Additional Alkylsilanization of Aminosilane-modified Glass Slide: Effect of Alkylsilane Structure for Enhancing Surface Amine Functionality

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Efficient amine-functionalized glass slides were developed by capping with alkylsilanes, which might reduce nonspecific interactions of amino groups with the surface silanol. We explored the types of alkylsilanes (used as silanol capping reagents) for designing high amine functionality on the glass slide.

Chemical modification of silica surfaces accomplished by reaction with aminosilanes has very important applications in different industrial fields such as chromatographic stationary phase,[1,2] advanced composite materials,[3,4] supports for catalysis,[5] and for covalent binding of biological molecules.[6–8] In general, amine-functionalized silica substrates are obtained through self-assembly of aminoalkoxysilanes in the solid/liquid interface. The alkoxy groups of silanes react with silanol groups on the silica matrix to form –Si–O–Si–R–NH_2 structure, providing amine functionality on the surface. However, the presence of surface silanol groups[9] on silica surface after postsilanization makes the amino groups not to be in free form such as R–NH_2 or R–NH_3^+ (in the equilibrium), but rather remain in the form of hydrogen bonding with remaining unreacted silanol groups, –Si–O–H···NH_2–R–, or in ion pairing, –Si–O^-···NH_3^+–R as shown in Figure 1.[10] In addition, protons from surface silanols are transferred to amino groups to form NH_3^+ forms which are not reactive.[11–13]

These nonspecific interactions between amino groups and surface silanols might reduce the chemical immobilization efficiency of biomolecules for biosensor preparation. We recently developed highly efficient amine-functionalized glass slides by capping with alkylsilanes, which might reduce nonspecific interactions of amino groups with the surface silanol.[14] In this study, we explored the silanol capping effect regarding the alkylsilane types to obtain enhanced amine functionality on the surface.

Amine functionalization of the glass slides (25.4 × 76.2 cm (±0.05 cm)) were performed by chemical treatment with 3-aminopropyltriethoxysilane (APS). First, glass slides were cleaned with acetone and freshly prepared Piranha solution (70% H_2SO_4, 30% H_2O_2). The resulting glass slides were treated with 2% APS in toluene at room temperature for 3 h, washed with toluene and baked at 110 °C for 1 h to generate amine-functionalized surface (APS glass slides). Additionally, the capping process was performed by following the same procedure as mentioned in aminosilanization procedure except for using alkylsilanes instead of APS. Several types of alkylsilanes having different numbers of alkoxy groups and different types of non-alkoxy alkylchains were used to cap surface silanols on APS glass slides, as illustrated in Figure 2 (butyltrimethoxysilane (BMS), trimethoxymethylsilane (TMS), methoxytrimethylsilane (MTMS), octadecyltriethoxysilane (ODTES), and so on).

To estimate the silanol-capping performance with alkylsilane, the contact angles of sessile drops (1 μL, distilled water) were measured with a goniometer (Kyowa Interface Science).[15] As shown in Figure 3, the capped amine-functionalized glass slides possess the increased contact angles compared with control APS surface (without capping) as well as plain and etched glass slides. Since the conversion of the surface silanol to Si–O–Si–R linkage increases the hydrophobic property on the glass slide, the contact angles might be used as an index for estimating capping efficiency. BMS treated APS glass slides (APS + BMS glass slides) presenting the butyl aliphatic chain showed higher contact angles (103.4 deg) compared to the capped amine-slides with TMS (70.7 deg) and MTMS (63.7 deg) with short alkylchains.

To evaluate amine functionality regarding alkylsilane types capped on amine-functionalized surface, the efficiency of chemical immobilization of a fluorescent label, Cy3-NHS (50 pmol/μL) was evaluated by spotting on each glass slide with an ink-jet spotter, washing and analyzing Cy3 intensity on the slide.[16]

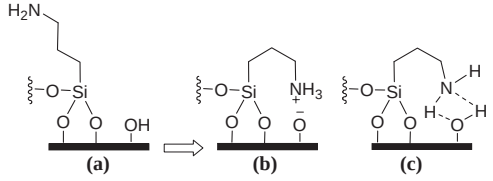

Figure 1. Schematic illustration of APS glass slide (a) and nonspecific interactions of amino groups with surface silanol groups on the modified surface (b), (c).

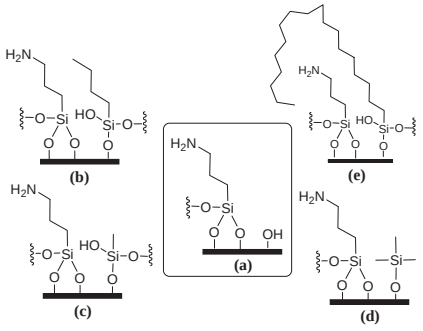

Figure 2. Schematic diagram of APS glass slide (a) and its alkylsilane-capped APS glass slides with BMS (b), TMS (c), MTMS (d), and ODTES (e).

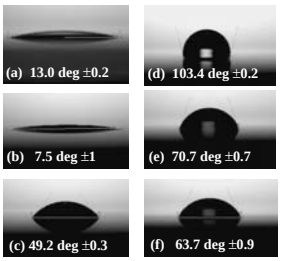

Figure 3. Contact angles of sessile droplets on plain (a), etched (b), APS (c), APS + BMS (d), APS + TMS (e), and APS + MTMS (f) slides.

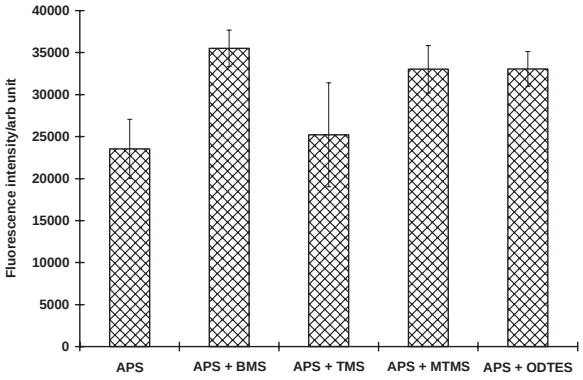

Figure 4. Comparison of Cy3 fluorescence intensity on APS (Control), APS + BMS, APS + TMS, APS + MTMS, and APS + ODTES glass slides.

All capped APS glass slides showed enhanced performance of Cy3-NHS immobilization than control APS glass slides (without capping). It was supposed that the effect of alkylsilanization on amine functionality enhancement might be related to the silanol-capping efficiency. Trialkoxy-type alkylsilanes with high silanization chance were found to be more effective capping reagents for obtaining high efficiency of Cy3-NHS immobilization compared with monoalkoxy-type alkylsilanes.

On the other hand, an exceptional tendency was also observed; that is, APS + MTMS glass slide (monoalkoxy type) was found to show higher efficiency than APS + TMS glass slide (trialkoxy types), as shown in Figure 4. Considering that less than two equivalents of alkoxy groups per one silane molecule react with the silanols to form Si–O–Si–R linkage, there is a large chance for one silanol to be left in the molecular layers.[17] Therefore, in the case of APS + TMS glass slide, some of liberated amines on the surface have chance to interact again with the silanol groups lowering the amine functionality on the surface. In contrast, unlike TMS, MTMS has a very low chance to leave one silanol group in the molecular layers, which could enhance the chemical immobilization.

Although similar low-immobilization efficiency could be expected in the case of APS + BMS or APS + ODTES glass slides, the presence of long alkylchains may provide a kind of steric hindrance to keep amino group away from interacting with the silanol groups. APS + BMS glass slides showed better amine functionality than APS + ODTES ones. The longer alkyl chain of ODTES than aminoalkyl chain of APS might reduce the accessibility of Cy3-NHS dye to the surface amino group thereby reducing the immobilization efficiency. At present, APS + BMS glass slide was found to be the best platform possessing high amount of amine functionality on the surface.

In present study, we investigated the types of alkylsilanes in terms of silanol-capping reagent for developing a highly efficient amine-functionalized surface. We found that, as well as capping performance of remaining silanol groups, the structure of alkylsilane is also an important factor influencing the enhancement of surface amine functionality. Therefore, we should consider alkylsilane type when the capping strategy is employed for obtaining high surface amine functionality. These results will be usefully employed for preparation of efficient amine-functionalized surface, which is essential platform for biomolecule-array or biosensor development.

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References and Notes

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15 Contact angle values obtained are the mean of tangents taken at both sides of each droplet at five different places on each glass slide (room temperature, 50% humidity).
16 A five by six spot format was used to spot fluorescent labels on the glass slides. Spotted glass slides were scanned with a DNA microarray scanner (Agilent Technologies), and fluorescent image intensity was analyzed by using mapping software (GenePixPro Ver 5.0 or 5.2, Molecular Devices). Fluorescence data form over 90 spots (three glass slides) were measured, and the statistical data obtained were used to evaluate the efficiency of chemical immobilization on the modified glass.
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