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Characterization of Novel Self Assembled Materials (SAM) for Surface Modification of Sensor Chip

Hyung Min Cho[1], Dae Ho Jang[1], Seung Kwon Lee[1], Su Jin Ku[1], Hyun Chul Kim[2], Kyu Sang Yu[1] & Joong Hwan Lee[1]

[1]Research Institute, Korea Materials & Analysis Corp. (K-MAC), Daejeon, Korea
[2]BioConvergence Technology Research Team, Daegu Gyeongbuk Institute of Science & Technology (DGIST), Daegu, Korea
Correspondence and requests for materials should be addressed to K.S. Yu (ksyu@kmac.to)

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Abstract

Recently, researchers have been frequently using a biosensor-based gold chip couple detection method. The gold surface is modified for various materials including self assembly monolayer (SAM), biotin, streptavidin, dextran, Prolinker, protein G, lipid bi-layer, etc. for use as a biosensor, diagnosis system. The modified gold surface can enhance the immobilization amounts, orientation, activity, and stability of the receptor, and decrease the non-specific binding. Surface plasmon resonance (SPR) is made of optical biosensors that are widely gaining recognition as a valuable tool to investigate biological interactions. The key features of SPR biosensors make this technology suitable for a wide range of applications. We have SPR system of fluidic type. Our SPR system can detect biomolecules-biomolecules or biomolecules-sensor surface interaction. A biosensor for the detection of small molecules or diagnostic markers requires high sensitivity. One of the methods for enhancing sensitivity is a surface modification to provide a bio-adaptable space. In this study, we modified the surface of a bare gold chip by using our NHS-SAM for enhancing of the detection sensitivity, and we compared its results with a commercial SAM. We also observed the antigen-antibody interaction and compared its results using an SPR analyzer.

Keywords: SPR, Biosensor, Biochip, SAM, Surface Modification

Introduction

The sensor chip is one of the main research fields in bio applications. Surface modification is an essential step for a sensor chip experiment due to such merits as oriented immobilization, activity enhancement, and prevention of non-specific adsorption. Researchers can modify the surface of a sensor chip using biotin, streptavidin, protein G, or an organic compound called SAM[1]. SAM is composed of a thiol group and functional group at both sides. The thiol group can bind to a gold surface, so SAM can assemble a monolayer there. Therefore, a sensor chip surface is modified to a specific functional group. Researchers can modify the surface with a specific purpose using SAM.

In this research, we synthesized a novel SAM. This material is composed of ethylene glycol as a spacer. This structure causes a non-specific adsorption of protein by hydrophilic increment. This SAM also caused an activity enhancement of immobilized biomolecules by flexibility increment[2].

The receptor binding site of SAM is composed of an NHS group. NHS groups are stable at pH 7 and easily bind to amine groups. Thus, this SAM shows stable binding and 1-step immobilization without buffer change, activation, etc[3]. We conducted an SPR (Surface Plasmon Resonance) experiment using the SAM. Many researchers use an SPR for sensor chip experiments. An SPR can analyze a bio interaction through the reflectance change of a sensor chip surface[4-6]. We modified the surface of an SPR sensor chip to NHS using our SAM. We analyzed an immunoassay using anti-mouse IgG and IgG on the surface-modified chip and compared our novel SAM with a commercial SAM to prove the commercialization possibility of our novel version. Also, the surface-modified chip was analyzed using a CRP (C-reactive protein) immunoassay. The CRP can diagnose infectious diseases and inflammation. We proved the development possibility of a diagnostic sensor chip using our surface-modified chip through CRP experiment.

Results and Discussion

Confirmation of NHS-SAM Synthesis and Surface Modification

A functional group of synthesized materials was confirmed by spectrum change using an FT-IR after

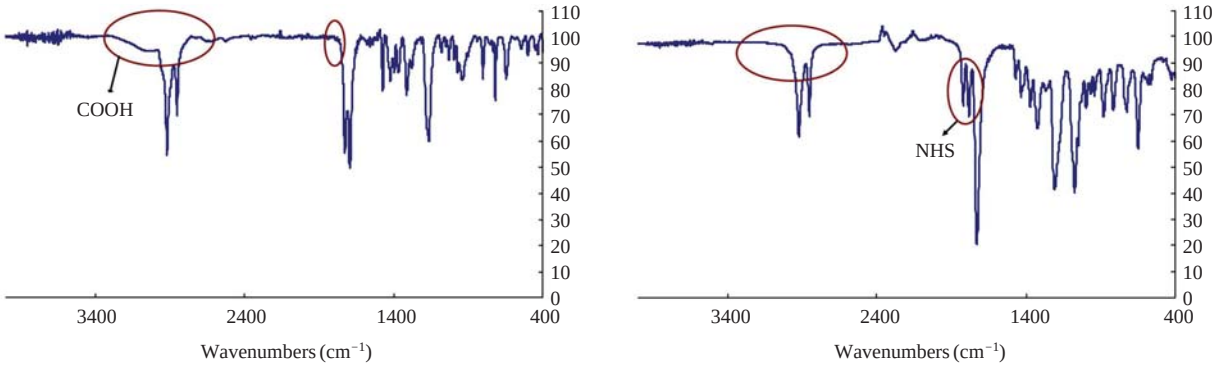

Figure 1. FT-IR spectrum change, (a) after synthesized carboxyl group, (b) after synthesized NHS group.

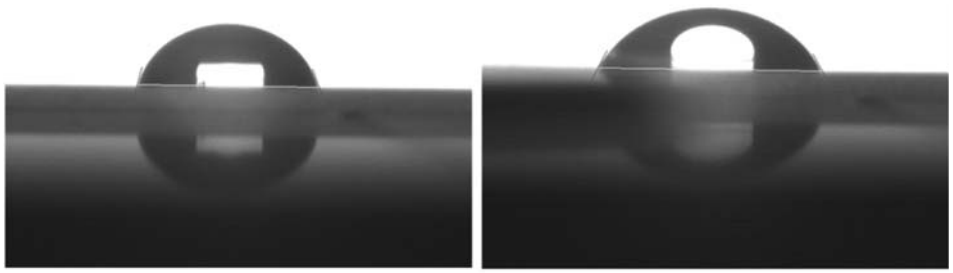

Figure 2. Comparison of the contact angles of a bare gold chip with a surface-modified chip.

being inducted with carboxyl and an NHS functional group. The results of the experiment showed a spectrum change near 1,800, 3,000 cm^{-1}. The X-axis of the spectrum graph is a wave number that indicates the existence of a specific functional group. 1,800 cm^{-1} indicates the existence of an N group in a synthesized SAM, and 3,000 cm^{-1} indicates the existence of a COO group in a synthesized SAM. A COO group was in existence after synthesized carboxyl, but it changed to an NHS group by chemical reaction with the NHS.

If the surface of a bare gold chip reacts with SAM, the surface will modify to a specific functional group. A gold surface has a comparatively hydrophobic character. But, the surface character will change to a hydrophilic state more than a bare gold surface after surface modification. We measured the contact angle before and after surface modification using our SAM. The bare gold chip was modified to an NHS surface at optimal conditions. Distilled water was dropped onto the bare gold chip and surface-modified chip. The contact angles of the bare gold chip and surface-modified chip are 79.9° and 64.1°. We confirmed that the surface character of the bare gold chip changed to a hydrophilic state by surface modification using our SAM. In this experiment, we confirmed the character change of a bare gold surface and the standard angle of a surface-modified chip using our SAM.

Optimization of K-MAC SAM Reactive Condition for Surface Modification

We optimized the reactive condition of our SAM for surface modification. Solvent and reactive times are set up factors in this experiment. SAM is an organic compound and in a solid phase, so it needs an organic solvent to dissolve. Chloroform and ethanol are mainly used for dissolving SAM. Our SAM was dissolved in chloroform and ethanol at the same conditions. The bare gold chip was taken in the SPR system, and two SAM solutions were then flowed into the SPR system. In the results of the experiment, the SAM solution in chloroform changed 0.5 R (%) more than the SAM in ethanol. The SAM solution in chloroform is suitable for surface modification due to the fact that the solubility of chloroform is higher than ethanol. Thus, we used chloroform as a solvent of our SAM[7].

Many researchers modified the sensor chip surface using a dipping method. Organic compounds need an exact time to stably bind with the sensor chip surface.

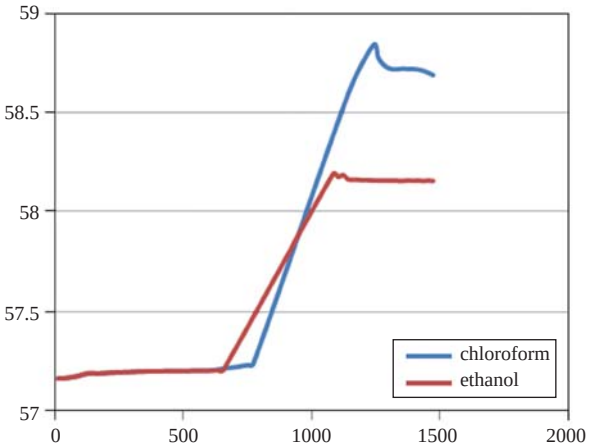

Figure 3. Effect on SAM reactivty of solvents (X axis: time (min), Y axis: Reflectance (%)).

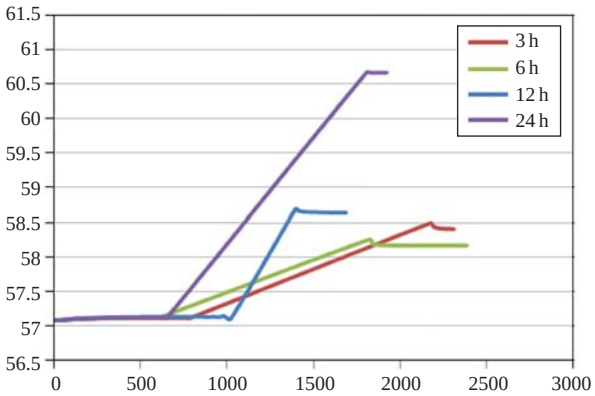

Figure 4. Effect on SAM reactivity of dipping time (X axis: time (min), Y axis: Reflectance (%)).

The bare gold chip was modified using our SAM at the same conditions, but the reactive time was a set-up factor in this experiment: 3, 6, 12, 24 hr. The surface-modified chip was taken in the SPR system, and 10 μg/mL anti-mouse IgG in 0.01 M PBS pH 7.4 was then injected into the SPR system. We measured the amount of immobilization in all cases. The results of the experiment show that the reflectance change of the surface-modified chip for 24 hr is 3.5%. It has a greater amount of immobilization than other cases. We confirmed that surface modification has an effect on the reactive time of SAM. When we modified the sensor chip surface for 24 hr or more of reactive time, we received a greater amount of immobilization. But, a greater reactive time is a non-efficient step for commercialization. Thus, we concluded that a 24 hr reactive time is suitable for optimal modification.

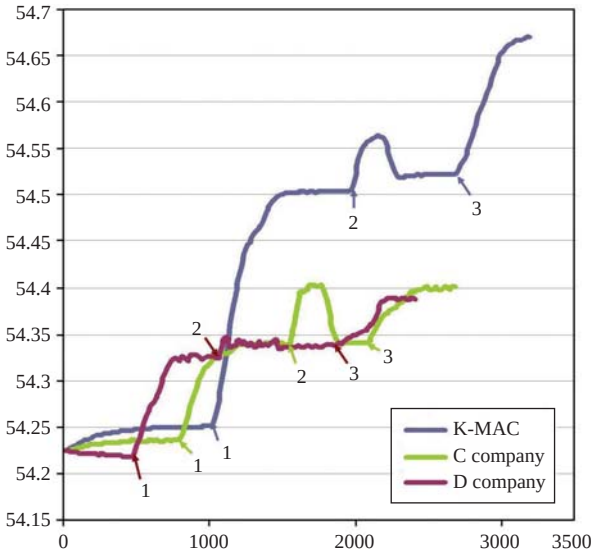

Figure 5. Comparison immunoassay of K-MAC SAM with commercial SAMs: 1-Anti-IgG, 2-BSA, 3-IgG injection (X axis: time (min), Y axis: Reflectance (%)).

Compare K-MAC SAM with Commercial SAM and Possibility of Detection as a Diagnostic Sensor Chip

We conducted a comparison experiment at same conditions using our SAM and a C. D. Corp. SAM. The SAMs were dissolved in chloroform, and all solution moles were 3 mM. The bare gold chip was modified in SAM solutions for 24 hr. And 10 μg/mL anti mouse IgG, IgG were immobilized on the surface-modified chips. The amounts of anti mouse IgG immobilization are 0.1 R (%) on the surface-modified chip using the C. D. Corp. SAM, but the amounts of anti mouse IgG immobilization are 0.25 R (%) on the surface-modified chip using our SAM. The surface-modified chip using our SAM has a greater amount of immobilization than the other commercial SAM. The amounts of mouse IgG binding are 0.06, 0.05, and 0.15 R (%) on the surface-modified chip using C. D. Corp. SAM and our SAM. The amounts of mouse IgG binding on the surface-modified chip using our SAM are also more than those using a commercial SAM due to the fact that the amounts of anti-mouse IgG on the surface-modified chip using our SAM is more than for other surfaces. In this result, our SAM has greater amounts of protein immobilization and higher protein capture capacity than the other commercial SAMs. We proved the commercial possibility and capacity of our SAM through this experiment.

The development of a diagnostic sensor chip is in the limelight of sensor chip research. We did an immunoassay experiment using CRP. CRP can diagnose

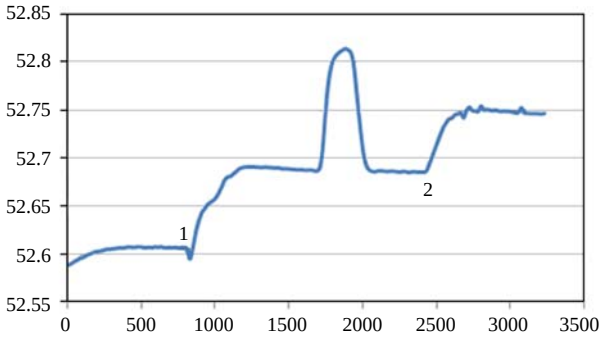

Figure 6. Possibility confirmation for the development of a diagnostic sensor chip using CRP: 1-Anti-CRP, 2-CRP injection (X axis: time (min), Y axis: Reflectance (%)).

inflammation and infectious diseases. Anti CRP and CRP were reacted on the surface-modified chip after the surface of a bare gold chip was modified using our SAM at the same condition as before. The amounts of immobilized anti-CRP and CRP are 0.095 and 0.055 R (%). The reactivity of anti-CRP on the sensor chip is lower than other proteins at real time, so 0.095 R (%) is a suitable change to the analysis of CRP interaction. The immunoassay ratio (CRP/anti-CRP) is 0.58; therefore, we confirmed the possibility of an immunoassay analysis such as in IgG using CRP. We proved the development possibility of a diagnostic sensor chip using our SAM through this result[8].

Conclusion

In this research, we synthesized a novel composition SAM transferred from DGIST. This was composed of an NHS functional group, 11 carbon chains, and ethylene glycol, so this SAM can proffer oriented immobilization, hydrophilicity, flexibility, and activity enhancement[9,10]. We modified the surface of an SPR sensor chip and then confirmed the surface modification using a contact angle analyzer. We also modified a bare gold chip using our SAM and commercial SAMs at the same condition, and an IgG immunoassay analyzed on the surface-modified chips. In the experimental results, the surface-modified chip using our SAM showed a greater amount of antibody immobilization and higher immunoassay ratio than surface-modified chips using commercial SAMs. CRP was reacted on a surface-modified chip using our SAM. In the results of this experiment, anti-CRP and CRP were suitably immobilized for analysis on a surface-modified chip using our SAM. We proved the commercialization possibility for the development of a diagnostic sensor chip using our SAM.

Materials and Methods

Synthesis and Confirmation of K-MAC SAM

We transferred the synthesis method of a novel SAM from DGIST. The synthesis process is composed of 3 steps: induce hydroxyl, carboxyl, and NHS functional group. 11-mercapto-1-undecanol (Sigma-Aldrich, USA) and sodium perborate monohydrate (Sigma-Aldrich, USA) were reacted in methanol and a distilled water mixed solution to induce the hydroxyl group. This solution was twice diluted by mixed chloroform after inducing the hydroxyl group. The solution separated two phases and we then accepted the lower phase. The solution was filtered and evaporated to remove the remaining chloroform and impurities. Succinic anhydride (Sigma-Aldrich, USA) and triethylamine (Sigma-Aldrich, USA) were a half weight added to the hydroxyl induced materials after the measured weight of synthesized materials. The synthesized materials are a dimer composition, so succinic anhydride and triethylamine were half weight added. 30 mL chloroform was added to the materials, and then reacted to induce the carboxyl group for 12 hr. The solution separated two phases, and we then accepted the lower phase. The solution was filtered and evaporated to remove remaining chloroform and impurities. N-(3-dimethylamino propyl)-N′-ethylcarbodiimide hydrochloride (EDC, Fluka, USA), N-hydroxysuccinimide (NHS, Sigma-Aldrich, USA) and 30 mL dichloromethane were added to carboxyl induced materials and then reacted to induce the NHS group for 12 hr. The solution separated two phases, and we then accepted the lower phase. The solution was filtered and evaporated to remove remaining solvents and impurities. The final product was measured to confirm the induced functional groups by using FT-IR[11].

Confirmation of Surface Modification using K-MAC SAM

Many researchers have experimented on the surface modification of sensor chips, so many confirmed methods also exist in sensor chip research; fluorescence detection, AFM (Atomic Force Microscopy), image ellipsometry, and contact angle analyzer[12]. In this research, we confirmed the surface modification through comparing a modified chip with an unmodified chip by using a contact angle analyzer. The contact angle analyzer can analyze the contact angle on a sensor chip through a formed image by dropping the liquid. The kind of surface, liquid type and mor-

phology of the surface have an effect on the contact angle. The contact angle also changes due to the kind of surface change according to the functional group after the surface of a sensor chip is modified using SAM.

Our SAM was dissolved in chloroform. The sensor chip was reacted in SAM solutions for 24 hr. The surface-modified chip was washed by ethanol and the remaining solution removed by nitrogen gas. The contact angle of a bare gold chip was also measured to compare the contact angle of the surface-modified chip. Distilled water was filled into a 1 mL syringe and the syringe connected to the contact angle analyzer (S.E.O., England). Distilled water of the same volume was dropped onto the surface of the sensor chips. The contact angle of each surface was measured by image analysis.

Optimization for Surface Modification of SPR Sensor Chip using K-MAC SAM

Reactive time, moles of SAM, temperature, and solvent have an effect on surface modification of sensor chips using SAM. In this research, the temperature and moles are fixed at room temperature and 3 mM because these are the general conditions at surface modification. So, time and solvent are the only freely set-up factors.

Ethanol and chloroform were used as a solvent for SAM. Our SAM was dissolved in ethanol and chloroform. A bare gold chip was taken in the SPR system (K-MAC, Korea), and the SAMs were then injected to analyze the adsorption reaction into the SPR system.

Our SAM was dissolved in chloroform to analyze the effect of reactive time. Four sensor chips were reacted in the same SAM solutions for 3, 6, 12, 24 hr. The surface-modified chip was washed by ethanol and the remaining solution removed by nitrogen gas. Surface-modified chips were taken into the SPR system, and 10 μg/mL of anti-mouse IgG, BSA, IgG (Sigma-Aldrich, USA) in 0.01 M PBS (Phosphate Buffered Saline) pH 7.4 was then injected into the SPR system.

Comparison of K-MAC SAM with Commercial SAM and Possibility Detection as Diagnostic Sensor Chip

Surface modification is an essential step for sensor chip research. Commercial SAMs exist in the sensor chip research field. We compared our SAM with commercial SAMs to appraise the commercial possibility of our SAM. We purchased commercial SAMs of C., D. Corporation. These SAMs are composed of 11 carbon chains and an NHS functional group as is our SAM.

The K-MAC and C., D. Corp. SAMs were dissolved in chloroform at the same condition. 3 bare gold chips were reacted in each SAM solution for 24 hr. The surface-modified chip was washed by ethanol and the remaining solution removed by nitrogen gas. The surface-modified chips were taken into the SPR system, and 10 μg/mL anti-mouse IgG, BSA, IgG in 0.01 M PBS pH 7.4 was then injected into the SPR system to analyze the amount of antibody immobilization and immunoassay.

The purpose of sensor chip research is estimate the basic character of a bio-molecule as an activity measurement using immobilization technology, and the development of diagnostic technology using an immunoassay. In this research, the development possibility of a diagnostic sensor chip was appraised using the SAM system.

CRP (C-Reactive Protein) is a target protein. CRP is composed of 260 amino acids, and its molecular weight is 115,000 Da. CRP is produced in the liver and exists in the human body. If we are infected with an infectious inflammation disease, the numerical value of CRP in our body will rise. Thus, many researchers know that CRP is a factor of inflammation and infectious disease[13,14].

Our SAMs were dissolved in chloroform at the same condition. A bare gold chip was reacted in SAM solutions for 24 hr. The surface-modified chip was washed by ethanol and the remaining solution removed by nitrogen gas. The surface-modified chips were taken into the SPR system, and 10 μg/mL anti-CRP, BSA, CRP (Sigma-Aldrich, USA) in 0.01 M PBS pH 7.4 was then injected into the SPR system to analysis the amount of antibody immobilization and immunoassay.

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