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Research Article

New Tool for Rapid and Accurate Detection of Interleukin-2 and Soluble Interleukin-2 Receptor α in Cancer Diagnosis Using a Bioresponsive Microgel and Multivalent Protein Binding

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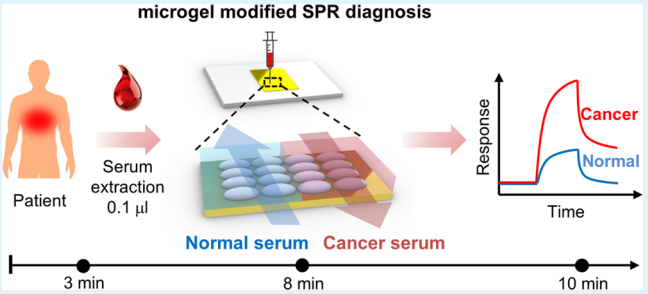
ABSTRACT: Interleukin-2 (IL-2) and its α receptor in soluble form (sIL-2Rα) are considered biomarkers for cancers and immune-related diseases. Enzyme-linked immunosorbent assay is the most common method used to evaluate biomarkers in clinical practice; it is precise but time-consuming and involves complicated procedures. Here, we have developed a rapid yet accurate modality for cancer diagnosis that enables on-site evaluation of cancer markers, that is, IL-2 and sIL-2Rα, without complicated pretreatment of cancer patient-derived blood samples. Surface plasmon resonance and bioresponsive microgels conjugated with IL-2 receptors, that is, IL-2Rβ and IL-2Rγ, were utilized to measure IL-2 and sIL-2Rα levels via multivalent protein binding (MPB) between the ligands and their receptors. Our results showed that this novel method enables us to perform cancer diagnosis with a 1000-fold dilution of serum in 10 min. The advantage of MPB-based cancer diagnosis originates from its great selectivity for a target molecule and tolerance to a myriad of nonspecific substances in serum, which allows on-site clinical evaluation. Importantly, our finding implies that MPB-based cancer diagnosis provides a new paradigm not only for improving cancer treatment but also for evaluating a target molecule in unpurified and complex solutions such as blood.



KEYWORDS: *hydrogel, multivalent protein binding, label-free, rapid detection, cancer diagnosis, biosensor, surface plasmon resonance*

INTRODUCTION

Interleukin 2 (IL-2) is a multipotent cytokine[1,2] that plays an important role in physiological functions,[3,4] and its alteration is related to immunologic disorders[5] and cancers.[6,7] For instance, the elevated level of IL-2 and its receptor, that is, soluble interleukin-2 receptor α (sIL-2Rα) in blood suggests the presence of cancer,[8,9] allowing its use as a cancer marker.[10] In addition, IL-2 has also been used for cancer immunotherapy[11] because of its role in the proliferation of immune cells to attack cancer cells.[12] These results imply that the measurement of IL-2[13] and IL-2/sIL-2Rα[14] in human blood is highly necessary in clinical practice. Currently, enzyme-linked immunosorbent assay (ELISA) has been considered the gold standard for the detection of biomarkers[15,16] in the blood including IL-2 and sIL-2Rα.[17,18] However, ELISA has the limitations of requiring complicated procedures and being time-consuming, limiting its use in rapid diagnosis of diseases on site.[19] Alternatively, real-time polymerase chain reaction (RT-PCR) has been shown to be highly sensitive for analyzing alterations of a target protein at the gene level,[20] but, like ELISA, RT-PCR also requires many hours to a day for the final result. Thus, a novel type of diagnostic tool enabling rapid and accurate analysis of such biomarkers could facilitate medical decisions for managing a number of diseases including cancers.

A number of hydrogel-based sensor systems have been demonstrated for specific target biomarkers including ions,[21,22] glucose,[23,24] pH,[25,26] DNA,[27,28] bacteria,[29,30] and proteins.[31,32] Recently, the surface plasmon resonance (SPR) technique has been intensively utilized to detect biomarkers because of its high sensitivity.[33−35] We also demonstrated that SPR and bioresponsive pNIPAm-*co*-AAc microgels can be employed to develop a highly sensitive biosensor by intensifying signals from multivalent protein binding (MPB) events of target molecules.[36] MPB-capable microgels on an SPR chip were exposed to either antibodies or small multivalent cations that generate MPB events by complexing with their binding moieties, while nonspecific and monovalent binding events

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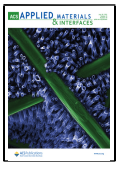

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yield significantly lower signals than MPB events. This result allows us to expand the application of this concept to label-free biosensing of a target molecule in complex media such as human serum, mainly because of its ability to distinguish specific MPB events from nonspecific or monovalent binding events. Thus, we suggest a new paradigm for clinical diagnosis via assessment of cancer markers using this MPB-based microgel SPR method.

Here, we report the development of a rapid yet accurate cancer diagnostic approach measuring IL-2 and sIL-2Rα in serum samples from cancer patients and healthy controls, with results having a strong correlation with those of conventional ELISA. The advantage of this method is the use of 1000-fold dilutions of serum without the need for further processing, which allows on-site evaluation of cancer markers in 10 min, that is, point-of-care testing (POCT). We found that the SPR signals from cancer markers in serum were amplified compared with those in phosphate buffered saline (PBS) buffer solution, which allowed us to detect IL-2 and sIL-2Rα more sensitively. Quantitatively, the fold increases in the values (cancer patients vs healthy controls) determined by microgel SPR and ELISA were comparable. In addition, the microgel SPR method in this application is superior for measuring kinetic values that are highly informative but unobtainable by ELISA as well as RT-PCR. The dissociation constant (K_D) values derived from our method were well correlated with the known values. The limitation of this proof-of-concept approach is that it was tested in patients with only one type of cancer, that is, myeloma. It will be highly desirable to advance this method to be useful for the diagnosis of different types of cancer by considering the observation that the level of sIL-2Rα in serum is elevated in most cancers. For instance, the median levels of serologic IL-2 and sIL-2Rα in chronic myeloma patients were reported as 190 and 1880 pg/mL, respectively, which are higher than those of the healthy control (IL-2: 23 pg/mL and sIL-2Rα: 1200 pg/mL).[37] In addition to its clinical application, this method will allow us to resolve a number of biological questions that are related to MPB of molecules in relevant pathways.

MATERIALS AND METHODS

Materials. All chemicals were used as received without further purification unless otherwise stated. *N*-Isopropylacrylamide (NIPAm), acrylic acid (AAc), *N*,*N*′-methylenebisacrylamide (BIS), ammonium persulfate (APS), polyoxyethylenesorbitan monooleate (Tween 80), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), cysteamine, and recombinant green fluorescent protein (rGFP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). IL-2 and IL-2 receptors were purchased from Acrobiosystems (Newark, DE, USA). The water used throughout this study was distilled, deionized to 18.2 MΩ cm (aquaMAX-Ultra 370 water purification system, Young In, Anyang, Korea), and filtered with a 0.22 μm filter to remove particulate matter. 2-(*N*-Morpholino)-ethanesulfonic acid (MES) and 0.9% sodium chloride buffer were purchased from Thermo Fisher Scientific (Waltham, MA, USA). PBS (1×) without calcium or magnesium was purchased from GE Healthcare (Chicago, IL, USA). The protocol of the clinical study was approved by the Institutional Review Board of the Korea University Anam Hospital (No. 2021AN0049). Blood samples from cancer patients were collected. The patients provided informed consent for the use of specimens, and all methods were performed in accordance with the relevant guidelines and regulations. Normal human serum was purchased from zenbio (Durham, NC, USA) and filtered with a 0.22 μm filter to remove the flocculent precipitate of serum.

Synthesis of the Hydrogel Microparticles. Hydrogel microparticles were prepared via aqueous free-radical precipitation polymerization with slight modifications from previously reported literature procedures.[36] Briefly, the total monomer concentration was fixed at 100 mM, with a total reaction volume of 100 mL. The AAc and BIS contents were adjusted to 10 and 2%, respectively, for optimal SPR responses, relative to the total monomer concentration. The solution mixture was prepared by dissolving NIPAm and BIS in deionized water and then adding Tween 80, yielding a total surfactant concentration of 0.5 mM. The monomer solution was filtered with a 0.22 μm syringe-driven filter and subsequently transferred to a 100 mL three-neck round-bottomed flask equipped with a reflux condenser, a thermometer, and a magnetic stirring bar. Once equilibrated to 70 °C, the reaction mixture was purged with Ar while stirring at 275 rpm. After 1 h, the solution of AAc was added to the mixture by pipetting, and Ar purging was maintained for 10 min before initiation of polymerization. Polymerization was initiated by syringe injection of a 1 mL aliquot of 1 mM APS into the reaction vessel. The reaction was allowed to proceed for 6 h at 70 °C under continuous Ar purging with stirring. After cooling, the resulting product was purified by dynamic dialysis (Spectra/Por Tube-A-Lyzer, MWCO 100 kDa, Repligen, Boston, MA, USA) against pure water for 24 h, and the water was changed at the 12 h mark.

Bioconjugation of IL-2 Receptors. Biosensing capability was imparted to the hydrogel microparticles by functionalization with IL-2 receptors. EDC/NHS coupling was performed to conjugate the amine groups of the receptors to the carboxyl groups of the hydrogel microparticles. The lyophilized hydrogel microparticles were suspended in MES buffer at a concentration of 10 mg/mL, and 10 μL of EDC (20 mg/mL) and 10 μL of NHS (40 mg/mL) were added to 40 μL of them and then incubated for 5 min at room temperature. For the preparation of IL-2Rα,β,γ-conjugated microgel, 12.5 μL (6.6 μM) of IL-2Rα, 12.5 μL (6.6 μM) IL-2Rβ, and 12.5 μL (6.6 μM) of IL-2Rγ were added to the reaction mixture to yield a final concentration of 0.8 μM followed by addition of DPBS to produce a final volume of 100 μL. For the IL-2Rβ,γ or IL-2Rα,γ-conjugated microgels, 12.5 μL (6.6 μM) of IL-2Rβ and 12.5 μL (6.6 μM) of γ proteins were added to the microgel mixture (same as the case of IL-2Rα and γ). The monoconjugated microgels were conjugated with only one IL-2 receptor protein (e.g., IL-2Rα or IL-2Rβ or IL-2Rγ) in the same reaction protocol. After 1 h incubation of each mixture, the reaction was stopped by removing unreacted EDC/NHS and proteins. Herein, the reaction mixtures were centrifuged and then resuspended in PBS buffer with repeating 3 times. The protein-conjugated microgels were stored at 4 °C and used the next day.

SPR Analysis. All SPR experiments were conducted with a Biacore T200 apparatus (Biacore GE Healthcare, Chicago, IL, USA) at 25 °C (Figure S1). In the modification of a gold sensor chip, water served as the running buffer for cysteamine functionalization, and 0.1 M MES buffer (0.9% sodium chloride, pH 5.6) served as the running buffer for the microgel attachment process. In all SPR analyses, PBS served as the running buffer. The sensor chip surface was activated with a 10 mM cysteamine solution in water for 600 s with a flow rate of 2 μL/min. The activated sensor chip was modified with a microgel solution in MES buffer 5 times of 60 s injection and 120 s of intervals at each cycle (flow rate of 2 μL/min). All the analytes were diluted in PBS buffer with various concentrations and injected into the chip for 60 s (flow rate of 2 μL/min). The 50 nM and 100 nM IL-2/sIL-2Rα sensorgrams obtained from IL-2Rβ,γ conjugated microgel were fitted using the Langmuir model[38] in the evaluation program (Biacore evaluation program version 2.0). The raw data and fitted results are shown in Figure S2.

sIL-2R ELISA. The blood samples were collected in tubes containing EDTA. The serum was separated by centrifugation at 2000 rpm for 20 min. The concentration of sIL-2Rα in the serum was measured using a Human sIL-2R Instant ELISA Kit (Invitrogen, USA) according to the manufacturer's protocol with minor modification. Briefly, 100 μL of distilled water was added to the sample wells. Each sample (50 μL) was added to each well, in duplicate, and incubated for 3 h at room temperature. For washing,

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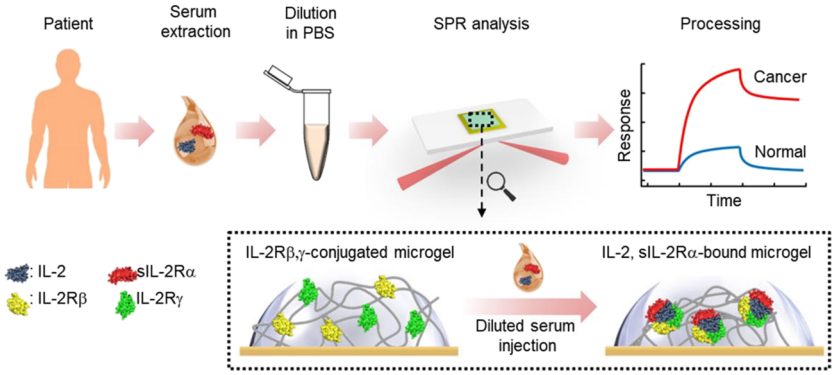

Figure 1. Schematic image of the cancer diagnosis method using the stimulus-responsive microgel-modified SPR sensor chip.

the solution of every well was removed, and 400 μL of washing buffer was added per well. This process was repeated 3 times. The tetramethylbenzidine substrate (100 μL) was added to each well and incubated for 30 min at room temperature. After adding 100 μL of stop solution, the sample was immediately measured by the absorbance at 450 nm.

Dynamic Light Scattering (DLS) and Electrophoretic Mobility Analysis. The hydrodynamic diameter and electrophoretic mobility of microgels were measured using a DLS instrument (Zetasizer Pro, Malvern Panalytical, Worcestershire, UK). Disposable polystyrene cuvettes and capillary cells were used for the DLS and electrophoretic mobility measurements, respectively. The neat gel and bioconjugated gel were diluted with DPBS (ionic strength: ~0.2 M) to 20 μg/mL each to measure the hydrodynamic size at 25 °C. The data reported were taken from the average of 15 individual measurements for measurement. The lower critical solution temperature (LCST) of the microgels was estimated by heating the microgel solution (10 mM NaCl) from 25 to 60 °C with an interval of 1 °C and equilibration time at each temperature for 2 min. The electrophoretic mobility was measured to validate the changes in surface charge of microgels upon protein conjugation. The samples were prepared by dissolving lyophilized microgels in 10 mM of NaCl solution (20 μg/mL), and the measurement was conducted by running 10 times for each sample. Estimation of the electrophoretic mobility was performed using the embedded software.

Microscopy. To obtain microscopic images of microgels, glass coverslips were first cleaned in a plasma cleaner for 10 min under oxygen supply. The coverslips were immersed in 1% of APTMS solution in ethanol (200 proof) for 2 h, then rinsed several times with ethanol, and blown with N_2 gas. The APTMS-functionalized coverslips were assembled with a silicon gasket, and then each well was dropped with 5 μL of 0.1 mg/mL microgel solution. A Nikon Ti2 inverted microscope (Nikon instrument Inc., Melville, NY, USA) equipped with a high numerical aperture, oil immersion 100× objective (NA = 1.25), and a CCD camera (DS-Qi2, Nikon instrument Inc., Melville, NY, USA) was used to capture the images of microgels. The diameter of the microgel was estimated by analyzing the capture images using image j 1.53a (Java 1.8.0_66, National Institutes of Health, Bethesda, MD, USA).

Quantitative Analysis of Protein Conjugation to Hydrogel Microparticles. rGFP was utilized to quantitatively estimate the protein conjugation to hydrogel microparticles via EDC coupling. Conjugation of rGFP was conducted under the same conditions as those of IL-2 receptors. First, 100 μL of rGFP conjugated microgel solution and various concentrations of rGFP solution (PBS buffer) were injected into 96-well black plates with a transparent bottom (Corning, NY, USA), respectively, and the fluorescence intensity of each solution was measured under appropriate conditions (excitation 395 nm and emission 509 nm) via a SpectraMax i3X plate reader (Molecular Device, Sunnyvale, CA, USA). Next, the mass of conjugated rGFPs at a given volume was estimated by fitting the measured fluorescence intensity to a standard curve obtained by measuring the fluorescence intensity of rGFP in PBS solution at various concentrations (Figure S3). Then, the number of microgels per unit volume (5 μL of 0.1 mg/mL microgel solution) was estimated by counting microgels attached on a glass slide via optical microscopy (100× oil immersion objective, Nikon instrument Inc., Melville, NY, USA) (Figure S4). Next, we divided the mass of conjugated rGFP by the number of microgels at a unit volume, resulting in the mass of conjugated rGFP per microgel. Finally, the number of rGFPs per microgel was estimated by dividing the obtained mass of conjugated rGFP per microgel by the molecular weight of rGFP (27 kDa) and multiplying by the Avogadro's number (6.02×10^{23}). In our estimation, there are about 28,900 of rGFPs conjugated per microgel.

RESULTS AND DISCUSSION

To develop a novel cancer diagnosis method, we utilized a stimulus-responsive pNIPAm-*co*-AAc microgel as a sensing element that enables the detection of MPB events.[39] Here, we hypothesized that the MPB-induced signal on the microgel is highly selective, allowing the detection of a cancer marker in complex media such as serum without the need for a complicated procedure (Figure 1). First, we focused on the binding mechanism of IL-2 by which it forms a tetrameric complex with its receptors IL-2Rα, β, and γ. Second, we explored the binding of IL-2 with sIL-2Rα. sIL-2Rα is known to be complexed with IL-2, enhancing the stability of IL-2 in the blood circulation. In previous studies, the levels of both IL-2 and sIL-2Rα were elevated in the blood of cancer patients and measured for the clinical diagnosis of cancers. In this study, we engineered hydrogel microparticles with IL-2Rβ and γ that allowed complexation with IL-2 and sIL-2Rα in serum, and their corresponding signals were measured by SPR analysis (Figure 1).

pNIPAm-*co*-AAc hydrogel microparticles were prepared as previously described.[36] Conjugation of IL-2 receptors to the hydrogel microparticles via EDC coupling was performed to generate the IL-2- and sIL-2Rα-responsive microgels (Figure 2a). The modification of the hydrogel microparticles was confirmed by measuring electrophoretic mobility of the microgels (Figure S5). We further characterized the protein modification of the hydrogel microparticles by measuring the fluorescence intensity and the number of rGFP conjugated microgels per unit volume (Figures S3 and S4). Approximately, 28,900 proteins were conjugated per microgel. The hydrodynamic diameters of the microgels were measured by DLS and were found to be about 1050 nm for the pNIPAm-*co*-AAc hydrogel microparticles and 1100 nm for the IL-2Rβ,γ-conjugated microgel, with polydispersity indices of 0.1 for neat hydrogel microparticles and 0.2 for IL-2Rβ,γ-conjugated microgels (Figure S6). The LCST of the neat hydrogel

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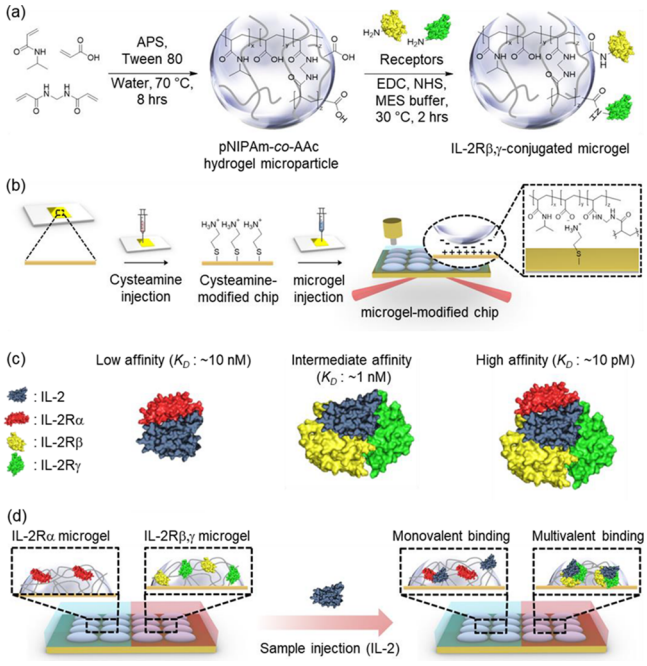

Figure 2. Overall process of MPB-based microgel-mediated SPR analysis. (a) Synthesis of responsive microgels through the conjugation of IL-2 receptors by EDC coupling. (b) Modification of the SPR sensor chip with the microgels in an SPR instrument. (c) Characteristics of IL-2 binding with its receptors. (d) Schematic diagrams of sample injection onto the microgel-modified sensor chip and the consequent deswelling of the microgels.

microparticles was characterized from 25 to 60 °C (Figure S7). Also, the pNIPAm-*co*-AAc hydrogel microparticles attached on the amine-functionalized cover glass were visualized using an optical microscope (Figure S8), which allows estimating the size of the microgels in PBS buffer (1030 nm ± 110 nm). Five types of IL-2 receptor-conjugated microgels were investigated to determine whether MPB-induced signals (IL-2Rβ,γ- and IL-2Rα,β,γ-conjugated microgels) were significantly higher than signals induced by nonspecific or monovalent binding (IL-2Rα-, IL-2Rβ-, and IL-2Rγ-conjugated microgels).

To investigate the MPB-enhanced SPR signals generated by IL-2 and sIL-2Rα binding via a label-free approach (Figure 2b), the surface of a bare gold SPR chip was first modified with a cysteamine solution that introduced a positive charge for coulombic interaction with the carboxyl groups of the microgels. Then, the microgel solution was dissolved in MES buffer (pH = 5.6) to enhance the coulombic force and injected using an SPR instrument (Figure S9). After sensor chip modification, SPR analysis was performed with each sample solution to detect target molecules based on MPB events between IL-2 and its receptors.

There are different types of IL-2 binding events with its receptors (Figure 2c). For instance, IL-2Rα alone has a relatively weak affinity (K_D: ~10 nM) for IL-2 via monovalent binding, while IL-2Rα significantly enhances the binding affinity (K_D: ~10 pM) between IL-2Rβ/γ and IL-2 via tetrameric binding, that is, a type of MPB.[40,41] In the absence of IL-2Rα, IL-2Rβ/γ binds to IL-2 with intermediate affinity (K_D: ~1 nM) via trimer-like binding that is, another type of MPB. MPB-assisted detection of IL-2 using this bioresponsive microgel and SPR system is illustrated (Figure 2d), where the IL-2Rβ,γ-conjugated microgels were more subjected to deswelling than were the IL-2Rα microgels because of MPB events. This MPB-induced deswelling of microgels can change the refractive index of the SPR sensor chip, as indicated by the sensorgram of the SPR instrument system.

To assess the application of the MPB-based microgel SPR method to cancer diagnosis, we examined whether microgels engineered with IL-2 receptors enabled the formation of complexes with the IL-2 ligand, enhancing SPR sensorgrams via MPB events (Figure 3). Herein, we prepared two types of

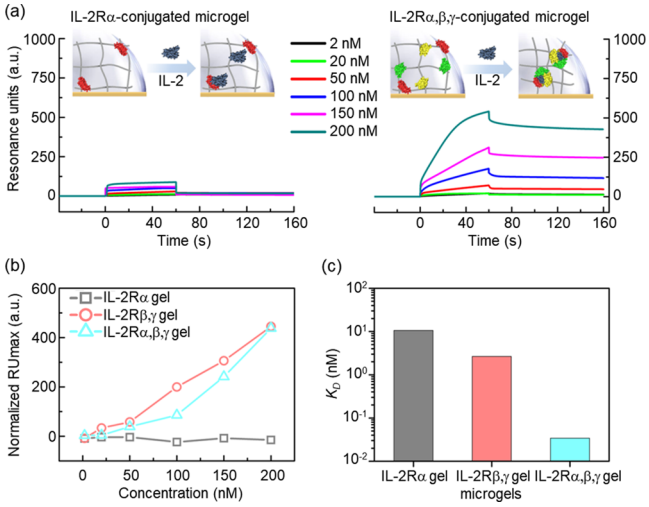

Figure 3. SPR analysis of IL-2 receptor-functionalized microgels upon IL-2 injection. (a) Sensorgrams of the IL-2Rα-conjugated microgel and IL-2Rα,β,γ-conjugated microgel upon IL-2 injection. (b) Normalized RUmax plot of the two MPB-capable microgels and the MPB-incapable microgel in response to IL-2 injection. (c) Estimation of the dissociation constant (K_D) for each type of complex using the MPB-based microgel SPR method.

MPB-capable microgels—that is, an IL-2Rβ,γ-conjugated microgel and an IL-2Rα,β,γ-conjugated microgel—and one type of MPB-incapable microgel—that is, an IL-2Rα-conjugated microgel. The representative SPR sensorgrams of these microgels are shown in Figures 3a and S10. By injecting an IL-2 solution into an SPR chip modified with each microgel, we were able to confirm MPB-enhanced SPR signals only for the MPB-capable microgels, while the MPB-incapable microgel showed sufficiently lower signals than the MPB-capable microgels (Figure 3b). Note that normalization of maximum resonance units (RUmax) upon IL-2 injection was conducted by subtracting the RUmax value of neat pNIPAm-*co*-AAc hydrogel microparticles from that of each receptor-conjugated microgel. We also estimated the dissociation constant (K_D) for each binding case (Figure 3c), and found that they were comparable to those in a previous report (Figure 1c). It is interesting to note that the RUmax values of the IL-2Rβ,γ- and IL-2Rα,β,γ-conjugated microgels in response to IL-2 injection were similar, probably because of MPB events. However, the dissociation constant (K_D) for each case differed, suggesting that the formation of tetrameric complexes between IL-2 and IL-2R$\alpha/\beta/\gamma$ is kinetically more favorable than the formation of trimeric complexes between IL-2 and IL-2Rβ/γ.

Next, we prepared microgel-based SPR biochips for the detection of IL-2 and sIL-2Rα as target molecules because of their elevated levels in the serum of most cancer patients.[42] In particular, the coexistence of IL-2 and sIL-2Rα facilitates the formation of tetrameric structures with IL-2Rβ/γ, which are

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physiologically important in the immune response and cancer progression. To evaluate the utility of our MPB-based microgel method in complexation of the tetramer, we prepared one type of MPB-capable microgel—that is, an IL-2Rβ,γ-conjugated microgel—and two types of MPB-incapable microgels—that is, IL-2Rβ- and IL-2Rγ-conjugated microgels. Representative SPR sensorgrams of the IL-2Rβ- and IL-2Rβ,γ-conjugated microgels, after injecting with a solution containing IL-2 and sIL-2Rα, are shown in Figure 4a (sensorgrams of the IL-2Rγ-conjugated microgel are shown in Figure S11). Enhanced SPR signals upon injection of the solution containing IL-2 and sIL-2Rα were only observable with the IL-2Rβ,γ-conjugated microgels, while significantly lower signals were measured with the IL-2Rβ- and IL-2Rγ-conjugated microgels (Figure 4b). This result suggests that IL-2 and sIL-2Rα induce the formation of a tetrameric structure with the IL-2Rβ/γ attached to the microgels and indicate that our MPB-based microgel method is efficient in identifying the tetrameric assembly of the IL-2 ligand and IL-2R$\alpha/\beta/\gamma$ proteins. Estimation of the dissociation constant (K_D) for each binding case (Figure 4c) was also conducted and indicated that the formation of the tetrameric complex is kinetically more favorable than the formation of trimeric complexes as previously reported.

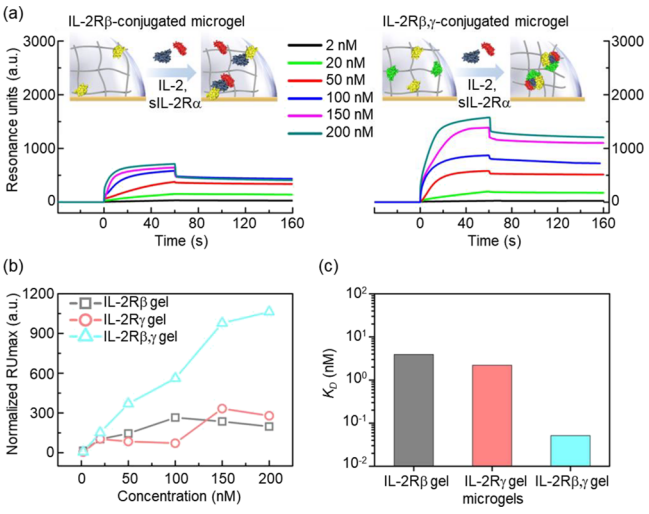

Figure 4. SPR analysis of IL-2 receptor-functionalized microgels upon injection of IL-2 and IL-2Rα. (a) Sensorgrams of IL-2Rβ- and IL-2Rβ,γ-conjugated microgels upon injection of IL-2 and IL-2Rα. (b) Normalized RUmax plot of one MPB-capable microgel and two MPB-incapable microgels in response to IL-2 and IL-2Rα injection. (c) Estimation of the dissociation constant (K_D) for each type of complex formation using the MPB-based microgel SPR method.

In developing a rapid cancer diagnosis method, we employed the capability of IL-2Rβ,γ-conjugated microgels, as illustrated in Figure 4, for the detection of IL-2 and sIL-2Rα in human serum samples. Overall schemes showing the process of cancer diagnosis by the MPB-based microgel SPR method and conventional ELISA are shown in Figure 5a. Serum samples were obtained from cancer patients who were previously diagnosed in the hospital and from healthy controls. For MPB-based microgel SPR analysis, the serum samples were serially diluted 100-, 1000-, 10,000-, and 100,000-fold and injected into IL-2Rβ,γ-conjugated microgel SPR chips and unmodified neat microgel SPR chips. Then, SPR analysis was conducted for the cancer patients and healthy controls. In parallel,

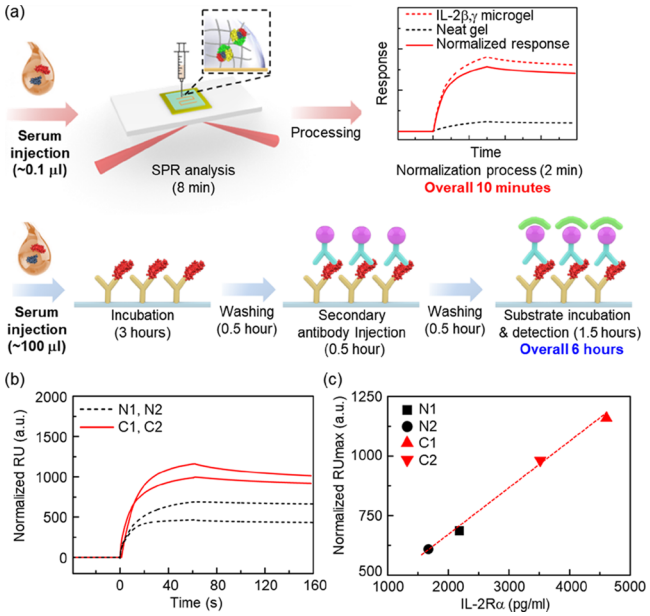

Figure 5. Comparison of the MPB-based microgel SPR analysis and ELISA methods for cancer diagnosis (a) overall procedure for MPB-based microgel SPR analysis and ELISA with cancer patients (C1 and C2) and healthy controls (N1 and N2). (b) Sensorgrams of C1, C2, N1, and N2 at dilution factor of 0.001×. (c) Correlation of normalized RUmax values with the concentration of IL-2Rα estimated by ELISA.

conventional ELISA was conducted with the same serum samples that were used for SPR analysis. It should be mentioned that ELISA was conducted with undiluted samples (~100 μL) because of the incapability of target detection with dilution samples (data not shown) and required more time (overall, 6 h) than MPB-based microgel SPR analysis using the prefunctionalized microgels (10 min). The SPR results suggested that the 1000-fold dilution of the serum samples was enough to yield contrasting signals between cancer patients (C1 and C2) and healthy controls (N1 and N2) (Figure 5b). Note that the RUmax value upon injection of each serum sample was normalized by subtracting the RUmax value of the neat microgel from that of the IL-2Rβ,γ-conjugated microgel (Figures S12 and S13). Figure 5c shows the good correlation with the results of the MPB-based microgel SPR analysis: the x-axis shows the results of conventional ELISA, and the y-axis shows the normalized RUmax values of 1000-fold diluted serum samples. It is also worthwhile to mention that the higher the dilution of serum, the lower the contrast in the signal between the cancer patients and healthy controls. This pattern could be explained by the observation that a higher dilution of serum decreases the concentrations of the target proteins, that is, IL-2 and sIL-2Rα, in the sample. Nevertheless, in correlation with ELISA, our method allows us to detect the difference of ~2 pg/mL of sIL-2Rα in serologic SPR analysis. These results suggest that our method potentially enables on-site diagnosis of cancers in a rapid and sensitive manner. In addition, it might be worthwhile to expand this MPB-based microgel SPR diagnosis to a large number of patients with different cancer types, such as lung and liver cancer.

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CONCLUSIONS

We developed the MPB-based microgel SPR method as a rapid and accurate cancer diagnosis tool by engineering pNIPAm-*co*-AAc hydrogel microparticles with IL-2Rβ/γ, which form tetrameric complexes with IL-2 and sIL-2Rα in patient serum. We demonstrated that MPB-dependent signals of bioresponsive microgels are suitable to determine the formation of trimeric and tetrameric structures by IL-2-induced receptor complexation. Our findings also indicated that the estimated dissociation constant (K_D) for each binding case was comparable to that previously reported, while the intensity of the SPR signal was significantly influenced by MPB events. The application of the MPB-based microgel SPR method for cancer diagnosis was successful, with results strongly correlated with those of conventional ELISA, which is currently used in hospitals. In addition, our method enables the identification of cancer patients with a 1000-fold dilution of serum in only a few minutes, superior to the ELISA, which requires undiluted serum and a much longer analysis time. In terms of cost, a bioconjugated microgel-modified SPR sensor chip can be manufactured with approximately US$ 1. In this study, we introduced a novel type of diagnostic platform for rapid and accurate clinical diagnosis. The lack of a requirement for complicated processing of clinical samples is a fundamental benefit of the MPB-based microgel SPR method for developing a POCT-type diagnostic tool for clinical use. Thus, it might be worthwhile to expand this method to other clinical assays that require rapid and accurate results in only a few minutes. There is a caveat that the high-quality SPR instrument (Biacore T200, used in this study) is expensive and only available at limited sites. Recently, inexpensive and portable SPR apparatuses have been introduced,[43] suggesting that the development of novel platforms for SPR biosensors would facilitate SPR's use and increase its accessibility. Detection of other cancer types related to elevated sIL-2Rα in serum[14,44] or viral RNA and DNA via DNA/RNA hybridization will be valuable for future studies using the MPB-based microgel SPR method.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.1c04827.

DLS result of the microgels, quantitative analysis of protein conjugation to microgels, SPR data of sensorchip modification, response of IL-2 receptor-functionalized microgels against IL-2, response of the IL-2 receptor-functionalized microgels against combination of IL-2 and sIL-2Rα, and response of the microgels against serums (PDF)

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Author Contributions

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Notes

The authors declare no competing financial interest.

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