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Crystallographic structure of Ni–Co coating on the affinity adsorption of histidine-tagged protein

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ABSTRACT

The principle of immobilized metal affinity chromatography (IMAC) has been recently implemented for protein microarrays for the study of protein abundance and function. Ni–Co film fabricated by electrodeposition is a novel microarray surface in an alloy type for immobilizing histidine-tagged proteins based on IMAC. In this paper, the effects of crystallographic structures and surface properties of Ni–Co coatings, with and without the annealing process, on the immobilization of histidine-tagged proteins were systematically investigated. The experimental results reveal that the stronger hcp texture, due to a higher Co content, results in better affinity adsorption for histidine-tagged biotin. Nevertheless, the allotropic phase transformation from hcp to fcc, due to the annealing process, leads to the decrease of affinity adsorption. The wettability property and the surface roughness of Ni–Co coating are, however, not important factors. Obviously, the crystallographic structure of Ni–Co coating is the dominant factor for the specific affinity adsorption of histidine-tagged protein.

1. Introduction

Proteins are essential for life because they participate in every process within cells. Understanding these complicated protein diversities in structures and functions will assist in developing effective therapies or potential new drugs for the treatment of disease. A protein microarray allowing high throughput study of protein abundance and function is the most common chip type presently developed and has become a powerful tool for clinical diagnostics, medical research and drug discovery [1]. The interface of the microarray surface immobilizing protein samples is a prerequisite for the investigation of molecular interactions and biological reactions. Moreover, specific binding of protein samples to the microarray surface is especially expected. To date, a variety of surface treatments and different types of substrates for protein attachment have already been explored. The nitrocellulose (NC) membrane is one of the most widely used films for protein analysis. With a porous fiber structure and electrostatic force, the membrane can provide nonspecific binding to capture the samples by entrapment. Besides, the chemical modification provides an effective method for protein binding [2,3]. Recently, much attention has been focused on plasma treatment [4–9]. Protein adsorption, based on the principle of immobilized metal affinity chromatography (IMAC), is an alternative approach. The technique of IMAC, originally used for the purification of histidine containing proteins or peptides, is based on the specific coordinate covalent bond of amino acids, particularly histidine, to transitional metals. Thus, most protein microarrays based on IMAC were fabricated using a chelator or compound of mono-metallic ions, such as Ni^{+2}, to capture the histidine-tagged protein [10–15].

The microarray must have a high binding affinity to prevent the proteins spotted on the surface from being rinsed away during the washing steps of the immunoassay and eventually to provide a precise experimental outcome. In our previous work, a protein chip with a Ni–Co layer fabricated by electrodeposition was developed [16]. It is an alloy type of protein chip. Since both nickel and cobalt are transitional metals, adding cobalt to form a codeposition of Ni and Co enhances the specific binding capability. Based on the principle of IMAC, the Ni–Co coating was found to have specific binding capability to immobilize functional proteins with a His-tag attached. Compared with the Ni-coated chip, this Ni–Co alloy chip is

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more sensitive because of stronger fluorescence intensity detected at the same concentration.

Nickel and cobalt can be easily codeposited on the surface of a substrate; however, different electroplating conditions result in distinct alloy composition. The binding affinity is then affected [17]. Therefore, a series of experiments were conducted in this study to systematically investigate the influence of the crystallographic structure of Ni–Co coating upon the affinity adsorption of His-tagged protein. The crystallographic structures and surface properties of Ni–Co coatings with or without an annealing process were characterized by using a scanning electron microscope (SEM), water contact angle measurement and atomic force microscopy (AFM). Subsequently, the protein adsorption behavior of Ni–Co coating was evaluated by bio-experiments. Electroplating is a well-developed technique. Acquiring in-depth knowledge of Ni–Co affinity binding to His-tagged protein allows us to fabricate an appropriate microarray surface with better immobilization ability by the electroplating process.

2. Materials and methods

2.1. Substrate and electroplating apparatus

Fluorine-doped tin oxide (FTO) glass (resistance: 7 Ω) was trimmed into small 2 cm × 2 cm pieces for use as the electrodeposition substrates. After a thorough cleaning, the FTOs were treated with oxygen plasma for surface modification. The process was conducted at a pressure of 10^{-2} torr for 15 min. This improved the hydrophile of the FTO surface, allowing the electroplated film to have better adhesion to the substrate. Then, Ni–Co electrodeposition was performed in a W18 cm × L25 cm × H18 cm electroplating tank. A 100 V/5 W pump equipped at the bottom of the tank provided a flow upward to the metal substrate in order to enhance the turbulence for promoting uniformity of the plating bath. To eliminate impurities and crystalline solids, a filter with 1 μm pores was installed above the pump. Furthermore, a digital power supply controlled by a PID controller supplied a 100 V/100 W output to a quartz heater for temperature control of the plating bath at 45 °C.

The necessary chemicals for electrodeposition included $Ni(NH_2SO_3)_2{\cdot}4H_2O$, $Co(NH_2SO_3)_2{\cdot}4H_2O$, $NiCl_2{\cdot}6H_2O$, H_3BO_3, $NiCo_3$ and HSO_3HN_2. The ratio of ion concentration Ni^{2+}/Co^{2+} in the electrolyte was 9:1. Among these chemicals, $NiCo_3$ (powder with grain size of 20 μm, purity: 100%) was used to control the pH of the plating bath to 5.4; while HSO_3HN_2 was used for pH adjustment to 3.1. Additionally, a low-stress additive (sodium saccharin, $C_6H_4SO_2NNaCO{\cdot}2H_2O$, 10 mL) was added to the electroplating bath (8 L). It is a surfactant to assist the progress of electroplating. The thickness of the coated Ni–Co film was 2 μm. After the electroplating process, the Ni–Co coated chips were preserved in a vacuum container for 24 h before usage.

2.2. Bio-experiments

In our previous work, the affinity between transitional metal and His-tagged protein has been demonstrated. The adsorption is through the histidine, based on the technique of IMAC and irrelevant to the species of proteins. Since the binding between biotin and streptavidin is the strongest non-covalent biological interaction known [18,19], we utilized this mechanism to investigate the influence of the crystallographic structure of Ni–Co coating on the adsorption of histidine-tagged protein. The Penta-His biotin conjugate was from Qiagen (Germany) and streptavidin-Cy5 was obtained from Life Technologies (NY, USA). All reagents were of analytical grade.

The 5× His-tagged biotin was diluted to a concentration of 2.5 μg/mL by 0.18 M of phosphate buffer saline (PBS, pH 7.0). On a Ni–Co coated chip, ten drops of His-tagged biotin were arrayed. The volume of each drop was 1 μL. In addition, a drop of streptavidin-Cy5 was added beside for comparison. Two-hour incubation for biotin immobilization in a 37 °C hot convection oven was carried out, followed by a wash in a washing buffer (PBS with 0.05% Tween-20, pH 7.4) for 2 min per wash in order to remove the redundant His-tagged biotin. Then, the chip was immersed in deionized (DI) water to rinse out the washing buffer. Next, Cy5 fluorescently labeled streptavidin was prepared with a dilute buffer (PBS) to the designed concentration and then, added onto the top of the biotin layer. The reaction of biological binding was again incubated in a 37 °C hot convection oven for 1 h, followed by the same washing procedure.

After the immunoassay, fluorescent detection was performed using a Gene TAC LS IV scanner manufactured by Genomic Solutions Inc. The excitation light wavelength was 635 nm. Once the fluorescence was detected, it revealed that the His-tagged biotin was bonded to the chip surface, and the assay was completed. That was due to the strong interaction between the biotin and streptavidin. The fluorescence intensities represent their respective binding capability between His-tagged biotin and Ni–Co film. The fluorescence intensity was analyzed by Gene Pix 4.1 software.

3. Results

To investigate and acquire more knowledge of the factors influencing the immobilization of histidine-tagged protein on Ni–Co coated surfaces, different electroplating conditions were designed. The current density increased from 0.5 A/dm^2 to 1 A/dm^2 while the pH value varied from 3.1 to 5.4. A total of 9 different deposition conditions were conducted. Moreover, the influence of the annealing with temperatures changing from 250 °C to 500 °C was studied. The annealing chamber was filled with nitrogen (N_2) gas with flow rate of 0.2 m^3/s to keep the chamber pressure at 80 psi. The N_2 gas was continuously introduced into the chamber until the annealing process was completed and the chamber was cooled down to the room temperature.

For each electroplating condition, ten Ni–Co chips were fabricated for repetitious bio-experiments and statistical analyses.

3.1. Ni–Co electrodeposition

3.1.1. Crystallographic structure

Fig. 1 shows the compositions of the codeposition. The cobalt content in the deposit was 66.76 wt% at an applied current density of 1 A/dm^2 with a bath of pH 3.1; while the content was 71.80 wt% at a current density of 0.75 A/dm^2 with bath of pH 3.1. Their SEM micrographs displayed a majority of hexagonal close packed (hcp) lattices, as shown in Fig. 1(b). This is in accordance with the results reported in [20], which presented that the crystal structure of the Ni–Co alloy forms an hcp phase if the Co content is greater than 60 wt%. For higher Co content, a stronger hcp texture can be observed [21]. Furthermore, the cobalt content decreases with increasing current density [22] and increases with the increase of the pH value [23]. That is, the electroplating condition with a lower current density and higher pH value will result in a stronger hcp texture. According to these conclusions and the measurement of the compositions, shown in Fig. 1, we can infer that the crystal structures of the 9 different deposition conditions designed in this study are all hcp lattice. As shown in Fig. 2, the alloys have a rather regularly branched structure, and all the surface morphologies of the Ni–Co coatings exhibit a majority of hcp lattice.