Protein Adsorption on the Nickel-Coated Glass Slide for Protein Chips

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The adsorption of proteins on the surface of glass slides is essential for the construction of protein chips. Here, we report that a Histidine (His)-tagged protein has been efficiently adsorbed on glass slides coated with nickel. A variety of nickel chloride-coated plates were prepared by the spin-coating method and adsorbed to the His-tagged protein. When the protein was adsorbed onto the surface of a variety of nickel chloride-coated glass slides, the efficiency of protein adsorption was dependent upon the coating conditions such as nickel chloride concentration, the spin speed and the drying temperature. The slides appropriate for protein adsorption were obtained when the slides were coated with 11%(w/w) of $NiCl_2$ at the spin speed of 4000 rpm for 20 sec and then dried at higher than 40 °C. The physical properties of their nickel chloride thin layer were characterized by scanning electron microscopy, x-ray diffraction and atomic force microscopy, finding that the nickel chloride particles were around 10 nm in diameter and uniformly crystallized at 101 faces. These results show that nickel chloride-coated slides prepared by the spin-coating method are utilizable for the construction of His-tagged protein chips.

Key Words : His tagged protein, Nickel-coated glass slides, Protein chip, Sol-gel

Introduction

In the genomic and proteomic era, the high-throughput assay systems are highly required for screening certain functional molecules, such as proteins, genes and metabolites, in a cell under a given set of physiological conditions. Protein chips are microarrays of proteins adsorbed on the surface of the glass slides to facilitate the high-throughput analysis.[1] In this system, it is possible to display a comprehensive set of proteins in the control or the experimental samples, so that we can compare protein profiles of the control with the target populations. Therefore, their applications expand potentially to the area of screening for new functional proteins, diagnosis and diseases therapy. Because of these possible applications, protein chips are highly focused and considered as valuable tools.

Protein chips are not as well established as DNA chips.[2,3] Compared with DNA chip fabrication, protein chip construction is more difficult not only because of the instability of the protein structure in retaining its functionality but also because of the lack of amplification tools for proteins like the polymerase chain reaction (PCR) for DNA.[1]

Recently considerable researches has been carried out on the construction of protein chips.[4-6] An important basic technique for protein chip fabrication is the binding of a set of protein populations on the surface of the glass slides. For the protein adsorption on the surface of the glass slides, the surface of the glass has to be treated with chemicals or metal ions, exposing the functional groups or metal ions. The exposed chemical groups are then utilized to bind proteins. The amine or aldehyde plates have been proved to facilitate the protein adsorption on the slide surfaces. On the aldehyde plates, proteins bind to the slides by the formation of Schiff base between aldehyde and free amines in proteins.[4] On the amine slides, proteins adsorb to the slide surface with the aid of a cross-linker.[7] Even though the above slides are well developed, there is a disadvantage in either case. When proteins are bound to either of the slides, the binding areas of the proteins are randomly selected. Then the functional domains of the proteins may be accidentally concealed by their linkage to the surface of the slides. The loss of functional domains could be fatal for screening a functional protein in many chip-based experiments.

To overcome the above problem, we used a histidine (His)-tagged protein to adhere on the surface of the slide. Since the His-tagged proteins contain the extra His residues that can be utilized for the adsorption to the slides, the target domain of the test proteins could be exposed to the outside without being involved in the adsorption to the slide. For this approach to be feasible, we developed nickel chloride coated slides that can bind to the His-tagged protein.

Experimental Section

Preparation of Nickel Chloride Coated Slides. The glass slides were cleaned up by sonication in ethanol and water alternatively and then completely dried before coating with nickel chloride. The cleaned slides were then put on the rotor of the spin coater, spread with 0.8 mL of various

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concentration of $NiCl_2$ and then spun at various speeds (1000 to 4000 rpm) for different time points. The coated slides were dried on the hot plates at various temperatures.

Protein Adsorption. The FITC-conjugated His-tagged p43 protein or the FITC-conjugated non-His-tagged p43 protein (8) was applied to the slides, incubated for 0.5h at room temperature, washed with PBS or methanol and dried for fluorescence detection.

Fluorescence Detection. FITC-conjugated proteins immobilized on the glass slide were detected by a Bio-Imaging Analyzer System (BAS; Fuji Photo Film Co.) at excitation and emission of 490 and 520 nm, respectively.

Assay for Physical Properties of the Nickel Chloride Coated Slides. Physical properties of the nickel chloride thin layer on the surface of the slides were assayed by X-ray diffraction (XRD; Scintag XDS 2000). The microstructure and formation of the crystal were also observed by Scanning Electron Microscopy (SEM; LEO 1530). The surface status of the coated slides was determined by Atomic Forces Microscope (AFM; DI Nanoscope 3A).

Results and Discussion

The glass slides were coated with $NiCl_2$ solution in a variety of conditions to obtain an optimal nickel chloride-coated slide for the protein chips. To investigate whether the nickel chloride concentration has an important effect on the protein adsorption, various $NiCl_2$ solutions (5-11%) were used for coating at the fixed spin speed (4000 rpm) and then the $NiCl_2$ coated slides were dried at 60 °C. As shown in Figure 1A and C, protein adsorption efficiently occurred in the nickel chloride-coated slides prepared at >11% (w/w) $NiCl_2$ solution. At a concentration lower than 11% (w/w), the propensity for protein adsorption decreases. We also tested whether the spin speed affects the protein adsorption. The glass slides were coated at three different speeds (4000, 2000 and 1000 rpm) and then dried at 60 °C. Protein adsorption increased with increasing spin speed, as a result the slides prepared at 4000 rpm were of best quality as shown in Figure 1B and C.

Other factors, such as coating time (5 to 30 sec), drying temperature (40-80 °C), and drying time (3 to 20 min), also varied when we coated the slides, showing that these factors were not critical for protein absorption (data not shown). The crucial points of the slide coating for the protein adsorption are the concentration of $NiCl_2$ and the spin speed.

We tested whether protein adsorption occurs specifically between histidine residues and nickel ions. We compared the protein adsorption of the His-tagged protein with the same protein untagged (Figure 1D). The protein adsorption of the His-tagged protein was about ten times higher than that of the untagged protein, indicating that the protein adsorption to the nickel chloride-coated slide is highly specific.

Immobilizing His-tagged proteins to a solid support, -has typically involved the use of Ni^{2+} ions chelated to imidodiacetic acid (IDA) or nitrilotriacetic acid (NTA).[9-12] The NTA sensor chip is now commercially available, but it is ill-suited

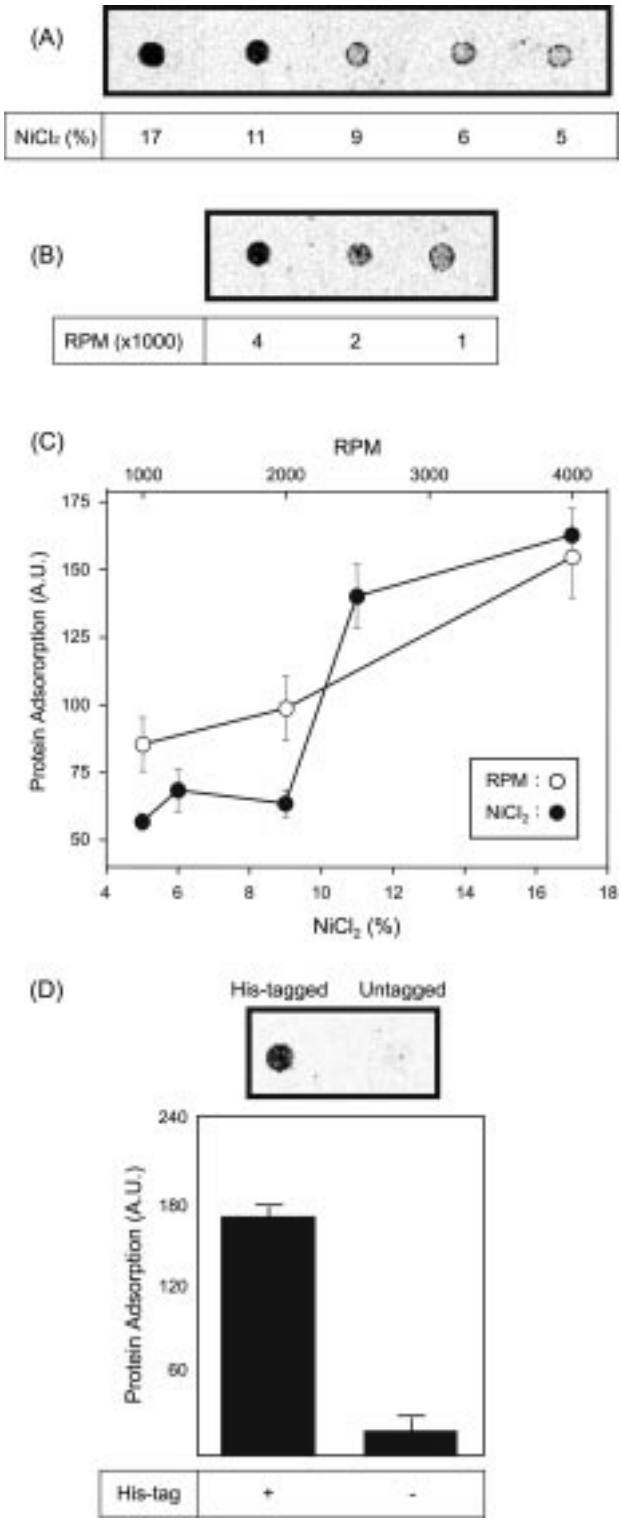

Figure 1. The His-tagged protein was specifically adsorbed to the $NiCl_2$-coated glass slides. The nickel coated slides were constructed by the spin-coating method using the indicated amount of $NiCl_2$ (A) and the indicated spin speed (B). Then the adsorbed proteins were detected and quantified by a BAS image analyzer. The quantification data (mean ± S.D., n = 3) were plotted as line graphs (C). The protein adsorption of the His-tagged protein was compared with that of the untagged same protein (D). The quantification was also performed and plotted as bar graphs (mean ± S.D., n = 7).

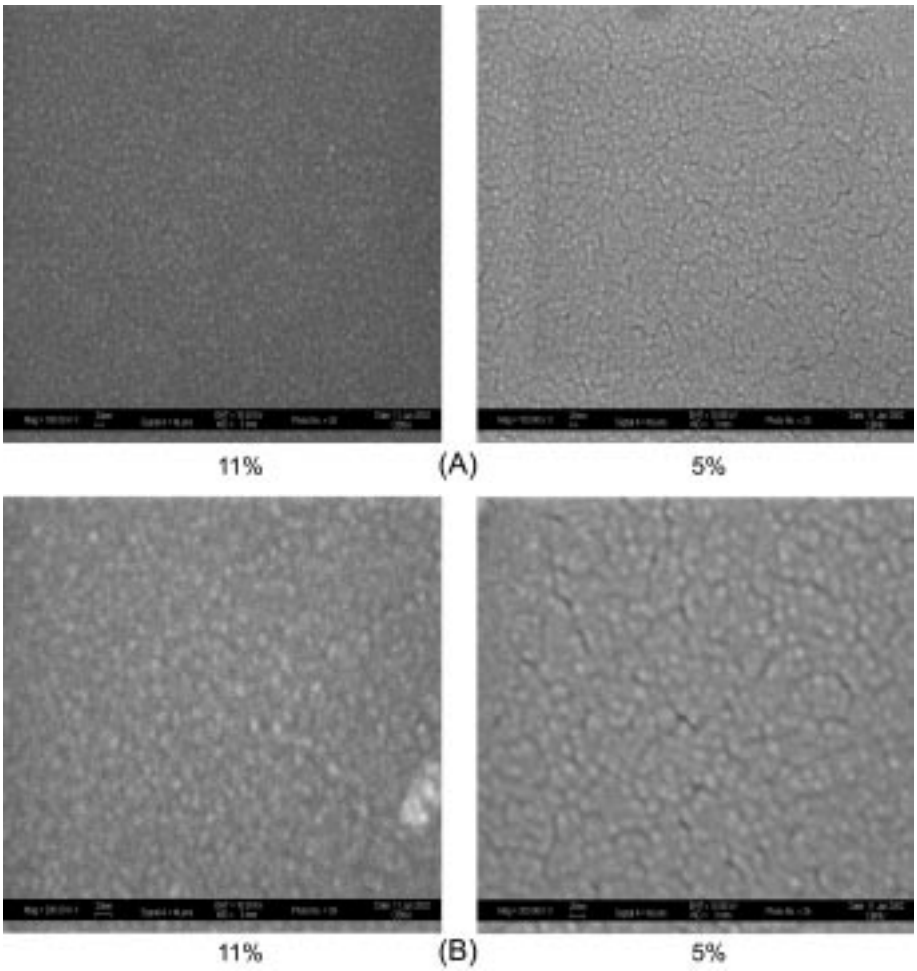

Figure 2. SEM analysis for the glass slides coated with two different $NiCl_2$ concentrations. The nickel chloride-coated slides were prepared as described in the Experimental Methods and then analyzed by SEM (A: × 100,000, B: × 200,000). Left panel: 11% $NiCl_2$, Right panel: 5% $NiCl_2$.

for high-throughput analytical assay.[9] Furthermore, the preparation of NTA or IDA coated glass slide is more complicated and the NTA or ITA coating is not necessarily employed for protein chip fabrication because the NTA or ITA coating is required for regeneration. Therefore our direct nickel chloride-coating method has advantages for protein chip fabrication.

Since the His-tagged protein was adsorbed to the nickel chloride-coated slide, we investigated the physical characteristics of the optimal nickel chloride slides. First, the nickel chloride thin layer on the slides was observed by SEM with different magnification (A: × 100,000 B: × 200,000) (Figure 2). As shown in the SEM pictures, tiny particles (10nm in diameter) are evenly coated on the surface of the slides with compact arrays. Interestingly, cracks were clearly detected in the slides prepared with 5% (w/w) $NiCl_2$, but the cracks dramatically decreased in the slides coated with 11% (w/w) $NiCl_2$. Since cracks reflect weak crystalline and/or irregular distribution of the particles, it is highly possible that better adsorption of protein on the surface results from even distribution of tight nickel chloride crystalline. Figure 3 shows SEM data of the two different slides prepared at 4000 rpm and 1000 rpm, respectively (A: × 100,000, B: × 200,000), indicating that tiny particles are arrayed on the surface. The slides prepared at 4000 rpm display more regular distribution and fewer cracks than those prepared at 1000 rpm and this result was consistent with the protein adsorption pattern in Figure 1. This result confirms the possibility that even distribution of crystalline is critical to bind His-tagged proteins.

Since the formation of crystalline may be a critical factor, XRD patterns were also determined (Figure 4). XRD patterns were obtained in glass slide coated with the indicated concentration of $NiCl_2$. The peak at ~31° becomes higher as the concentration of $NiCl_2$ increases. The peak at ~31° represents an XRD signal for the (101) face of $NiCl_2$ crystalline (13). Although we expected multiple peaks in XRD, crystallization occurred only at the face of (101) during the nickel coating process, possibly resulting from the biased crystallization that may be elicited by centrifugal force of the spin-coater. Interestingly, protein adsorption occurred more efficiently in the slides retaining higher amount of $NiCl_2$ crystalline. This may be caused by the regular arrangement of Ni^{2+} ion in the crystalline, but it remains to be studied.

We also observed the surface shape of the nickel chloride-

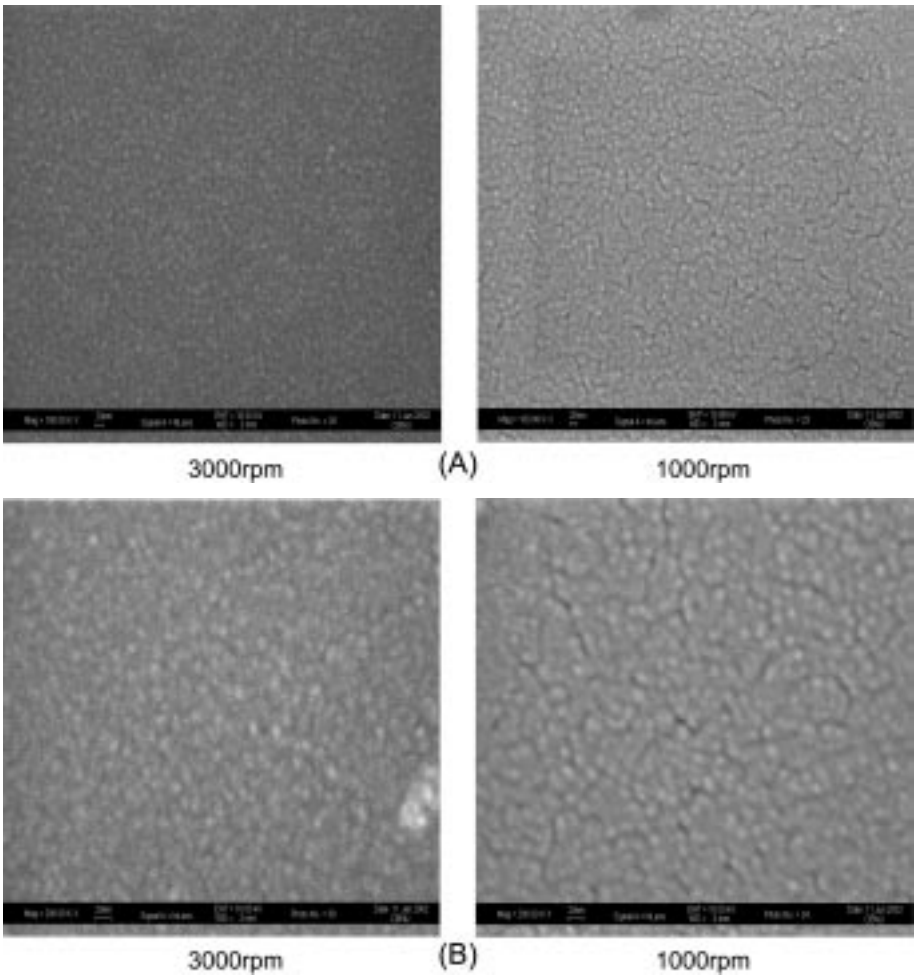

Figure 3. SEM analysis for the glass slides prepared at two different spin speeds. The nickel chloride-coated slides were prepared as described in the Experimental Methods and then analyzed by SEM (A: × 100,000, B: × 200,000). Left panel: 4000 rpm, Right panel: 1000 rpm.

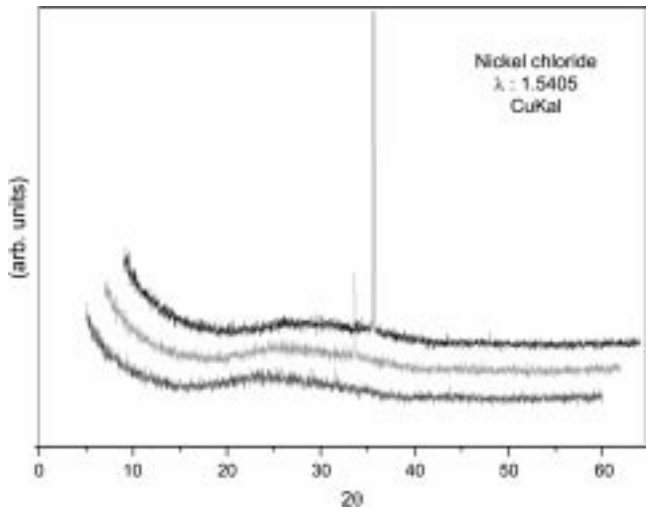

Figure 4. XRD patterns for the glass slides coated with various concentrations of $NiCl_2$. The nickel chloride-coated slides were prepared as described in the Experimental Methods and then analyzed by XRD. Bottom: 5% $NiCl_2$, Middle: 7% $NiCl_2$, Top: 11% $NiCl_2$.

coated slides by using AFM (Figure 5). While the slides prepared with 5%(w/w) $NiCl_2$ shows multiple patterns of crystal and sparse in crystal density, the crystalline forms more densely and the surface patterns are more regular as the concentration of $NiCl_2$ increases. The AFM results also imply that the surface or crystal patterns created in the spin-coating process determine the binding properties of the His-tagged proteins.

Conclusions

The glass slides were coated with nickel ion in various conditions by the spin-coating method and successfully bound to the His-tagged protein. The protein adsorption properties were specifically determined by the condition of the nickel chloride coating. The His-tagged domain binds most efficiently to nickel ions in the nickel chloride-coated plate prepared with 11-17%(w/w) $NiCl_2$ at the spin speed of 4000 rpm, indicating that the most important factors for protein adsorption are the concentration of $NiCl_2$ and the spin speed in the spin coating process. Interestingly, the

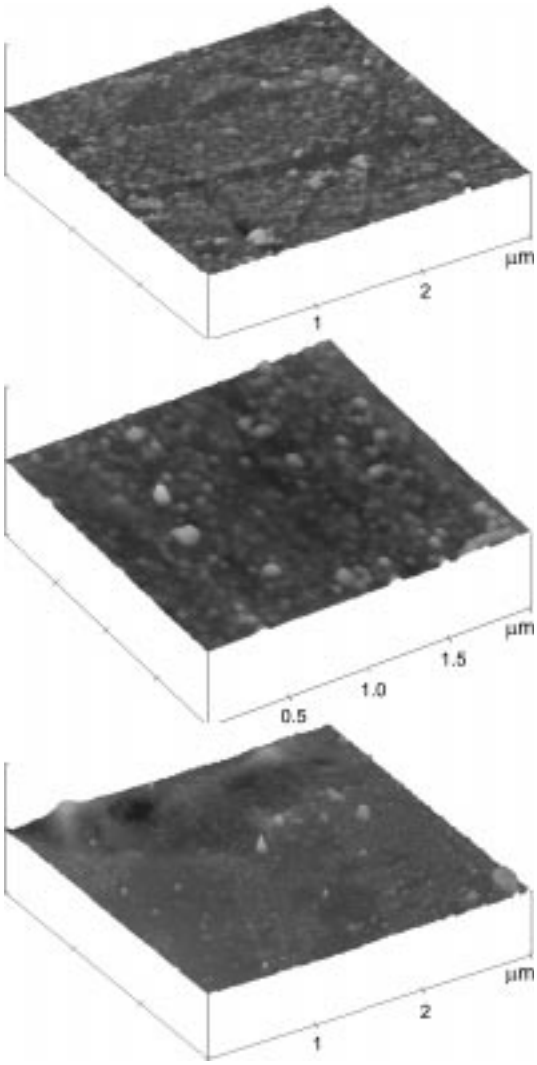

Figure 5. AFM Micrograph for the glass slides coated with various concentrations of $NiCl_2$. The nickel chloride-coated slides were prepared as described in the Experimental Methods and then analyzed by AFM. Bottom: 11% $NiCl_2$, Middle: 9% $NiCl_2$, Top: 5% $NiCl_2$.

slides prepared in the above condition display regular crystallization (the diameter of crystalline is ~10 nm), the even and dense distribution of nickel crystalline, and a singular surface pattern. Thus we conclude that the protein adsorption to the nickel chloride-coated slides is critically determined by the organization and density of the nickel crystalline in the thin layer coated on the glass slides.

The nickel chloride-coated slides prepared here by the spin-coating method have many advantages for protein chip fabrication; 1) low cost, 2) simple constructing process, and more importantly, 3) maintenance of the functional domains of test proteins during protein chip fabrication.

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