Article

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Polydiacetylene (PDA) Liposome-Based Immunosensor for the Detection of Exosomes

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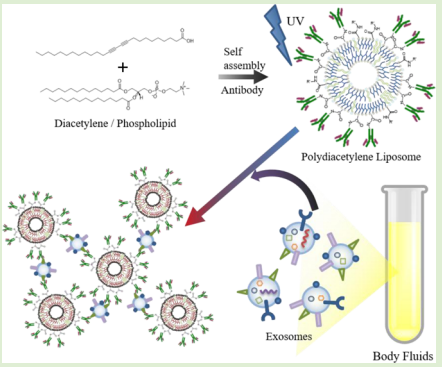
Supporting Information

ABSTRACT: Exosomes are extracellular vesicles (EVs) that have attracted attention because of their important biological roles in intercellular communication and transportation of various biomolecules, including proteins and genetic materials. However, due to difficulties in their selective capture and detection, further application of exosomes remains challenging. To detect EVs, we fabricated a liposomal biosensor based on polydiacetylene (PDA), a conjugate polymer that has been widely used in sensing applications derived from its unique optical properties. To confer selectivity and sensitivity to the sensory material, antibodies targeting CD63, a membrane protein exclusively found in exosomes, were attached to the PDA liposomes and phospholipid molecules were incorporated into the PDA vesicles. Signal analysis derived from PDA liposomes for exosome detection and quantification was performed by observing colorimetric changes triggered by the ligand−receptor interaction of PDA vesicles. Visual, UV−visible, and fluorescence spectroscopic methods were used to obtain signals from the PDA lipid immunosensor, which achieved a detection limit of 3×10^8 vesicles/mL, the minimum concentration that can be used in practical applications. The strategies used in the system have the potential to expand into the field of dealing with exosomes.



INTRODUCTION

The treatment for lethal degenerative diseases such as Alzheimer's and Parkinson's diseases has become insufficient, leading to a shift from a therapeutic approach to an incurable disease to a preventive and management approach. These changes have extended to the field of disease prevention, especially early diagnosis, to prevent the symptoms of incurable diseases from deteriorating. Among the various ways of diagnosing diseases, methods for analyzing extracellular vesicles as a means of diagnosing diseases have been emerging.

Exosomes, which is are kind of extracellular vesicle, refers to a class of cell-derived vesicles with sizes of several tens to 150 nm and are released via the exocytosis of multivesicular bodies (MVBs).[1] Before an MVB is released from the origin cell, the MVB inside the cell is intruded, leading to the formation of small vesicles carrying molecules such as proteins, DNA, and RNA from the cytoplasm. The exosomes produced by MVB are released from the plasma membrane by exocytosis and circulate in the body fluid to perform intercellular communication.[2,3] Because exosomes transmit various biomaterials, they have been used as important tools for diagnosing[4−10] or treating various diseases.[11−13] However, because of their small size, low density, and scarcity, current methods for selective isolation and screening of exosomes require highly sophisticated equipment or complex experimental procedures such as deposition of gold film through lithography, fabrication of flow cell, introduction of a receptor onto the detection chamber, and detection of exosomes with electrical or optical measurements.[6,10,14−18] Since this limits their further applications and increases the need for devices that require simple instrumentation and experimental procedures, a prompt and facile exosome detection system for exosomes is needed.

Polydiacetylene (PDA) can be a versatile material that has been utilized in the fast and simple sensor application field because of its unique optical properties derived from the ene−yne-conjugated backbone in its structure.[19,20] The conjugated backbone of PDA can be formed via a 1,4-addition reaction of diacetylene (DA) induced by ultraviolet or γ irradiation of self-assembled DA. When the backbone of PDA formed by the aligned monomer is disturbed by external stimuli, the energy gap between the highest occupied and lowest unoccupied molecular orbitals of overlapped p-orbitals increases, resulting the absorption pattern of the conjugated backbone and a blue to red colorimetric change.[21] In addition to the color change, the distorted backbone of PDA exhibits fluorescence, enabling the optical properties of PDA to be characterized by changes in the absorption spectrum and intensity of fluorescence.[22]

PDA has attracted attention in sensing applications for various reasons. First, because of their self-assembly ability, aligned monomers can form various shapes such as crystals, films, filaments, and vesicles. Polymerization can be easily

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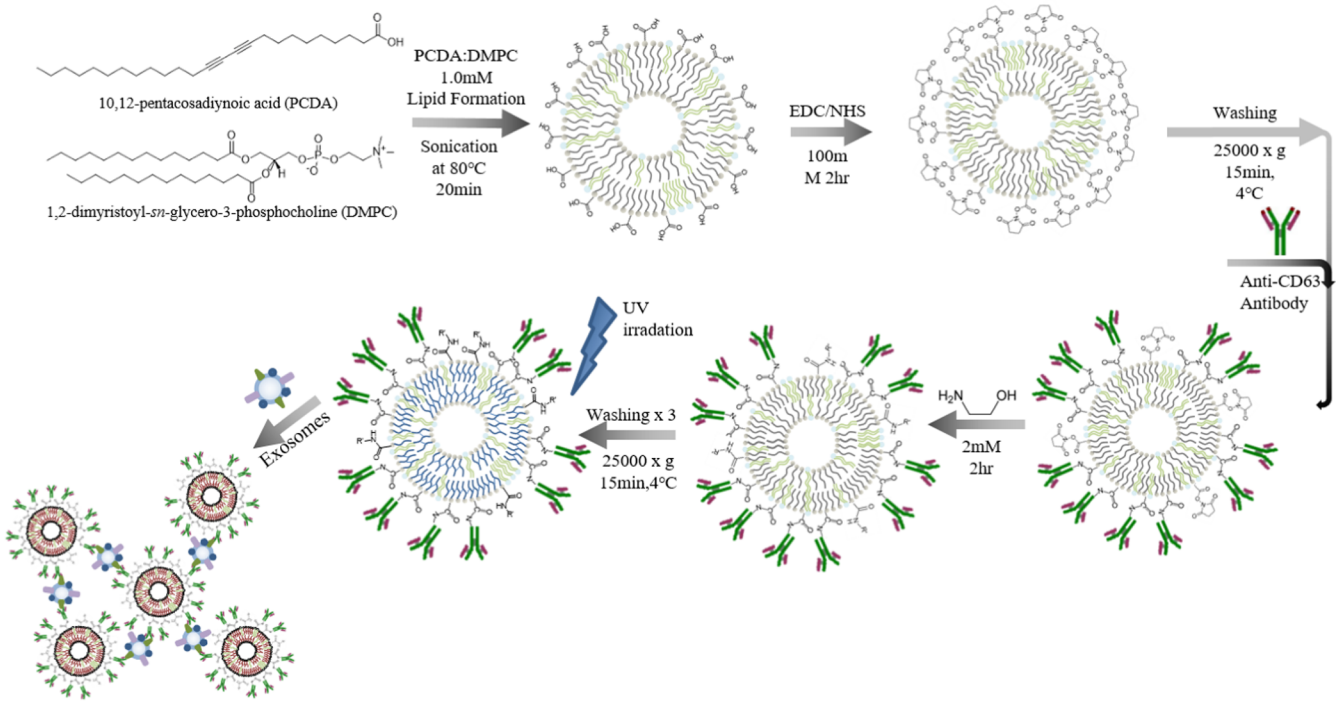

Figure 1. Schematic illustration of the fabrication of the PDA liposome immunosensor for exosome detection. The liposome consisted of a PCDA monomer and a DMPC phospholipid. PDA liposomes, composed of PCDA and phospholipid, were activated by EDC/NHS chemistry, and antibodies were attached to the PDA liposome, conferring selectivity to the PDA liposome for exosome detection. After adding exosomes, the PDA vesicle solution undergoes color change and fluorescence.

induced by irradiating UV light at 245 nm without using a UV absorber, resulting in the production of a high-purity sensing material.[21,23−28] Second, factors such as pH, temperature, solvent, electrical stress, mechanical stress, and ligand−receptor interactions confer PDA with optical characteristics, which are required for the sensing process.[26−34] Another advantage is that PDA is a label-free material; it can change color or emit fluorescence without any dye or fluorophore, unlike many other sensing methods.[34,35] Despite these advantages, low quantum efficiency and limit of detection are typical drawbacks of PDA sensors. To overcome these disadvantages, phospholipids or cholesterol can be introduced into the PDA membrane. The inserted material imparts fluidity to the film of PDA, resulting in improved color change and fluorescence.[36,37]

In this study, we developed a label-free PDA/phospholipid composite immunosensor constructed by introducing an antibody against CD-63, an exosome marker protein, for selective exosome detection. We observed clear optical changes in color and fluorescence spectrum upon detection of exosomes isolated from human plasma in the same liquid phase. The liquid-phase polydiacetylene liposomes do not require chip manufacturing, so the chip preparation process can be omitted as compared with the existing assays, and have the versatility of the measurement equipment.

MATERIALS AND METHODS

Materials. The diacetylene monomer used was 10,12-pentacosadionic acid (PCDA), which was purchased from GFS Chemicals (Powell, OH). *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) were obtained from Tokyo Chemical Industry (Tokyo, Japan). Ethanolamine, *N*-hydroxysuccinimide (NHS), and fibrinogen extracted from human plasma were purchased from Sigma-Aldrich (St. Louis, MO). The anti-CD-63 monoclonal antibody [TS63] was purchased from Abcam (Cambridge, U.K.), and exosome solution was prepared from lyophilized exosomes from human plasma purchased from HansaBioMed Life Science (Tallin, Estonia). Bovine serum albumin (BSA) was obtained from MP Biomedicals (Illkirch, Santa Ana, CA). Phosphate-buffered saline (PBS) was purchased from Welgene, Inc. (Gyeongsan-si, Korea). The 30% acrylamide solution [acrylamide-bisacrylamide 29:1], Tris−HCl buffer [pH 6.8 and 8.8], sodium dodecyl sulfate (SDS) solution, and Tris−glycine running buffer used for SDS-polyacrylamide gel electrophoresis (PAGE) were purchased from ELPIS Biotech (Deajeon, Korea). Ammonium persulfate and tetramethylethylenediamine were obtained from Sigma-Aldrich. The staining solution for protein detection [PageBlue] was acquired from Thermos Scientific. All organic solvents used were obtained from Samchun Chemicals (Seoul, Korea).

Preparation of PCDA/DMPC Liposome Composite. The procedure used to prepare the PDA/phospholipid composite sensor is shown in Figure 1. PCDA and DMPC were individually dissolved in chloroform in glass vials, and the solutions were blended at a 4:1 molar ratio of PCDA and DMPC. The mixture solution was slowly dried under gentle nitrogen flow, and distilled water was added to the thin layer to achieve a total lipid concentration of 1.0 mM. To prepare uniform vesicles with the nanometer scale, the lipid solution was sonicated with a probe sonicator (Sonics & Materials, Newtown, CT) at 150 W for 20 min at 80 °C, resulting in a translucent cloudy suspension. After sonication process, the vesicle solution was immediately filtered using a disposable 0.45 μm syringe filter (Advantec, Dublin, CA) to remove aggregated material or large particles. The filtered solution was slowly cooled to room temperature around 25 °C and stored at 4 °C to crystallize the lipid vesicles for at least 4 h.

Conjugation of Anti-CD-63 mAb on the Liposome Composite. The steps used to prepare the PDA/phospholipid composite sensor are illustrated in Figure 1. EDC and NHS solutions separately dissolved in deionized water were added to 1 mL of vesicle solution at 100 mM each, and the NHS activation reaction was carried out for 2 h at room temperature. To remove residual EDC/NHS after

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Biomacromolecules 2019, 20, 3392−3398

the reaction, the NHS-activated vesicles were precipitated by centrifugation (Gyrozen, Seoul, Korea) at 25 000*g* for 15 min at 4 °C and the supernatant was removed, followed by resuspension of the vesicles in PBS. The anti-CD63 monoclonal antibody was added to the activated vesicle solution (0.1 mg/mL final concentration) with gentle stirring for 2 h at room temperature, and ethanolamine in PBS (2.0 mM final concentration) was added to deactivate the remaining NHS-activated vesicles for 2 h. The antibody-conjugated vesicle solution was centrifuged at 25 000*g* for 15 min at 4 °C and washed with PBS three times. Prior to use, the PCDA/phospholipid vesicles were polymerized by 254 nm UV irradiation at 400 $\mu W/cm^2$ for 30 min using a UV ramp (Vilber, Marne-la-Vallée, France), giving a blue polymerized PDA vesicle solution.

Structural and Chemical Analyses of the PDA Liposome Composite. The size and morphology of the PCDA/DMPC vesicles were characterized with a high-resolution transmission electron microscope (JEM-3010, JEOL, Tokyo, Japan) at an accelaration voltage of 300 kV. The transmission electron microscopy (TEM) samples were prepared by dropping the diluted vesicle solution onto a copper grid (300 mesh), which was covered with carbon film and dried in a desiccator. Dynamic light scattering analysis (Zetasizer nano ZS, Malvern Instruments, Malvern, U.K.) was conducted to confirm the size distribution and surface charge of the PDA vesicles. Dynamic light scattering (DLS) measurements were performed using samples diluted to approximately 0.05 mM.

Chemical analysis of the PCDA/DMPC vesicles was analyzed by ATR-FTIR (Alpha, Bruker, MA). The liposomes in each step were washed with pure water and centrifuged at 25 000*g* for 15 min at 4 °C. After removing the supernatant, the precipitate vesicle was lyophilized in the precipitated state. The lyophilized samples (PCDA/DMPC vesicles and NHS-activated PCDA/DMPC vesicles) were collected in a solid state, and their transmittance spectra were recorded at a resolution of 4 cm^{-1} through the Fourier transform infrared (FTIR) spectrometer.

SDS-PAGE Analysis of the PDA Vesicle Complex. To confirm the binding between the PDA vesicle and the antibody, a protein detection technique using SDS-PAGE was used. SDS-polyacrylamide gel electrophoresis was performed using a Mini-PROTEAN tetravertical electrophoresis cell (Bio-Rad Laboratories, CA). Stacking gel and running gel were prepared with 5 and 6% acrylamide concentrations, respectively, according to reference,[38] and stored at 4 °C before use. Samples for protein detection were treated with the same amount of sample buffer, then incubated at room temperature for 5 min, and finally heated at 95 °C for 5 min. PDA vesicles were polymerized before they are processed in the sample buffer to prevent them from passing through the gel. Each sample was loaded in an electrophoresis cell filled with SDS-glycine running buffer at 15 μL in a gel-loaded chamber. The sample was run at 80 V for about 20 min until it reached the running gel boundary and then electrophoresed at 180 V for about 40 min through the running gel. After electrophoresis, polyacrylamide gel was stained with a protein staining solution (PageBlue, Thermo Scientific) according to manufacturer's protocol. Briefly, the gel was rinsed with distilled water, immersed in 100 mL of distilled water, exposed to microwave for 1 min, and stirred for 5 min. The following washing procedure was carried out three times, the protein staining solution was added with microwave treatment for 30 s, and then the mixture was stirred for 20 min to stain the gel. The dyed gel was soaked in distilled water for 1 h to perform destaining and identify the remaining stained area. The PDA vesicles filtered at the top of the polyacrylamide gel were observed with a fluorescence microscope (ZEISS, Carl Zeiss AG, Germany) and fluorescent excitation and emission of PDA were performed by 560 and 640 nm filters, respectively.

Isolation and Quantification of Exosomes. Exosomes were separated by analytical ultracentrifugation (ProteomeLab XL, Beckman, Brea, CA). Human plasma was centrifuged at 3000*g* for 30 min to remove the remaining massive cell flakes, and the pellet and the suspended material in the plasma were removed by filtering with a disposable 0.2 μm syringe filter. The supernatant was ultracentrifuged at 170 000*g* for 90 min at 4 °C to obtain the exosome pellet. The supernatant was removed, and the pellet was thoroughly redispersed in PBS. The high-speed ultracentrifugation step was repeated, and the resulting exosomal pellet was redispersed in PBS. Characterization and quantification of the exosome solution were performed by TEM, DLS, and nanoparticle tracking assay (NTA). TEM images and the size distribution of isolated exosomes were obtained as described for PDA liposomes, and NTA was performed by measuring a 100-fold diluted exosome solution with NanoSight LM10 (Malvern Instruments).

Spectrophotometric and Fluorescence Analyses of the PDA Composites. Different concentrations of exosome solution (30 μL) were added to the PDA vesicle solution (30 μL) and incubated at 37 °C for 30 min. UV−visible and fluorescence spectra were recorded to measure the colorimetric changes and fluorescence intensity using a microplate reader, SYNERGY H1 (BioTek, Winooski, VT). Quantification of the change in the color of PDA vesicles interacting with exosomes from blue to red, the colorimetric response (CR), was performed as follows

$$\mathrm{CR}(\%) = \frac{(\mathrm{PB}_{\mathrm{bef}} - \mathrm{PB}_{\mathrm{aft}})}{\mathrm{PB}_{\mathrm{bef}}} \times 100,\ \mathrm{PB} = \frac{A_{\mathrm{blue}}}{A_{\mathrm{blue}} + A_{\mathrm{red}}}$$

where A is the relative absorbance of the blue or red element in the UV−vis spectrum at 640 or 540 nm, respectively, PB_{bef} is the relative ratio of the blue and red elements before exosome addition, and PB_{aft} is the relative value of blue and red elements after adding the exosome. The fluorescence spectra of PDA vesicles were recorded at an excitation wavelength of 480 nm, and the maximum fluorescence level of the sample was recorded. The final fluorescence intensity was obtained by subtracting the base intensity of the control sample.

RESULTS AND DISCUSSION

Principle. Exosomes are extracellular vesicles secreted by cells and have sizes of several tens to 150 nm. Because the vesicles are directly secreted from the cells and present in body fluids, they contain biomarkers derived from their cell of origin, making them an important tool for disease diagnosis. Exosomes are present in the human blood at approximately 1×10^{10} vesicles/mL. Because they also have similar properties to cell membranes, tetraspanin (CD-9, CD-63, CD-81), a class of exosome biomarker, can be used to impart exosome selectivity to biosensors. As shown in Figure 1, the PCDA monomer was mixed with DMPC, a phospholipid, to enhance the charge stability and membrane flexibility of PDA vesicles, and an exosomal marker antibody was conjugated to the vesicle to induce antigen−antibody interactions. The addition of phospholipid to PCDA showed the change in stability of liposome when EDC/NHS was added (Figure S2).

The remaining charge prevents aggregation because the phospholipid, which does not have a carboxylic group, retains the charge constantly during the reaction. The optical characteristics of the PDA such as the length of the conjugated backbone of the PDA were altered by these interactions.

Since the inserted phospholipid affects the sensitivity of the PDA, the molar ratio of PCDA to DMPC was optimized by the value of the PDA liposome signal. As the molar ratio of DMPC in the liposome increases, the exosome-derived color change of PDA liposome increases, accompanied by the increased color change due to the nonspecific reaction by BSA. Therefore, the molar ratio of PCDA to DMPC was determined by the ratio of the selective signal to the nonselective signal (Figure S3).

The EDC/NHS chemistry of the PCDA/DMPC vesicle was confirmed by FTIR spectra. The NHS-activated vesicle is presented by three peaks at around 1800 cm^{-1}. The carbonyl stretch of NHS and symmetric and asymmetric stretches of NHS carbonyls correspond 1814, 1783, and 1740 cm^{-1},

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respectively. The N−C−O bond of NHS matches with 1067 cm^{-1} (Figure S4).

PAGE Analysis of PDA Vesicles with Antibodies. To confirm the binding between the PDA liposome and the antibody, SDS-PAGE for protein detection was performed. Four samples of pure PDA/phospholipid vesicles, PDA vesicles and antibody mixtures, and antibody-conjugated PDA vesicles before and after washing were stained by gel electrophoresis. In the sample of pure PDA liposome, no band was observed, meaning that the PDA contained no protein involved in the band formation (Figure 2). On the other hand, in the PDA-

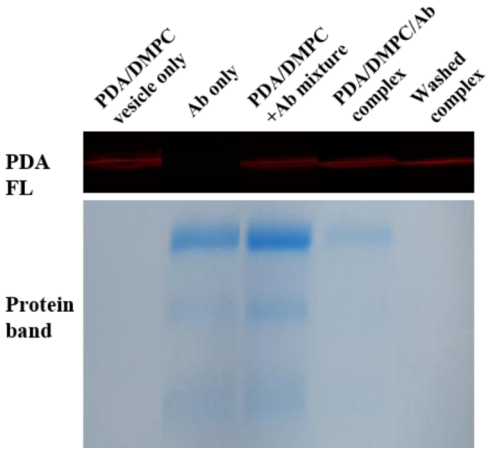

Figure 2. SDS-PAGE analysis for characterization of PDA liposomes. Fluorescent image of the upper part of polyacrylamide gel at the sample loading area (top) and SDS-PAGE image of the remaining protein in solution (bottom).

antibody mixtures, a clear band (nonreduced form at the upper part and reduced forms at the middle and lower parts; 150, 50, and 25 kDa, respectively) of the same size as that of the antibody sample was observed, meaning that the pure PDA exists independently and did not interact with the antibody. Unlike a simple mixture of PDA vesicle and antibody, antibody samples bound by NHS-activated PDA liposomes had a significantly reduced band strength due to chemical bonding. In general, polymerized PDA vesicles are filtered at the top of the gel because the shape-retaining PDA vesicles are not small enough to pass through the polyacrylamide gel during electrophoresis (Figure 2, top). When the NHS-activated PDA vesicle is mixed with the antibody, the antibody binds onto the PDA liposome to form a structure, and most of the bound antibody is filtered together with the PDA vesicle. This confirms that the antibody is attached to the surface of the PDA vesicle. Finally, to achieve a higher binding efficiency, it was confirmed that the remaining antibody was not detected in the washed PDA vesicle solution.

Structural Transition of PDA Vesicles due to Interaction with Exosomes. The colorimetric transition and fluorescence properties of PDA vesicles are caused by modification of the conjugated backbone produced by the polymerization of diacetylene monomers. Because the length variation of the conjugated backbone can be confirmed by size, shape, and aggregation, it is important to identify the structural deformation of PDA vesicle triggered by antigen−antibody interactions.

First, antibody conjugation of PDA vesicles was confirmed by measuring the surface charge of the vesicle. The surface charge before initial antibody binding was strong (−27.1 mV) because of the carboxyl group present on most PCDA monomers. However, after antibody conjugation, the negative charge of PDA vesicles was greatly reduced (−1.4 mV) and the charge was evenly distributed over a wide range (Figure S5).

The structural changes in PDA vesicles before and after spiking with exosomes were characterized by TEM and DLS to verify the interaction between PDA vesicles and exosomes. PDA vesicles were found to be aggregated in a line in the TEM images (Figure 3a). The initial liposome was found to increase

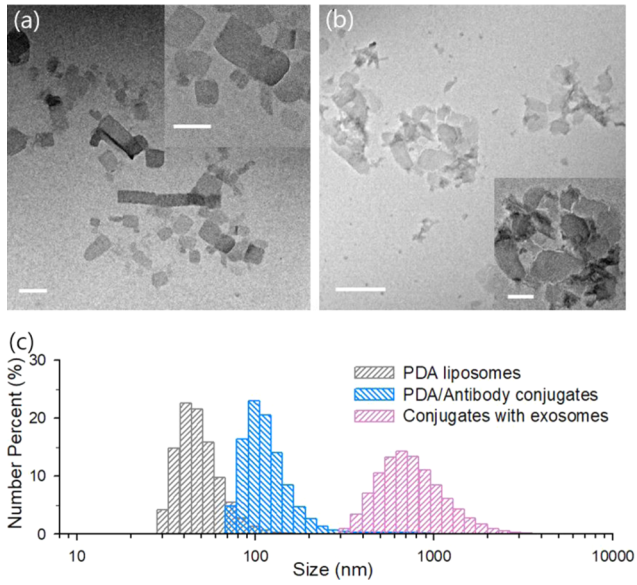

Figure 3. TEM images of the PDA liposome immunosensor before (a, scale = 0.2 μm and 100 nm of inset image) and after exosome addition (b, scale = 1.0 μm and 200 nm of inset image). The size distribution change of initial PDA vesicles (black), antibody-introduced PDA liposomes (blue), and PDA−antibody conjugates with exosomes (pink).

in size by several tens of nanometers through the reaction with the antibody, and the size of PDA vesicles before exosome addition was approximately 150 nm. Then, the particle size of PDA vesicles increased dramatically to approximately 820 nm after adding exosomal solution at 1×10^{10} vesicles/mL (Figure 3c). When exosomes are added to the polydiacetylene vesicles linked with antibodies, the ligand−receptor interaction between the tetraspanin on the surface of the exosomes and the antibody results in a chain reaction and eventually to the formation of large clusters. The polydiacetylene−exosome clusters in the TEM image are approximately 1 μm in size, which is similar to the average size of the DLS data (Figure 3b). Changes in the PDA size seen in the TEM and DLS distort the conjugated backbone of the PDA and cause a colorimetric change in the PDA liposome.

Spectrophotometric and Spectrofluorometric Detection of Exosomes with PDA Vesicles. To confirm the optical property changes caused by structural changes in PDA, UV−vis spectra and fluorescence were recorded. In the UV−vis absorption spectrum, the blue control PDA vesicle solution showed the maximum absorption at approximately 640 nm. As the exosome concentration increased, the absorbance at this wavelength band gradually decreased, while the absorbance at approximately 540 nm increased (Figure 4a). Because of the change in absorbance, the blue PDA solution gradually

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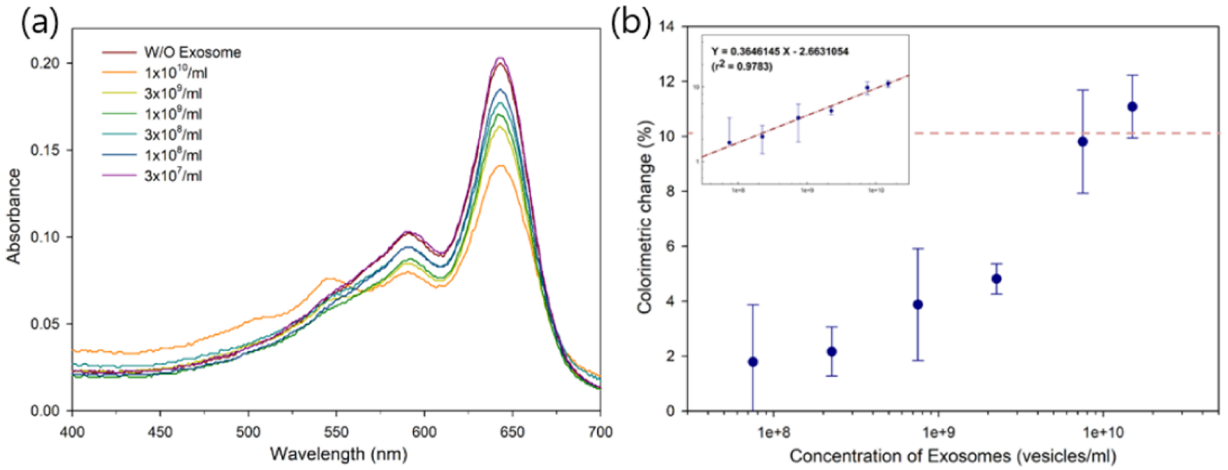

Figure 4. (a) UV−visible spectrum of the PDA liposome with addition of different concentrations of exosomes. (b) Relationship between colorimetric changes (%) of the PDA vesicle solution and the concentration of the exosome. The pink dotted line indicates the degree of colorimetric change that can be observed with the naked eye (b, inset). Regression line for CR and exosome concentration in the PDA sensor solution (b, inset). The x and y axes of the inset graph were both changed to a logarithmic scale.

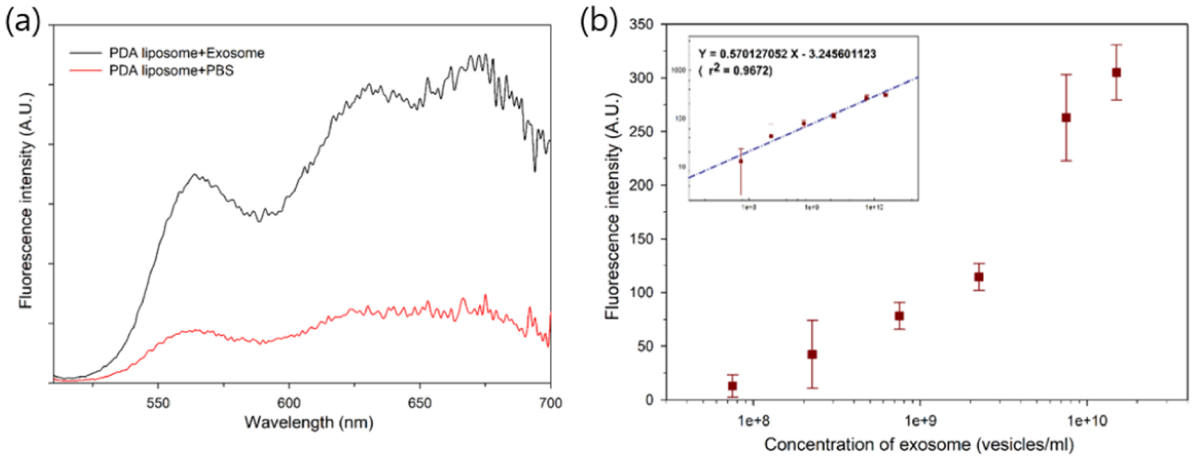

Figure 5. (a) Fluorescence spectrum of the PDA solution with PBS and exosome. (b) Relationship between the intensity of fluorescence and the amount of exosomes (n = 4, the fluorescence intensity was calculated at the maximum values ranging from 500 to 700 nm under 480 nm of excitation wave). Regression line for CR and exosome concentration in PDA sensor solution (b, inset). Derived intensities are the values obtained by subtracting the signal from the baseline signal in PBS. The x and y axes of the inset graph were both changed to a logarithmic scale.

changed to red depending on the external stimulus. The color change of PDA vesicles can be quantified as CR (%), which reveals the degree of colorimetric change in the visible light region of the solution after exosome addition. The CR value of the PDA vesicle solution increased with the exosomal concentration, indicating that interactions between PDA vesicles and exosomes had increased. At an exosome concentration of 1×10^{10} vesicles/mL, the CR value was greater than 10% (Figure 4b), which was the level that could be detected by direct observation (Figure S6). At a high concentration of exosomal solution, the colorimetric change of PDA liposome solution is observable with the naked eye, so rapid detection of exosomes is possible. In addition, if the accuracy of the device observing the color change is high, not only the presence of exosomes but also their concentrations can be deduced.

Additionally, because PDA modified by external stimuli was fluorescent and showed a color transition, we measured the fluorescence intensities of PDA vesicles containing exosomes. Although the weak fluorescence of the PDA itself is measured without target substance, the fluorescence of the PDA vesicle is greatly increased when the exosome is added (Figure 5a). For this reason, the fluorescence of the PDA vesicle solution with different concentrations of exosomes was measured and each fluorescence value derived from the exosome interaction was obtained by subtracting the baseline value from the reaction with PBS. Addition of PBS to the PDA solution resulted in a slight increase in fluorescence intensity; the fluorescence intensity except for the value due to PBS was increased as the exosome concentration was increased (Figure 5).

Specificity of PDA Liposome for Exosome Detection. To confirm the selectivity and specificity of the PDA vesicle sensor targeting exosomes, we determined the CR values of the PDA vesicle solution using various nontarget proteins abundant in the blood such as human immunoglobulin G, BSA, and fibrinogen. The test was carried out in the same manner as that used for solution-based exosome detection with a 1× concentration of PBS (pH 7.4), human immunoglobulin G, BSA, and fibrinogen solution. Each nontarget protein was injected into the PDA liposome sensor solution in the absence of exosomes to a final concentration of 0.1 mg/mL, and the CR value was measured based on the absorption spectrum of

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the CR value from the 1× concentration of PBS. The CR value of the measured nontarget protein was significantly lower than that of the exosomes, which was similar to the CR value of PBS. In contrast to the interaction between exosomes and PDA liposomes, the interaction between various proteins and PDA liposomes was considerably lower, suggesting that the PDA immunosensor system has sufficient selectivity for exosomes (Figure 6).

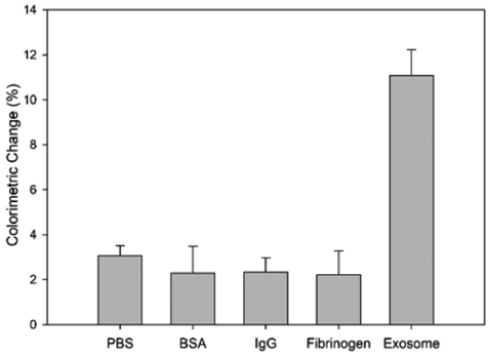

Figure 6. Specificity of the PDA liposome immunosensor for exosome detection with fibrinogen, BSA, and hIgG (concentration of BSA, fibrinogen, and IgG was 0.1 mg/mL).

As previously mentioned, the selectivity of the PDA liposome to exosomes is determined by the introduced antibody, so no ligand−receptor reaction occurs when a functionalized PDA liposome does not have the desired target. In other words, PDA vesicles do not undergo colorimetric change unless a ligand−receptor-mediated cluster formation occurs. For example, when a mixture of BSA or fibrinogen solution in the exosome-targeting PDA vesicle does not form a cluster of PDA vesicles (Figure S7a,b). Conversely, when the solution of the BSA-blocked PDA vesicle is mixed with an exosome solution or a fibrinogen solution, none of the PDA-mediated clusters due to ligand−receptor interactions are formed (Figure S7c,d). Based on these results, it can be suggested that the ligand−receptor interaction between the antibody on the PDA vesicle and exosomes forms a PDA vesicle cluster, inducing a colorimetric change of the PDA vesicle.

CONCLUSIONS

We developed a simple, fast-responding, and sensitive sensor for selectively detecting exosomes isolated from the human plasma. Colorimetric changes and fluorescence of the PDA immunosensor were observed based on interactions with external exosomes by conjugating an antibody to the PDA vesicle, conferring specificity to the exosome. This enabled the detection of the exosome solution at a concentration of 3×10^8 vesicles/mL. Compared to the existing exosome detection sensors, our system can be used to detect exosomes simply and rapidly without using labeling materials. In addition, since the liquid-phase PDA liposome sensor does not require functional chips or devices, the lithography and chip fabrication processes for fluidic chip fabrication can be omitted. The estimate of the cost is 450−500$/mL, and fabrication of the total assay of polydiacetylene immunosensor takes about 18 h. The manufacturing cost can be decreased by changing the antibody that takes up most of the price or using a measuring device that requires a small sample volume. This newly developed PDA vesicle sensor has potential for further applications using exosomes as diagnostic tools by diversifying the antibodies or aptamers targeting the biomarkers for various diseases.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biomac.9b00641.

Characterization of exosome extracted from human plasma and additional experiments to determine the reactivity and stability of the PDA liposome composite. PCDA/DMPC molar ratio optimization, IR spectrum of PCDA/DMPC vesicle and NHS-activated PCDA/DMPC vesicle, size distribution and ζ-potential of the PDA vesicle, naked-eye detectable colorimetric change of the PDA vesicle, and TEM images of the PDA sensor with BSA and fibrinogen for identification of the sensor's selectivity (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

PDA, polydiacetylene; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; BSA, bovine serum albumin

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Biomacromolecules 2019, 20, 3392−3398