sensors require extra analytic tools such as a fluorescence spectrophotometer and/or fluorescence microscope due to low signal intensity. Further, immobilized PDA vesicles are easily detached from the solid surface during washing steps. To ameliorate these issues affecting immobilized PDA sensors, chemically conjugated PDA−silica microbead complexes have recently been developed by taking advantage of liquid-phase as well as immobilized PDA sensors.[23,24] In addition, physical encapsulation of PDA vesicles in a host matrix such as an agar scaffold, poly(vinyl alcohol) (PVA), alginate, electrospun microfiber, or poly(ethylene glycol) diacrylate (PEG-DA)[25] was carried out in order to improve the limitations of liquid-phase PDA sensors.[26−29]

To overcome the limitations of liquid-phase as well as immobilized PDA sensors, we devised a novel immunohydrogel bead system using PEG-DA hydrogel as a host matrix as well as antibody-functionalized PDA vesicles as a transducing material. In this system, PDA vesicles are encapsulated at high concentrations within the PEG-DA hydrogel matrix, resulting in generation of PEG-DA hydrogel beads, and both processes are carried out in a fully automated manner by use of a microfluidic T-junction channel.[30] The size of the PEG-DA hydrogel beads can be readily controlled by manipulating the channel dimensions. The automated microbead generation system allows the immunohydrogel beads to be separated from the solution without loss of any PDA vesicles. Additionally, the hydrogel beads can be removed from the sample solution without any contamination.

To demonstrate the proposed sensor system, we performed the detection of phosphinothricin acetyltransferase (PAT), which is a marker protein in GMOs. PAT protein is expressed in genetically engineered plants by the *bar* and *pat* genes from *Streptomyces hygroscopicus* and *Streptomyces viridochromogenes*, respectively.[31] Further, PAT-expressing transgenic plants display tolerance to herbicides such as glufosinate through acetylation of phosphinothricin.[32] Here, we used our sensor system to detect PAT proteins at various concentrations by the blue−red color transition of hydrogel beads, which is visible to the naked eye, and numerical data were conveniently obtained by simple red−green−blue (RGB) analysis of digital images. In addition, target molecules were quantitatively detected with heightened sensitivity (as low as 20 nM) or a limit of detection (LOD) of 0.14 μg/300 μL. Therefore, our biosensor system is more rapid and sensitive for the detection of biomolecules than existing liquid-phase PDA sensors and solid surface-immobilized sensors.

MATERIALS AND METHODS

Materials. 10,12-Tricosadiynoic acid (TCDA) was purchased from GFS Chemicals (Columbus, OH). 1,2-Dimyristoylphosphatidylcholine (DMPC) was purchased from Avanti Polar Lipids (Alabaster, AL). *N*-Ethyl-*N*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), ethanolamine, *N*-hydroxysuccinimide (NHS), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) powder, poly(ethylene glycol) diacrylate of molecular weight (MW) average M_n 700 (PEG-DA 700), 2-hydroxy-2-methylpropiophenon (Darocur 1173), Span 80, and Tris−ethylenediaminetetraacetic acid (EDTA) (1× , pH 7.4; TE buffer) were purchased from Sigma−Aldrich (St. Louis, MO). Chloroform was purchased from Junsei Chemical (Tokyo, Japan). Anti-PAT antibody and PAT proteins were prepared as described previously.[23] Water used in all experiments was doubly distilled.

Preparation of PDA Vesicles and Conjugation of Antibodies onto Surface of PDA Vesicles. PDA vesicles were prepared as described in our previous work.[23] Two types of lipid monomer, TCDA and DMPC, with a molar ratio of 8:2, were dissolved in chloroform to a final concentration of 2 mM in a glass vial. After chloroform was evaporated under a gentle stream of Ar gas, the vial was placed in a vacuum overnight to completely remove residual solvent. HEPES buffer solution (5 mM, pH 7.4) was then added to the glass vial to obtain a final lipid concentration of 2 mM. The lipid suspension was heated in a water bath for 1 h at 80 °C, followed by sonication (probe sonicator, Sonics & Materials, Inc., VCX 500) for 15 min. In order to remove lipid aggregates, the vesicle solution was filtered through a 0.8 μm syringe filter, and the filtered solution was cooled at 4 °C.

In order to conjugate antibodies onto the surface of PDA vesicles, EDC/NHS-coupled reaction was performed. Carboxylic acids of the TCDA headgroup were converted into succinimidyl active esters in the presence of excess NHS and EDC. First, the PDA vesicle solution was reacted with 20 mM EDC and 40 mM NHS in 5 mM HEPES buffer (pH 7.4) for 2 h. Extra NHS and EDC were then removed by centrifugation at 13000*g* (Eppendorf 5415R, Eppendorf Geratebau, Hamburg, Germany). To conjugate antibodies to succinimidyl active esters on the surface of PDA vesicles, the activated PDA vesicle solution was resuspended in HEPES buffer containing anti-PAT antibodies (300 μg/mL), after which the solution was incubated at 4 °C overnight. To remove unconjugated antibodies, the solution was washed twice with HEPES buffer and a centrifugal filter (MWCO 30 000 Da, Microcon, Millipore, Bedford, MA) was used. Remaining succinimidyl active esters were inactivated with 2 mM ethanolamine. Antibody-conjugated PDA vesicles were then irradiated with 254 nm UV light for a few minutes, resulting in a color change to blue.

Colorimetric Quantitative Detection of PAT Protein. In order to perform quantitative analysis, PAT protein at various concentrations was reacted with anti-PAT antibody-conjugated PDA vesicles suspended in solution or embedded within the hydrogel matrix. In detail, solution containing antibody-conjugated PDA vesicles (1 mM) was reacted with 0−2 μM PAT protein dissolved in 5 mM HEPES buffer at 37 °C for 1 h, and the final volume of the reaction mixture was 200 μL. For reaction test of PDA vesicles embedded in hydrogel matrix, hydrogel precursor solution containing antibody-conjugated PDA vesicles (1 mM) was polymerized by 365 nm UV light in a 96-well plate. Polymerized hydrogel was then reacted with 0−2 μM PAT protein dissolved in 5 mM HEPES buffer at 37 °C for 1 h, and the final volume of the reaction mixture was 200 μL.

To predict the amount of PAT protein, the degree of red color intensity of reacted sensor material was quantitatively calculated by the RGB module in Image J software (NIH, Bethesda, MD), which is widely used for characteristic analysis in analytical bioassay.[33] Furthermore, fluorescence intensities were measured on a plate reader (Safire, Tecan, Grodig, Austria) for further quantitative analysis. For fluorescence measurement, excitation and emission wavelengths were fixed at 540 and 620 nm, respectively.

DOI: 10.1021/ac501795x
Anal. Chem. 2015, 87, 2072−2078

Fabrication and Setup of PDMS Microfluidic Chip. The microfluidic device was fabricated according to standard photolithographic technique.[34] Channel depth was 500 μm, and the widths of oil and aqueous channels were 500 and 300 μm, respectively. Particularly, the structure of the oil channel widened from 500 to 700 μm. To prepare an SU-8 PR mold, negative photoresist (PR) was first coated onto the surface of a 4-in. Si wafer to a thickness of 500 μm by use of a spin coater. The PR-coated wafer was then prebaked at 95 °C and covered by a microchannel (CAD/Art Services, Ind. Bandon, OR) patterned film mask. The unblocked section of the PR-coated wafer was cured by UV light at 365 nm (570 mJ/cm^2). After removal of the film mask, the wafer was postbaked. The resulting SU-8 PR mold was then subjected to a development process that removed uncured PR from the surface of the wafer.

PDMS precursor solution, mixed with curing agent at a ratio of 10:1 (w/w), was poured into the SU-8 PR mold. Bubbles found in PDMS solution were removed by a vacuum pump before the PDMS was cured in an oven at 70 °C for 2 h. The cured PDMS replica was detached from the mold, and inlet and outlet channel entrances were punched. The PDMS replica was then attached to a slide glass (1 mm thick) cleaned by oxygen plasma (CUTE-100LF, FEMTO Science) for 1 min.[35] To restore the hydrophobicity of the channel surface, the device was stabilized in an oven at 65 °C overnight.

Preparation of Immunohydrogel Beads. Immunohydrogel beads consisted of PEG-DA hydrogel matrix encapsulating antibody-conjugated PDA vesicles. PEG-DA solution containing 50% PEG-DA and 5% Darocur 1173 (photoinitiator) mixed in 1× TE buffer was prepared first.[25,36] The PEG-DA precursor solution was prepared from a mixture of PEG-DA solution and antibody-conjugated PDA vesicle solution. In order to determine the optimal ratio of the mixture, five different mixtures were prepared at volumetric ratios of 4:1, 2:1. 1:1, 1:2, and 1:4.

To create the immunohydrogel beads, droplets of hydrogel precursor solution were made in the T-junction, which is the intersection of aqueous and oil channels in the microfluidic chip. Oil and aqueous phases were then injected into the main channel and subchannel, respectively. The flow rate of each stream was adjusted by use of syringe pumps (22 I/W, Harvard Apparatus Inc., Holliston, MA) with an oil-to-water flow rate ratio of 10. The oil phase was *n*-decane containing 2% Span 80 (w/w), whereas the aqueous phase was the hydrogel precursor solution of PEG-DA. Span 80 was added to the oil phase to prevent droplets from fusing with each other. The aqueous droplets containing hydrogel precursor were flowed through to the outlet tube connected to the main channel, upon which they were photopolymerized by UV illumination (λ = 365 nm) to produce immunohydrogel beads. A spot UV curing system (Ommicure S2000, Lumen Dynamics Group Inc., Ontario, Canada) was used with a UV light source (37.34 W/cm^2, 320−500 nm). In order to optimize curing conditions, the UV exposure energy was adjusted by tuning the UV intensity of the light source (100, 200, 300, and 370 J/cm^2) at a fixed exposure time of 10 s. Immunohydrogel beads were subsequently separated from the oil phase at the end of the outlet tube, which was soaked in a HEPES buffer-filled vial (see Supporting Information).

Immunoassay of Immunohydrogel Beads. To test the reactivity of the immunohydrogel beads, the same amount of immunohydrogel beads as PDA vesicles above was reacted with 0−2 μM PAT protein dissolved in 5 mM HEPES buffer at 37 °C for 1 h, and the final volume of the reaction mixture was 300 μL. To calculate numerical values, red color intensities in all reaction mixtures were calculated with the RGB module of ImageJ software (NIH, Bethesda, MD).

RESULTS AND DISCUSSION

Colorimetric Assay Using Polydiacetylene Vesicles. In this study, we prepared a chromatic biosensor consisting of PDA vesicles for the detection of phosphinothricin acetyltransferase (PAT) protein, which is one of the most important marker proteins in GM crops.[37] PDA vesicles were made from TCDA and DMPC at a ratio of 8:2, and they are known to display increased sensitivity in the color transition upon external stimulation due to noninterference of DMPC during the polymerization process.[38] Anti-PAT antibodies were conjugated onto the surface of PDA vesicles via EDC/NHS chemistry. When anti-PAT antibody-conjugated PDA vesicles were reacted with PAT protein for 1 h at 37 °C, no measurable color change was observed in the reaction mixture up to 0.4 μM PAT, but the CR (%) rapidly increased when more than 2 μM PAT protein was added (Figure 1A). The results shown here do not satisfy the limit of detection (LOD) required for GM biosensors, precluding the use of PDA vesicles as sensor materials. In addition, PDA vesicles were suspended in the reaction mixture, resulting in low color intensity. Thus, an additional centrifugation step was required to amplify the color intensity to suitable levels for on-site diagnosis.[23]

Signal Amplification by PDA Vesicles Embedded in Hydrogel. To overcome the disadvantages described above, we have embedded antibody-conjugated PDA vesicles at high density within a hydrogel matrix in order to amplify the signal ratio in the color transition. To select a proper host hydrogel, we tested the stability of PDA vesicles when encapsulated in various host matrixes including other commonly used hydrogels such as agarose, alginate, etc. Alginate triggered color change of PDA vesicle during the curing process, and agarose monomers were released during the detection process (Figure S1, Supporting Information). However, PEG-DA does not affect color change of PDA vesicles nor release host monomers. In addition, PEG-DA is suitable for an automated bead formation system with a microfluidic channel because it can be polymerized by UV illumination in a controlled manner. Thus, PEG-DA was used as proper host hydrogel matrix in our work. As shown in Figure 1B, color changes in PDA vesicles within the hydrogel were observable by the naked eye in response to various concentrations of PAT protein (0−2 μM). Furthermore, signal ratios in the color transition were enhanced in comparison to PDA vesicles suspended in solution. On the other hand, hydrogels containing PDA vesicles did not respond to bovine serum albumin (BSA) protein, as in Figure 1C, thereby demonstrating selectivity for target proteins.

Although the colorimetric response to PAT protein was still distinguishable by the naked eye, red color intensity was quantified by the RGB module in ImageJ software. Figure 1D shows the relative intensities of the colorimetric transitions at various analyte concentrations. The signal intensities of PDA vesicles embedded within hydrogel were higher than those of PDA vesicles suspended in solution, whereas PDA vesicles remained sensitive to PAT protein. Amplification of the signal was attributed to macromolecular confinement effect,[39] which means that immobilization of biological molecules in a confined area such as hydrogel matrix had a positive effect on stability and folding of biological molecules, resulting in enhanced

DOI: 10.1021/ac501795x
Anal. Chem. 2015, 87, 2072−2078

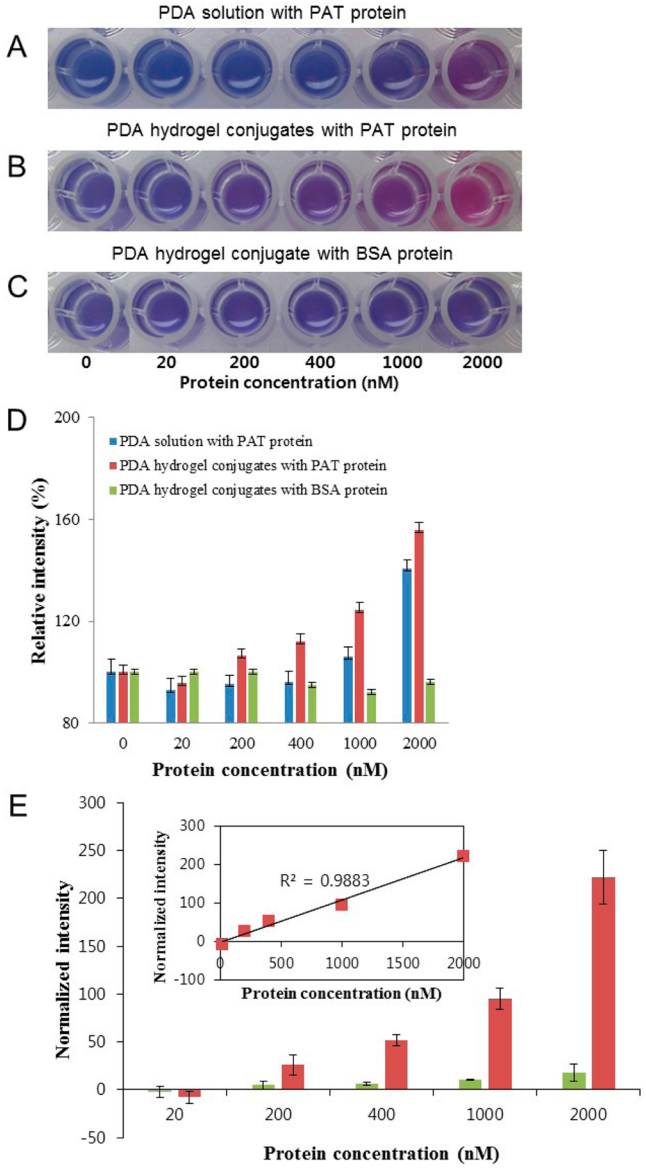


Figure 1. (A–C) Comparison of colorimetric changes of PDA vesicles with under various conditions and (D, E) plots of relative red color and fluorescence intensities. (A) Free PDA vesicle suspension reacted with various concentrations of PAT protein. (B) PDA vesicles embedded within hydrogel matrix reacted with various concentrations of PAT proteins. (C) PDA vesicles embedded within hydrogel matrix reacted with various concentrations of BSA proteins as a control. (D) Plot of relative intensities of red color in PDA vesicles and PDA vesicles embedded within hydrogel containing different concentrations of analyte. (E) Normalized intensity of red fluorescence from PDA vesicles embedded within hydrogel in response to various concentrations of PAT and BSA proteins.

activity of biological molecules. As such, activity of antibodies on the PDA vesicles in hydrogel matrix was far more enhanced than that in solution.

Measurement of the characteristic fluorescence emission of PDA vesicles provides another method to quantitatively analyze signals from corresponding immunoreactions. Red PDA vesicles were excited by fluorescence at 540 nm, while emission occurred at 620 nm. Fluorescence emission from red PDA vesicles was measured on a microplate reader after the immunoreactions. As shown in Figure 1E, the red fluorescence intensity from PDA vesicles embedded in hydrogel increased linearly with the amount of PAT protein, whereas no change in fluorescent emission was observed from reaction with BSA protein.

Preparation of Hydrogel Precursor and Automated Fabrication of Immunohydrogel Beads. To enhance the signal to target materials as well as enable storage and transportation for onsite testing, we developed PDA vesicles embedded in hydrogel in the form of microparticles with a higher surface area to volume ratio (Figure 2A). The

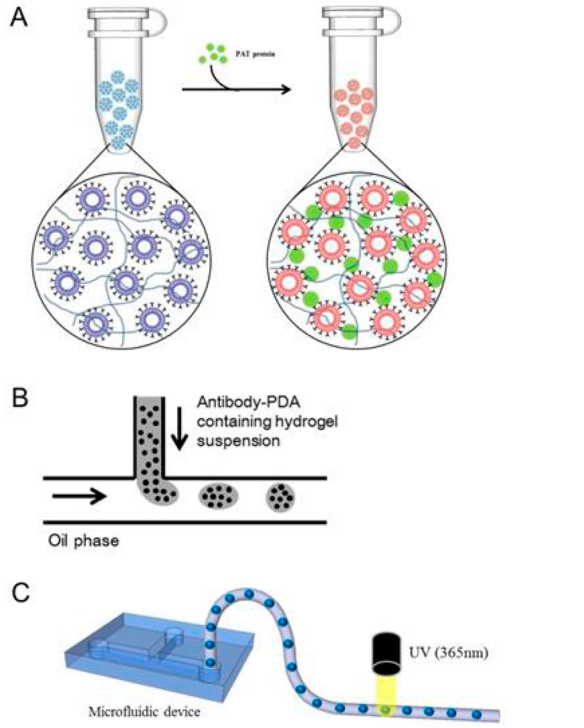


Figure 2. Schematic illustrations of (A) colorimetric detection of PAT proteins by use of PDA vesicles embedded in immunohydrogel beads, (B) immunohydrogel bead formation in T-junction microchannel, and (C) synthesis of immunohydrogel beads and photopolymerization.

construction of immunohydrogel beads was automated by use of a microfluidic T-junction device. First, we prepared hydrogel precursor solution by mixing PEG-DA solution and antibody-conjugated PDA vesicles at a 1:4 ratio. To maximize sensitivity, the hydrogel matrix must contain a large quantity of PDA vesicles. Therefore, various concentrations of both hydrogel precursor solution and PDA vesicles were tested. Hydrogel precursor solution consisted of PEG-DA solution, whereas PDA vesicles were made of TCDA and DMPC at a ratio of 8:2. Ratios of PDA vesicles to hydrogel precursor mixture were 4:1, 2:1, 1:1, 1:2, and 1:4 (v/v). The 1:4 mixture was subjected to color change measurement prior to reaction with the target substances, as PEG-DA solution has been shown to interact with the eneyne-conjugated backbone of PDA vesicles.[25] When the PDA vesicle and hydrogel precursor mixtures exceeded a 4:1 ratio, PEG-DA solution became relatively diluted, whereas hydrogel did not form under UV irradiation. Therefore, the 4:1 mixture of PDA vesicles and hydrogel precursor was able to maximize the concentration of PDA vesicles, thereby enhancing sensitivity of the immunohydrogel beads.

DOI: 10.1021/ac501795x
Anal. Chem. 2015, 87, 2072–2078

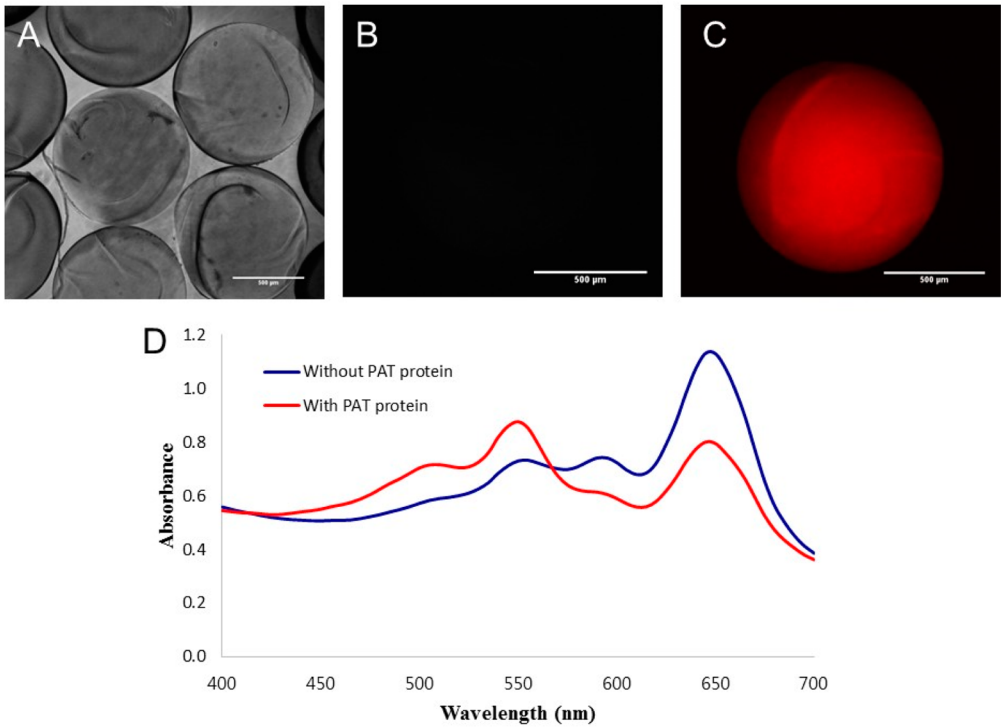


Figure 3. (A) Bright-field image of immunohydrogel beads containing antibody-conjugated PDA vesicles. (B, C) Fluorescence images of immunohydrogel beads before and after reaction with PAT protein. (D) Spectroscopic result of immunohydrogel bead with and without PAT protein.

The aqueous phase, including the optimized hydrogel precursor solution, was subsequently injected into the inlet located in the T-junction branch, whereas the oil phase was injected into the main channel. Uniform-sized droplets were continuously formed when flows of two immiscible phases encountered each other at the T-junction (Figure 2B). The size of the droplets was readily adjustable by controlling the flow velocity and the dimension of each channel. The appearance of large-sized droplets can be explained by the principle of continuity in fluid dynamics, as the outlet they passed through was connected to tubes having different inner diameters. To photopolymerize the hydrogel precursor solution into microparticles, the droplets in contact with the hydrogel precursor solution were immediately subjected to UV irradiation at 365 nm (Figure 2C). PDA vesicles turned red when overexposed to UV irradiation at high energy. Thus, the appropriate time and intensity of UV irradiation were shown to be important factors since PDA vesicles were suspended and traveled through a microfluidic channel. When the PEG-DA precursor solution was exposed to UV irradiation for more than 20 s, PDA vesicles turned red, whereas UV irradiation for only 10 s was enough to completely cure the hydrogel precursor solution. At the same time, four different UV intensities (100, 200, 300, and 370 J/cm^2) were tested to determine the optimal UV energy to generate hydrogel particles. The results show that 300 J/cm^2 gave most stable hydrogel particles, displaying a clear blue color.

Immunoassay of Immunohydrogel Beads. Immunohydrogel beads with ∼700 μm diameter were automatically generated from the device shown above (Figure 3A). The immunohydrogel beads showed no fluorescence before reaction with PAT protein, as shown in Figure 3B. On the other hand, immunoreaction with PAT protein was confirmed in Figure 3C by red fluorescence from PDA vesicles. Although PDA vesicles showed a visible red color and strong fluorescence intensity in response to external stimuli, their suspension in sample solution reduced their sensitivity. Thus, additional instrumentation or further analysis is required to obtain satisfactory results. Here, our immunohydrogel beads encapsulated PDA vesicles at high density within a hydrogel matrix; thereby the detection of target molecules was enabled by the unaided eye without any need for additional instrumentation. In addition, sensing materials were made in the form of large-sized particles, which makes the materials readily collectable and portable. Figure 4A demonstrates that target molecules could be detected for single hydrogel beads with the unaided eye. Each well contained six immunohydrogel beads corresponding to the same volume (10 μL) of PDA vesicle solution. Each reaction was carried out at various concentrations of PAT protein ranging from 0 to 2 μM at 37 °C for 1 h. Figure 4A, row 1, illustrates immunoreaction of the same amount of suspended PDA vesicles as there are PDA vesicles encapsulated within hydrogel beads. The immunoreactions in each well were not detectable since PDA vesicles were diluted to very low concentrations in the solution, resulting in almost no visible color. On the other hand, the immunohydrogel beads underwent very clear color changes as observed by just the naked eye (Figure 4A, row 2). The higher signal ratio of immunohydrogel beads to target materials was further analyzed, as shown in Figure 4B.

Negative control experiment was conducted in order to demonstrate specific detection ability of the immunohydrogel bead. To verify nonspecific interaction of PAT protein with PDA vesicle surface, hydrogel beads encapsulating antibody-unconjugated PDA vesicle were prepared. The hydrogel beads were reacted with high concentration of PAT protein (2 μM) under the same conditions of immunoreaction test described

DOI: 10.1021/ac501795x
Anal. Chem. 2015, 87, 2072−2078

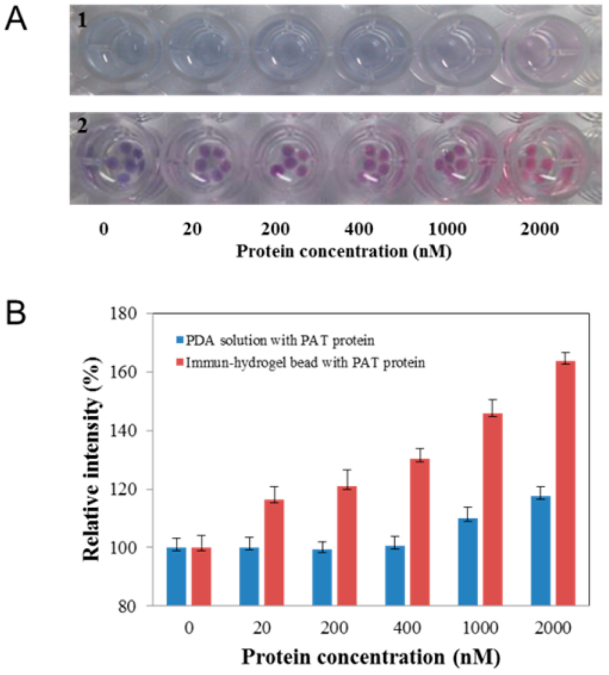


Figure 4. (A) Colorimetric changes of free PDA vesicles (row 1) and immunohydrogel beads (row 2), containing the same volume of PDA vesicles as in row 1, in response to various concentrations (0–2 μM) of PAT protein. Immunohydrogel beads with a diameter of 2 mm were used. (B) Plot of relative intensity of red color from free PDA vesicles and immunohydrogel beads with different concentrations of PAT protein.

above. As a result, the color of the hydrogel beads did not change (Figure S2, Supporting Information). Therefore, we demonstrated that PAT protein does not interact with PDA vesicles through nonspecific binding. To additionally verify nonspecific interactions of PAT protein with other antibodies, immunohydrogel beads encapsulating anti-hIgG antibody-conjugated PDA vesicles were also prepared. The prepared immunohydrogel beads were incubated with a high concentration of PAT protein under the same conditions described above. Again, the color of the immunohydrogel beads did not change (Figure S3, Supporting Information), indicating that PAT protein does not specifically interact with other antibodies. Thus, through negative control experiments, we demonstrated that PAT protein can be specifically detected by color change of the immunohydrogel beads encapsulating anti-PAT antibody-conjugated PDA vesicles (Figure 4A).

The immunohydrogel beads shown in this work could detect PAT protein at concentrations as low as ~20 nM. The sensitivity of our immunohydrogel beads is favorable in comparison with those of S-ELISA and CI-ELISA, which have LOD of 0.01 and 1.0 μg/mL for general immune reactions, respectively.[40] It was reported that the typical concentration of transgenic materials in plant tissues is higher than 10 μg/g.[41] For instance, the concentration of PAT protein in GM pepper is ~100–170 ng/300 μL of tissue solution.[40] Therefore, the LOD of our system (140 ng/300 μL reaction) is sufficiently low to detect typical target materials present in GMOs. Therefore, our immunohydrogel beads provide an excellent sensing platform compared to conventional PDA-based sensors. Other advantages of our system are that hydrogel beads are easily collectable and transportable.

CONCLUSIONS

In this study, we developed a novel sensing platform using hydrogel in the form of microparticles. The microparticles include PDA vesicles that are reactive to PAT protein, which is one of the most abundant marker proteins in GM crops. To demonstrate our biosensing platform, PAT proteins were reacted with immunohydrogel beads as well as PDA vesicles suspended in solution. The immunohydrogel beads showed much greater sensitivity than PDA vesicles in solution. Moreover, color changes in the immunohydrogel beads could be observed by just the naked eye, potentially widening their applicability. By encapsulating PDA vesicles within a hydrogel matrix at high density, we were able to achieve an enhanced signal ratio to target molecules as well as overcome the drawbacks of conventional sensing platforms based on PDA vesicles suspended in liquid phase. Other advantages include the simple and rapid detection of target molecules, and the reacted hydrogel beads are collectable by gravity only with no sample contamination. Furthermore, the immunohydrogel beads can be automatically generated by a microfluidic system and made in a ready-to-use form. Our novel PDA platform is also highly sensitive in both solution and solid forms, thereby enabling the detection of target molecules at low concentrations. Therefore, we believe that our sensing system will be a promising biosensing platform for onsite testing as well as for rapid and accurate diagnosis in a number of bioanalytical applications.

ASSOCIATED CONTENT

Supporting Information

Three figures, showing comparison of host hydrogel matrices and immunoreaction tests with antibody-unconjugated PDA vesicle and anti-hIgG antibody-conjugated PDA vesicle encapsulating immunohydrogel bead, and two video clips, showing formation of hydrogel bead precursors from the T-junction microfluidic device and polymerized hydrogels collected in a small vial. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail tjjeon@inha.ac.kr.

Author Contributions

▽S.-H.J. and H.J. contributed equally to this work.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Professor Moon Il Kim for valuable discussions on the macromolecular confinement effect and suggestions for appropriate references. This work was supported by grants from the Next-Generation BioGreen 21 Program (PJ007995) of the Rural Development Administration and National Research Foundation (NRF-2014R1A1A2059341) as well as by the Pioneer Research Center Program (NRF-2012-0009575) of the National Research Foundation of Korea. This work was also partially supported by Inha University Research Grant.

REFERENCES

(1) Singh, O.; Ghai, S.; Paul, D.; Jain, R. *Appl. Microbiol. Biotechnol.* **2006**, *71*, 598–607.

DOI: 10.1021/ac501795x
Anal. Chem. 2015, 87, 2072–2078

(2) Haslberger, A. G. *Science* **2000**, *287*, 431−432.
(3) Miraglia, M.; Berdal, K. G.; Brera, C.; Corbisier, P.; Holst-Jensen, A.; Kok, E. J.; Marvin, H. J. P.; Schimmel, H.; Rentsch, J.; van Rie, J. P. P. F.; Zagon, J. *Food Chem. Toxicol.* **2004**, *42*, 1157−1180.
(4) Holst-Jensen, A.; Bertheau, Y.; de Loose, M.; Grohmann, L.; Hamels, S.; Hougs, L.; Morisset, D.; Pecoraro, S.; Pla, M.; den Bulcke, M. V.; Wulff, D. *Biotechnol. Adv.* **2012**, *30*, 1318−1335.
(5) Kim, J.-H.; Jeong, D.; Kim, Y.-R.; Kwon, Y.-K.; Rhee, G.-S.; Zhang, D.; Kim, H.-Y. *Food Control* **2013**, *31*, 366−371.
(6) Erickson, B. E. *Anal. Chem.* **2000**, *72*, 454A−459A.
(7) Mazzara, M.; Paoletti, C.; Corbisier, P.; Grazioli, E.; Larcher, S.; Berben, G.; Loose, M.; Folch, I.; Henry, C.; Hess, N.; Hougs, L.; Janssen, E.; Moran, G.; Onori, R.; Eede, G. *Food Anal. Methods* **2013**, *6*, 210−220.
(8) Stave, J. W. *J. AOAC Int.* **2002**, *85*, 780−786.
(9) Posthuma-Trumpie, G.; Korf, J.; Amerongen, A. *Anal. Bioanal. Chem.* **2009**, *393*, 569−582.
(10) Reppy, M. A.; Pindzola, B. A. *Chem. Commun.* **2007**, 4317−4338.
(11) Cantow, H.-J.; Enkelmann, V. *Adv. Polym. Sci.* **1984**, *63*, 91−136.
(12) Jeong, S.; Ha, S. H.; Han, S.-H.; Lim, M.-C.; Kim, S. M.; Kim, Y.-R.; Koo, Y.-M.; So, J.-S.; Jeon, T.-J. *Soft Matter* **2012**, *8*, 5501−5506.
(13) Kew, S. J.; Hall, E. A. H. *Anal. Chem.* **2006**, *78*, 2231−2238.
(14) Phollookin, C.; Wacharasindhu, S.; Ajavakom, A.; Tumcharern, G.; Ampornpun, S.; Eaidkong, T.; Sukwattanasinitt, M. *Macromolecules* **2010**, *43*, 7540−7548.
(15) Wu, W.; Zhang, J.; Zheng, M.; Zhong, Y.; Yang, J.; Zhao, Y.; Wu, W.; Ye, W.; Wen, J.; Wang, Q.; Lu, J. *PLoS One* **2012**, *7*, No. e48999.
(16) Yoon, J.; Chae, S. K.; Kim, J.-M. *J. Am. Chem. Soc.* **2007**, *129*, 3038−3039.
(17) Ahn, D. J.; Kim, J.-M. *Acc. Chem. Res.* **2008**, *41*, 805−816.
(18) Kim, Y. R.; Jung, S.; Ryu, H.; Yoo, Y. E.; Kim, S. M.; Jeon, T. J. *Sensors* **2012**, *12*, 9530−9550.
(19) Park, C. H.; Kim, J. P.; Lee, S. W.; Jeon, N. L.; Yoo, P. J.; Sim, S. J. *Adv. Funct. Mater.* **2009**, *19*, 3703−3710.
(20) Su, Y.-l.; Li, J.-r.; Jiang, L. *Colloids Surf., B* **2004**, *38*, 29−33.
(21) Park, M.-K.; Kim, K.-W.; Ahn, D. J.; Oh, M.-K. *Biosens. Bioelectron.* **2012**, *35*, 44−49.
(22) Kim, J.-M.; Lee, Y. B.; Yang, D. H.; Lee, J.-S.; Lee, G. S.; Ahn, D. J. *J. Am. Chem. Soc.* **2005**, *127*, 17580−17581.
(23) Lim, M. C.; Shin, Y. J.; Jeon, T. J.; Kim, H. Y.; Kim, Y. R. *Anal. Bioanal. Chem.* **2011**, *400*, 777−785.
(24) Jun, B.-H.; Baek, J.; Kang, H.; Park, Y. J.; Jeong, D. H.; Lee, Y.-S. *J. Colloid Interface Sci.* **2011**, *355*, 29−34.
(25) Lee, N. Y.; Jung, Y. K.; Park, H. G. *Biochem. Eng. J.* **2006**, *29*, 103−108.
(26) Chae, S. K.; Park, H.; Yoon, J.; Lee, C. H.; Ahn, D. J.; Kim, J. M. *Adv. Mater.* **2007**, *19*, 521−524.
(27) Kim, J. M.; Lee, Y. B.; Chae, S. K.; Ahn, D. J. *Adv. Funct. Mater.* **2006**, *16*, 2103−2109.
(28) Lee, J.; Kim, J. *Chem. Mater.* **2012**, *24*, 2817−2822.
(29) Silbert, L.; Shlush, I. B.; Israel, E.; Porgador, A.; Kolusheva, S.; Jelinek, R. *Appl. Environ. Microbiol.* **2006**, *72*, 7339−7344.
(30) Jang, H.; Hu, P. C.; Jung, S.; Kim, W. Y.; Kim, S. M.; Malmstadt, N.; Jeon, T. J. *Biotechnol. J.* **2013**, *8*, 1341−1346.
(31) Lutz, K. A.; Knapp, J. E.; Maliga, P. *Plant Physiol.* **2001**, *125*, 1585−1590.
(32) De Block, M.; Botterman, J.; Vandewiele, M.; Dockx, J.; Thoen, C.; Gossele, V.; Movva, N. R.; Thompson, C.; Van Montagu, M.; Leemans, J. *EMBO J.* **1987**, *6*, 2513−2518.
(33) Acevedo, C. A.; Skurtys, O.; Young, M. E.; Enrione, J.; Pedreschi, F.; Osorio, F. *LWT–Food Sci. Technol.* **2009**, *42*, 1444−1449.
(34) Duffy, D. C.; McDonald, J. C.; Schueller, O. J.; Whitesides, G. M. *Anal. Chem.* **1998**, *70*, 4974−4984.
(35) Yu, S.; Jeon, T. J.; Kim, S. M. *Chem. Eng. J.* **2012**, *197*, 289−294.
(36) Appleyard, D. C.; Chapin, S. C.; Srinivas, R. L.; Doyle, P. S. *Nat. Protoc.* **2011**, *6*, 1761−1774.
(37) Herouet, C.; Esdaile, D. J.; Mallyon, B. A.; Debruyne, E.; Schulz, A.; Currier, T.; Hendrickx, K.; van der Klis, R. J.; Rouan, D. *Regul. Toxicol. Pharmacol.* **2005**, *41*, 134−149.
(38) Park, C. K.; Kang, C. D.; Sim, S. J. *Biotechnol. J.* **2008**, *3*, 687−693.
(39) Zhou, H. X.; Rivas, G. N.; Minton, A. P. *Annu. Rev. Biophys.* **2008**, *37*, 375−397.
(40) Shim, Y. Y.; Shin, W. S.; Moon, G. S.; Kim, K. H. *J. Microbiol. Biotechnol.* **2007**, *17*, 681−684.
(41) Kuiper, H. A. *Food Control* **1999**, *10*, 339−349.

DOI: 10.1021/ac501795x
Anal. Chem. 2015, 87, 2072−2078