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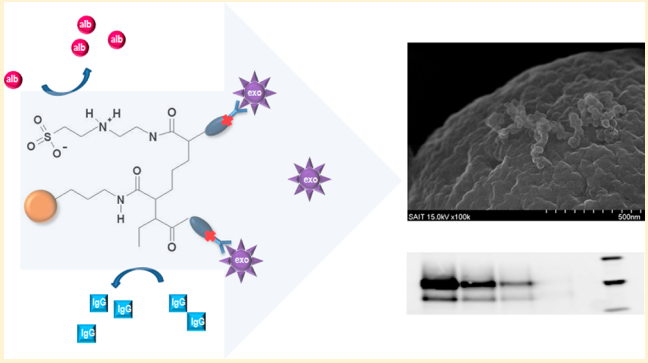
Noble Polymeric Surface Conjugated with Zwitterionic Moieties and Antibodies for the Isolation of Exosomes from Human Serum

Gahee Kim, Chang Eun Yoo, Myoungsoon Kim, Hyun Ju Kang, Donghyun Park, Myoyong Lee,* and Nam Huh

Bio Research Center, Samsung Advanced Institute of Technology (SAIT), Mt. 14-1, Nongseo-dong, Giheung-gu, Yongin-si, 446-712, Gyeonggi-do, South Korea

Supporting Information

ABSTRACT: New zwitterionic polymer-coated immunoaffinity beads were developed to resist nonspecific protein adsorption from undiluted human serum for diagnostic applications of exosomes. A zwitterionic sulfobetaine monomer with an amine functional group was employed for simple surface chemistry and antifouling properties. An exosomal biomarker protein, epithelial cell adhesion molecule (EpCAM), was selected as a target molecule in this work. The beads were coated with polyacrylic acids (PAA) for increasing biorecognition sites, and protein G was then conjugated with carboxylic acid groups on the surfaces for controlling EpCAM antibody orientation. The remaining free carboxylic acid groups were modified with sulfobetaine moieties, and anti-EpCAM antibody was finally introduced. The amount of anti-EpCAM on the beads was increased by 40% when compared with PAA-uncoated beads. The surfaces of the beads exhibited near-net-zero charge, and nonspecific protein adsorption was effectively suppressed by sulfobetaine moieties. EpCAM was captured from undiluted human serum with almost the same degree of efficiency as from PBS buffer solution using the newly developed immunoaffinity beads.



INTRODUCTION

Since Norwegian scientist John Ugelstad was first able to create polystyrene beads, research on bead-based isolations was extensively conducted for biomedical applications such as clinical diagnosis,[1] drug targeting,[2] cell isolation[3] and purification,[4] nucleic acid purification,[5] and detection.[6]

Especially, the utility of magnetic beads in biological fluids is tremendous due to the simple and fast separation process of beads. In general, it is difficult to isolate target biomolecules present in complex media with high purity. Therefore, magnetic bead-based immunoaffinity purification has been envisaged as a strategy to enhance the effective isolation of a target molecule from the media. However, an immunoaffinity bead-based method is not easily applicable to high-protein-containing body fluids such as serum or plasma due to nonspecific adsorption of serum proteins. In the case of serum, the primary serum proteins are albumin, immunoglobulin, and fibrinogen, and these proteins represent over 90% of total serum protein.[7,8] Because these proteins are adsorbed onto the surfaces of immunoaffinity beads very fast and thus interfere with specific binding, control of the nonspecific protein adsorption plays a key role in achieving the successful isolation of target molecules. Therefore, it is critical to develop immunoaffinity beads with antifouling surface materials on the surfaces which are effective enough in undiluted human serum. The most common materials used for surface coatings to prevent nonspecific protein adsorption are poly(ethylene gylcol) (PEG) and oligo(ethylene glycol)s (OEG).[9] However, PEG and OEG can decompose in the presence of oxygen or transition metal ions,[10,11] resulting in limited application of these materials in biological fluids.

Recently, zwitterionic moieties such as phosphorylcholine, sulfobetaine, and carboxybetaine are being studied intensively for reducing nonspecific protein adsorption in various applications.[12−15] Zwitterions are excellent candidates as nonfouling materials because of balanced charge, minimized dipole moment, and strong hydration capacity through electrostatic interactions as well as highly hydrophilic characteristics.[7] Target proteins and chemicals in serum or plasma were effectively detected by sensors of which the surfaces were coated with zwitterionic moieties such as carboxybetaine.[16] The sensors in undiluted serum showed almost the same sensitivity as in buffer solutions. Therefore, it is expected that immunoaffinity magnetic beads modified with zwitterionic moieties will have a great deal of utility in serum-based diagnostics.

In general, zwitterionic polymer-based surface platforms are prepared by surface grafting polymerization through surface-

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initiated atom transfer radical polymerization (ATRP) or self-assembled monolayer (SAM) formation through thiolating monomers of zwitterionic monomers on the surface, which limits the full exploitation of a zwitterionic surface chemistry in various diagnostic applications. Even though one study reported that a zwitterionic polymer could be synthesized as a coatable type of polymer, the preparation process was highly complicated and polymerization reactions were not efficiently achieved.[7] Therefore, immunoaffinity magnetic beads using a zwitterionic surface chemistry have not yet been studied to the best of our knowledge.

In this study, we developed zwitterionic polymer-coated magnetic beads having a high degree of biorecognition sites. We designed and synthesized the sulfobetaine monomer with an amine functional group for simple conjugation with carboxylic acid groups on bead surfaces, enabling a simple antibody incorporation process in the bead preparation. The number of specific binding sites was increased by coating the surfaces with PAA to maximize the efficiency of target molecule isolation, and protein G was conjugated with PAA for controlling antibody orientation. We analyzed the surface characteristics of the prepared beads and evaluated the antifouling properties by comparing target detection in PBS buffer with that in undiluted human serum.

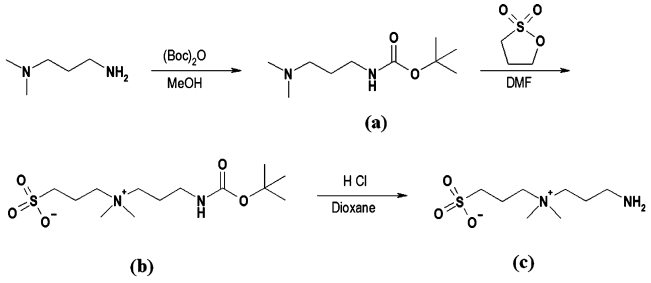
Exosomes are membrane vesicles with a size of 40−100 nm that are released from many different cell types such as red blood cells, platelets, lymphocytes, dendritic cells, and tumor cells[17] and are found to occur in body fluids such as breast milk, serum, plasma, malignant ascites, and urine.[18,19] Recently, a number of studies have demonstrated that exosomes carry specific proteins, mRNA and miRNA,[18,20] which can be utilized as diagnostic markers of malignancy, including cancer. In this work, an exosomal tumor marker protein, epithelial cell adhesion molecule (EpCAM) was selected as a model protein to demonstrate an antifouling surface chemistry of the zwitterionic polymer-coated immunoaffinity beads.

EXPERIMENTAL SECTION

More detailed information on the materials and methods used can be found in the Supporting Information.

Synthesis of Zwitterionic Moiety, Sulfobetaine (SB) (Scheme 1). **N-Boc-dimethylaminopropylamine** (A): Di-*tert*-butyldicarbonate was added to a solution of *N*,*N*-dimethylaminoproylamine in methanol at 0 °C and the solution was warmed to RT. The solvent was then evaporated and DI water was added. After extracting the product with ethyl acetate, the solvent was evaporated. The white powder product was obtained and used in the next step without further purification. **3-((3-((*tert*-Butoxycarbonyl)amino)propyl)-dimethylammonio)propane-1-sulfonate** (B): Sultone was added to the solution of N-Boc-dimethylaminopropylamine in anhydrous *N*,*N*-dimethylformamide, and this solution was stirred for 2 days at RT. After evaporating the solvent, the product was dissolved in ethyl ether. By withdrawing the solvent, the excess sultone was removed. The remaining solvent was evaporated under high vacuum. **3-((3-Aminopropyl)-dimethylammonio)propane-1-sulfonate** (sulfobetaine, C): 4 M HCl in 1,4-dioxane was added to the solution of 3-((3-((*tert*-butoxycarbonyl)amino)propyl)dimethylammonio)propane-1-sulfonate in methylene chloride at 0 °C, and this solution was then warmed to RT. After being stirred for 20 min, the solvent was evaporated. The final product was obtained as a white solid by repeated recrystallization in methylene chloride/isopropylalcohol/methanol.

Scheme 1. Synthesis of the Zwitterionic Moiety, Sulfobetaine



Preparation and Characterization of Immunoaffinity Beads. The immunoaffinity beads (B-PGSE*) was prepared through five stepwise reactions. Those are polymer incorporation, protein G conjugation, zwitterionic moiety modification, antibody immobilization, and cross-linking of antibody with protein G. The experimental details on the preparation and surface characterization are provided in the Supporting Information.

RESULTS AND DISCUSSION

Preparation of Immunoaffinity Beads with Multifunctional Groups. The preparation of immunoaffinity beads comprises five steps (Scheme 2): (i) PAA coating for increasing antibody reactive site (B-P), (ii) protein G conjugation for controlling orientation of antibodies (B-PG), (iii) zwitterionic moiety modification for preventing nonspecific protein adsorption (B-PGS), (iv) antibody introduction for target specific binding (B-PGSE), (v) cross-linking of antibodies with protein G for preventing replacement with human immunoglobulins (B-PGSE*) in serum. PAA was introduced onto magnetic beads via EDC and NHS amide coupling for increasing reactive sites. Additionally, PAA can lead to reduce steric hindrance caused by bulky proteins such as protein G and antibodies. PAA is a type of anionic polymer. In a water solution at neutral pH, many of the side chains of PAA will lose their protons and acquire a negative charge. Due to these polyelectrolyte properties, PAA is hydrophilic and resistant to nonspecific protein adsorption.[21] Major proteins of human serum are albumin and human IgG. The pI value of albumin is 4.5−5.0 and that of human IgG is 7.0−7.5. At neutral pH, they have a slightly negative charge or neutral charge; therefore, negatively charged PAA is more efficient than positively charged PAA for nonspecific protein adsorption. Protein G was conjugated with carboxylic acid groups of PAA via EDC and NHS coupling reactions for orientation of antibodies.[22] The protein G used in this study was a genetically engineered truncated protein which retained its affinity for the Fc region of IgG, but which lacked albumin- and Fab-binding sites and membrane-binding regions.[23] The sulfobetaine (SB) moieties were also coupled with the remaining carboxylic acid groups of PAA via EDC and NHS coupling reaction for suppressing nonspecific protein adsorption. The representative zwitterionic moieties are phosphorylcholine (PC), carboxybetaine (CB), and sulfobetaine (SB). PC has been heavily studied because of its biomimetic characteristics,[7,12,24] but PC is relatively less effective at resisting nonspecific protein adsorption in physiological conditions than CB and SB.[15] Most recently, CB is having been greatly investigated because of its nonfouling

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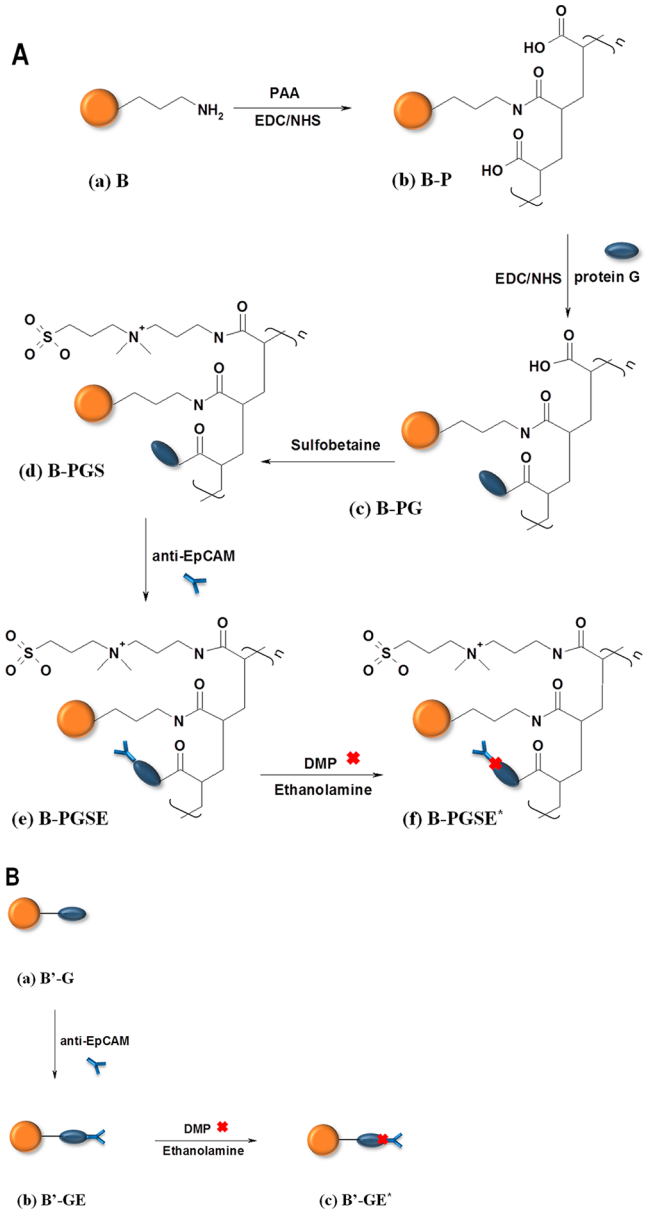
Scheme 2. Schematic Representation of the Immunoaffinity Beads Preparation Process[a]



[a](A) Sulfobetaine-containing bead. (B) Commercial bead.

characteristics and functionalities. The carboxylate group itself of CB could be both a reactive site to be coupled with another molecule and the anionic part of a zwitterionic moiety.[25] Therefore, as other molecules are coupled to the carboxylic acid groups of CB polymer, the zwitterionic characteristics of CB will be lessened, which limits the use of CB as a surface modifier in a simple process of surface chemistry exploiting carboxylic acid functionality. SB moieties are effective at resisting nonspecific protein adsorption in physiological conditions but do not have reactive sites to be coupled with other molecules.[26] Sulfobetaine (SB) was thus chosen as a zwitterionic moiety in our surface treatment study using PAA-coated beads. The bead surface modification by SB moieties was confirmed by ToF-SIMS, as shown in Figure 1. The intensity of other molecules such as CH, CN, and O was not changed, but that of sulfur-containing molecules which are the

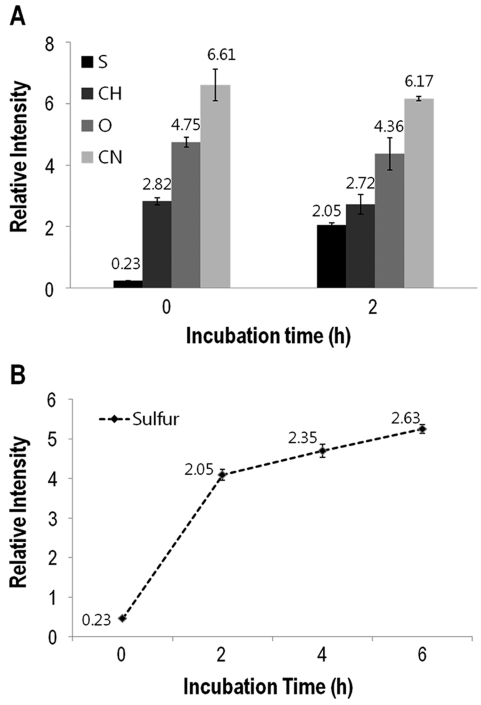


Figure 1. Identification of sulfobetaine (SB) on B-PGSE* by ToF-SIMS. (A) Relative intensity of molecules S, CH, CN, and O after 0 h (initial) and 2 h incubation. The relative intensity of sulfur was the sum of S, SO, SO_2, and SO_3. (B) Relative intensity of sulfur molecules with incubation time.

sum of S, SO, SO_2 and SO_3 originated from sulfate groups in sulfobetaine increased significantly (Figure 1A). The intensities of sulfur-containing molecules were not changed significantly after 2 h of reaction time (Figure 1B). Although the amounts of SB coupled on beads were not assessed directly, it could be concluded that the beads used in our study were saturated with SB from the results. The antibody for capturing a specific target protein was introduced to the protein G via bioaffinity interaction. We chose the EpCAM (epithelial cell adhesion molecule) as a target protein, which is a transmembrane protein. The amount of anti-EpCAM introduced on beads was assessed by measuring the protein content of the antibody solution before and after binding, as described in the Experimental Section. To check the maximum amount of antibody introduced into beads, solutions with various amounts of antibody were mixed with beads (B-PGS) and the amount of antibody in solution before and after mixing was measured. The result is shown in Figure S1 in the Supporting Information. The amount of antibody bound to beads did not increase as the input amount of antibody was over 80 μg. The maximum amount of antibody bound to B-PGSE* was 80 μg per 100 μL bead. The same experiment was done with commercial protein G bead (B′-GE*) and the maximum amount of antibody bound to B′-GE* was 56 μg per 100 μL bead (Table 1). This amount corresponds to ca. 65% of that for B-PGSE*. Therefore, the

Table 1. Comparative Data on B-PGSE* and B′-GE*

bead ID	bound antibody (per 100 uL of beads)	zeta potential (mV, in pH 8.0)	nonspecific protein adsorption (ng/cm^2)
B-PGSE*	80 μg (±2.0)	−0.55 (±1.8)	775.0 (±28)
B′-GE*	56 μg (±1.2)	−4.04 (±0.2)	1576.1 (±90)

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amount of antibody bound to B-PGSE* increased by 1.4 times, compared with that on B′-GE* (According to the manufacturer's manual, both types of beads have the same size (2.8 μm) and concentration (2×10^9/mL). Therefore, direct comparison of antibody amount is reasonable). It is thought that the increased amount of antibody on B-PGSE* may have resulted from more reactive sites and possible reduction of steric hindrance by introducing PAA on the surfaces of beads as described above. The cross-linking was conducted between protein G and anti-EpCAM to prevent an exchange of mouse EpCAM antibody for human immunoglobulins (IgG) in serum. The equilibrium constant (K) of protein G and human IgG interaction is smaller than that of protein G and mouse IgG (6.70×10^{-11} M^{-1} vs 3.96×10^{-10} M^{-1}).[27] The association constants are almost the same for these two reactions, but the dissociation constants differ by 1 order of magnitude. Considering these values, it may be assumed that anti-EpCAM which is not cross-linked with protein G will be exchanged with human IgG if the reaction is conducted in serum. This assumption was confirmed by incubation of B-PGSE both in buffer and in serum, followed by Western blot analysis. The Western blot band of anti-EpCAM when incubated in undiluted human serum decreased about 40% due to the exchange of antibody with IgG whereas that in buffer almost retained (see Figure S2 in the Supporting Information). Therefore, cross-linking of antibody with protein G must be needed to prevent exchange of target-specific antibody for human serum IgG. Dimethyl pimelimidate (DMP) was used as a cross-linker because the imidoester functional group is one of the most specific acylating groups available for the modification of primary amines and has minimal cross reactivity toward other nucleophilic groups in proteins. In addition, the imidoamide reaction product does not alter the overall charge of the protein, potentially retaining the native conformation and activity of the protein.[28] Completion of cross-linking was confirmed by disappearance of antibody band in Western blot assay after reaction (see Figure S2 in the Supporting Information).

Surface Properties of Prepared Immunoaffinity Bead (B-PGSE*). There are four main interactions at the interface between protein and surface. Those are hydrophobic, electrostatic, hydrogen bonding, and van der Waals interactions. In order to reduce nonspecific protein adsorption onto a surface, the control of these interactions is very important. Van der Waals interactions cannot be controlled because they exist between molecules universally. The control of hydrogen bonding interaction between surface and nontarget proteins is also limited, because all biomolecules act in the medium of water including the human body. As a result, the control of hydrophobic and electrostatic interactions between surface and nontarget proteins is needed to resist nonspecific protein adsorption.[7,29,30] This could be done by incorporation of zwitterionic groups on a surface. Zwitterionic surfaces induce more water molecules to bind to the surface via ionic interactions in addition to hydrogen bonding, resulting in the thicker hydration layer and large amount of free water in the hydration layer.[16,31] Therefore, zwitterionic surfaces are very hydrophilic, and nonspecific protein binding can be minimized.[13] Zwitterionic surfaces have also balanced (net-zero) charge and minimized dipole, and these characteristics are not changed by biological pH range. In general, electrostatic interactions between balanced charge surfaces and proteins can be minimized if the pH of media is not significantly different from pI values of proteins.[32] Considering the pH of serum (7.35−7.45) and pI value of main proteins in serum (IgG, 7.4; albumin, 4.7), it may be expected that nonspecific protein adsorption by electrostatic interaction in serum can also be minimized. The charge properties of bead surfaces could be identified by measuring zeta potential. The zeta potential of B-PGSE* was determined by streaming potential in sodium borate buffer (pH 8.0) and shown in Figure 2. The zeta

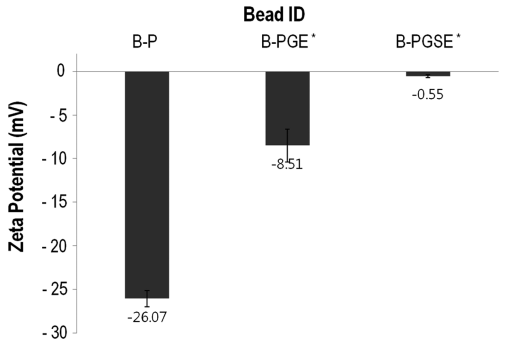

Figure 2. Zeta potential of B-P, B-PGE*, and B-PGSE* in sodium borate buffer (pH 8.0) at RT. Error bars show the standard deviation of three experiments.

potential value of B-PGSE* was −0.55 mV, almost zero charge. On the other hand, those of B-P (only PAA coated beads) and B-PGE* (beads prepared without SB) were −26.07 mV and −8.51 mV, respectively. The value of B′-GE* (beads prepared by using commercial protein G beads) was −4.04 mV (Table 1). The highly negative charge of B-P was caused by carboxylic acid groups of PAA. The net negative charge of B-PGE* may be due to remaining carboxylic acid and conjugated proteins on the beads when considering pK_a value of carboxylic acid groups (4.5) and pI value of protein G (4.2) and anti-EpCAM (7.4). This result indicates that, in the case of B-PGSE*, almost all carboxylic groups are coupled with protein G and SB, and SB moieties are highly effective for neutralizing the surface charge. From these data, we could conclude that the immunoaffinity beads with almost net zero surface potential were prepared successfully by incorporating SB moieties on the bead surface.

A measurement of water contact angle on the bead surface was not technically feasible. However, it may be expected that the beads with sulfobetaine have a high degree of hydrophilicity when considering water contact angle value of 15° reported for the flat surface coated with sulfobetaine.[15] This high degree of hydrophilicity of SB modified surface was due to the formation of a thicker hydration layer via charge−charge (electrostatic) interaction as well as hydrogen bonding, as mentioned previously.[15,16] These hydrophilic and electrically neutral characteristics are thought to contribute to the resistance of nonspecific protein adsorption on the bead surface.

Assessment of Nonspecific Protein Adsorption with B-PGSE*. We used B′-GE* (beads prepared by using commercial protein G beads) as references to evaluate isolation capacity of our beads system. We evaluated the nonspecific resistance properties of B-PGSE* by reacting in undiluted human serum, and the level of serum protein adsorption was compared with that of B′-GE*. The result was shown in Table 1. The amount of bound protein onto B-PGSE* was 775.0 ng/cm^2 and there was only about 50% of B′-GE* nonspecific binding (1576.1 ng/cm^2). Nonspecific protein adsorption was highly suppressed in the zwitterionic moiety modified beads (B-

PGSE*) as compared to control beads (B′-GE*). As previously mentioned, charge balanced and highly hydrophilic surfaces are the most important factors to reduce nonspecific protein adsorption. These two factors were embodied on bead surfaces by employing sulfobetaine, and consequently, nonspecific adsorption of protein was reduced by about 50% when compared with the control beads which have hydrophilic but negatively charged surfaces.

In our results, the absolute amount of nonspecific protein adsorption was rather higher than those reported in other references.[7,14] This discrepancy results from the different detection systems used in the studies. In the most studies, the nonspecific protein adsorption was measured by SPR spectroscopy. The SPR spectroscopic study of protein adsorption is very restricted. The SPR spectroscopy is a flow cell detection system in which a contact time between surfaces and analytes is limited. Even though, the flow rate can be controlled, the contact time is within a very restricted range, several tens of minutes because of systematic limitation. As described in the Experimental Section, our beads were incubated in human serum overnight and the contact condition between bead surfaces and human serum proteins is not flow system but mixed system. It allows us to adjust contact time between surfaces and analytes as much as we want. Considering these factors mentioned above, it is a matter of course that nonspecific protein adsorption on bead surface is much higher than that on flat surface, even if the same functional group such as sulfobetaine are on both surface.

The comparative data of B-PGSE* and B′-GE* are summarized in Table 1.

Isolation of Exosome with B-PGSE*. To isolate exosome from serum, we selected EpCAM as a specific protein biomarker of exosome. EpCAM is the cell surface molecule that is known to be highly expressed in many primary and metastatic epithelial cancers.[33] Recently, some reports review that the cancer-derived exosomes contain a higher level of EpCAM than normal exosomes.[33] In this research, serum of cancer patients was mimicked by spiking EpCAM expressed exosomes derived from a cancer cell line into normal human serum. The normal human serum was purchased as sterile-filtered solutions, and EpCAM was undetectable our assay. The spiked samples were reacted with B-PGSE* and B′-GE*, and exosomal EpCAM was then detected (Figure 3). The EpCAM capture by the beads increased depending on the concentrations of spiked exosomes. The exosomes were also spiked into PBS buffer, and the binding was compared with that in undiluted human serum. Figure 3A showed the captured amount of EpCAM by B-PGSE* from the buffer and undiluted human serum. The relative intensities of detected EpCAM were confirmed by Western blot band (Figure 3C,D) and were calculated by using *ImageJ*.

The amounts of EpCAM bound to B-PGSE* in serum were the same as that in PBS within the experimental errors over a concentration range of 4.8 ng to 32 ng of exosomal EpCAM was used. In contrast, the amount of EpCAM bound to B′-GE* in serum were decreased by about 25−57% compared to that in PBS over the same range of concentrations (Figure 3B). It was reported that, in immunoaffinity reactions, a decrease in nonspecific adsorption could reduce nonspecific interaction of antibody with nontarget proteins.[26] In our study, nonspecific protein adsorption on beads decreased by incorporation of sulfobetaine, and thus, anti-EpCAM might interact with EpCAM more effectively on B-PGSE*. Even though B-

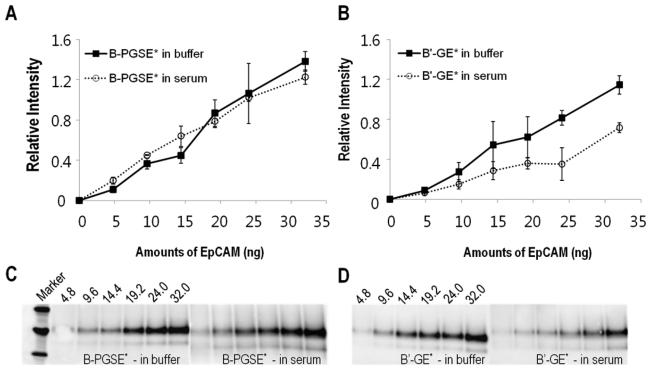

Figure 3. Detection of captured of EpCAM by B-PGSE* and B′-GE*. (A) Relative band intensity of captured EpCAM by B-PGSE* after incubation with various amounts of EpCAM positive exosomes in buffer and in undiluted human serum. (B) Relative band intensity of captured EpCAM by B′-GE* after incubation with various amount of EpCAM positive exosomes in buffer and in undiluted human serum. The relative band intensity is calculated by dividing intensity of input exosome amounts band. Error bars show the standard deviation of three experiments. (C) Western blot image of (A). (D) Western blot image of (B).

PGSE* captures EpCAM more efficiently than B′-GE* due to a higher degree of antibody on the surface, the amount of antibody conjugated on beads does not explain the difference entirely. We compared the capture efficiencies of B-PGE* and B-PGSE* with the same amount of antibody to validate the zwitterionic effect of sulfobetaine moieties. When reacted with ca. 20 ng of exosomal EpCAM, B-PGE* showed 30% lower capture efficiency than B-PGSE* in undiluted serum. Therefore, the relatively lower decrease of captured amount of EpCAM in serum by B-PGSE* may be largely due to the decrease of nonspecific protein adsorption by sulfobetaine. As mentioned in the Introduction, these results are parallel to results in ref 16 in which the sensors coating the surface with zwitterionic moieties in undiluted serum showed almost the same sensitivity as in buffer solutions.

Exosomes used in this experiment were prepared by ultracentrifugation of cell culture media. Pellets obtained by ultracentrifugation contain not only exosomes, but also various proteins.[34] EpCAM might also exist as a soluble form or membrane debris. Therefore, it is useful to identify that the signal of EpCAM is largely from exosomes for the possible utilization of exosomes in diagnostic applications. We determined that the captured protein fractions would have another exosomal marker protein, integrin β, as well as EpCAM,[35] in order to determine that EpCAM was not recovered in a soluble form. The result was shown in Figure 4A. Both proteins could be detected in the same sample, and the captured amounts of both proteins were proportional to the total protein amounts of exosome fractions used for spiking. Additionally, the same experiments were carried out with another two kinds of beads which had antimouse IgG (B-PGSI*) and no antibody (B-PS) instead of anti-EpCAM (Figure 4B,C). Although amount input of total protein in exosomes increased, EpCAM and integrin β were barely detected in both beads, excluding the possibility of nonspecific interaction or adsorption of integrin β.

Last, the visualization of exosomes bound on beads was performed by using field emission scanning electron microscopy. Figure 5A is an image of exosomes itself, Figure 5B is an image of exosomes captured by beads in buffer, and Figure 5C

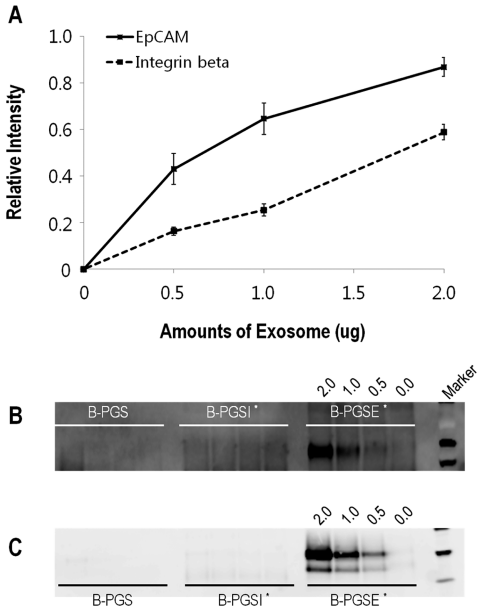

Figure 4. Detection of captured EpCAM and integrin β expressed on exosome surface by B-PGSE*, B-PGSI*, and B-PS in undiluted human serum. (A) Relative band intensity of captured EpCAM and integrin β by B-PGSE*, B-PGSI*, and B-PS after incubation of each bead with various amount of exosomes. Relative band intensity is calculated by dividing intensity of 2 μg exosome band into that of each band. Error bars show the standard deviation of three experiments. (B) Western blot image of captured integrin β by B-PGSE*, B-PGSI*, and B-PS. (C) Western blot image of captured EpCAM by B-PGSE*, B-PGSI*, and B-PS.

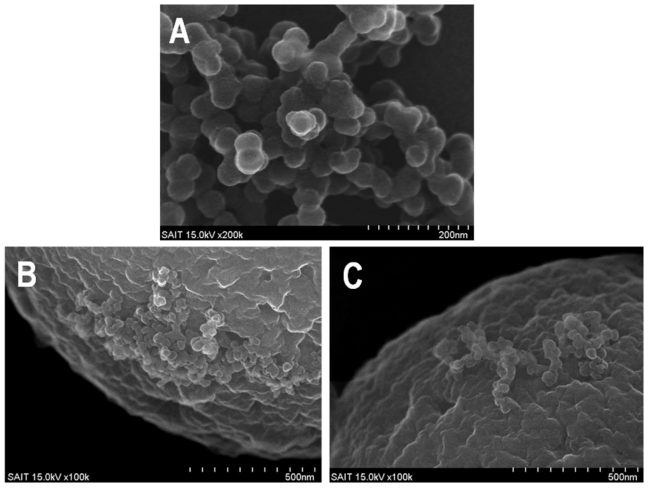

Figure 5. SEM images of the exosome as free and bead bound form: (A) Free exosome. (B) Exosome captured by B-PGSE* in buffer. (C) Exosome captured by B-PGSE* in undiluted human serum.

is an image of exosomes captured by beads in serum. The exosomes were rather aggregated in both free and bound forms. However, in this study we could not confirm that this aggregation already existed in the solution or arose from the sample preparation for SEM measurement. From these two experiments, we could conclude that the captured EpCAM on beads originated from exosomes.

CONCLUSIONS

We have successfully prepared immunoaffinity beads with zwitterionic moieties for controlling nonspecific protein adsorption. The surface characteristics of beads were determined by ToF-SIMS, BCA assay, and zeta potential measurements. We also demonstrated that our novel beads could successively suppress nonspecific protein adsorption and effectively capture a target protein, EpCAM expressed on exosomes in undiluted human serum. This study described that our novel beads were very useful for isolating specific protein molecules from body fluids such as human blood and could be readily applicable for in vitro diagnostics use.

ASSOCIATED CONTENT

Supporting Information

Additional experimental section and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: +82 31 2806943. Fax: +82 31 2806816. E-mail: myoyong.lee@samsung.com.

Notes

The authors declare no competing financial interest.

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The first two authors (G. Kim and C. E. Yoo) contributed equally to this work. We would like to acknowledge Kyunghee Park, Sungwoo Hong, Dr. Yeryoung Yong, and Jongmyeon Park for helpful discussion and technical assistance.

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