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# Tracking of Cell Adhesion Process to Self-Assembled Monolayer of Alkanethiols with Various Functional Groups

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## 1. Introduction

Cell adhesion to synthetic materials is critically important in the various aspects of the development of biomedical devices, artificial organs, and biosensors. Cell adhesion behaviors onto surfaces have been determined for various surfaces with different properties including wettability, roughness, surface charge, and chemical functionality. When the substrate is exposed to a suspension of cells in a culture medium containing serum, serum proteins rapidly adsorb on the surface and then cells subsequently approach and adhere to the surface. Although it has been accepted that surface properties of materials affect cellular behavior through the adsorbed protein layer, there are yet many uncertain aspects to be resolved for detailed processes of cell adhesion to material surfaces and its correlation to surface properties.

Most of the synthetic materials that have been examined suffer either from a lack of surface homogeneity or instability. Model surfaces with well-controlled properties are required to study interactions between cells and synthetic materials. Alkanethiols, denoted as $HS(CH_2)_nX$, chemisorb on surfaces of gold and silver, and spontaneously form stable and well-defined self-assembled monolayers (SAMs). Different functional groups, X, at the other terminal position of the alkanethiol make it possible to control the surface structure of different properties at the molecular scale.[1] SAMs of alkanethiols on gold, therefore, afford useful model surfaces to study the effect of surface chemistry on protein adsorption and cell adhesion.

Several studies using alkanethiol SAMs have been performed to determine the effects of surface properties on cell attachment, spreading and proliferation. Most of these studies examine cell adhesion by the number of adherent cells, morphology, and immunofluorescent staining after several hours of incubation. Further understanding of the biophysical mechanism of cell adhesion requires real-time tracking of protein adsorption and cell adhesion since composition of the adsorbed protein layer and cell morphology change with incubation time.

The objective of this study was to track real-time events including protein adsorption and cell adhesion behavior, and to elucidate determinants of cellular response on artificial substrates with a wide variety of surface properties. We employed a SAM of alkanethiols as a model surface, surface plasmon resonance (SPR) to examine protein adsorption behavior, and total internal reflection fluorescence microscope (TIRFM) to observe initial cell adhesion behavior.

## 2. Materials and method

*Surface preparation*

The S-LAL10 glass plates ($n$ = 1.72) were coated with a chromium underlayer of 1 nm and then a gold layer of 10 nm in thickness. The gold-coated glass plate was then immersed in a 1 mM ethanol solution of alkanethiols terminated with methyl ($CH_3$; 1-dodecanethiol), hydroxyl (OH; 11-mercapto-1-undecanol), carboxylic acid (COOH; 11-mercaptoundecanoic acid), and amino groups ($NH_2$; 11-amino-1-undecanethiol hydrochloride) for 24 h to form the SAM.

*Observation of cell adhesion using TIRFM*

We used a homemade, prism-type TIRFM to observe cells adhering to the SAM surface (Fig. 1).[2] The glass plate modified with SAM was optically coupled to a triangular prism using a refractive index matching fluid. The laser beam ($\lambda$ = 532 nm) was directed into the prism at an incident angle of 54.2˚. A cell culture medium supplemented with 2% fetal bovine serum (FBS) was infused into a chamber built on the SAM surface, and then suspensions of human umbilical vein endothelial cells (HUVECs), whose membrane was labelled with a fluorescent dye PKH26 (Sigma), were injected into the chamber. Fluorescent images were acquired with a CCD camera at an interval of 5-60 min. The fraction of adherent cells and cell adhesion area were calculated from TIRFM images using Scion Image 4.02 (Scion Corp.).

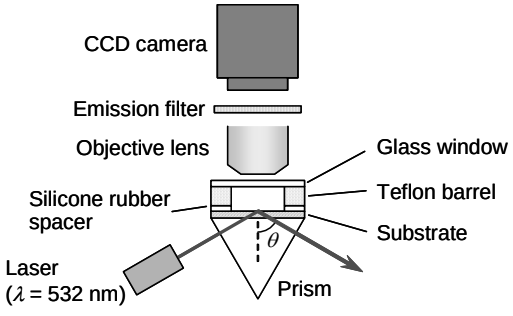


Fig. 1 Setup of a total internal reflection fluorescent microscope

*Protein adsorption measurement using SPR*

Protein adsorption onto the SAMs was examined by surface plasmon resonance (SPR) apparatus.[3] A cell culture medium containing 2% FBS was exposed to the SAMs for 1 h and phosphate buffered saline (PBS) was subsequently flowed for 15 min to wash out the medium, including weakly adsorbed serum proteins. Change in reflectance during exposure to the medium was monitored.

## 3. Result and discussion

We employed TIRFM to observe adhesion behavior of HUVECs, whose membranes were labeled with a fluorophore, PKH26, on the SAMs presenting different functional groups. TIRFM utilizes an evanescent field, which is generated at a water/glass interface due to the total internal reflection at the interface. The distance in which fluorophores are irradiated by the evanescent field in our TIRFM system is estimated to be about 100 nm. Figure 2 shows representative TIRFM images of HUVECs plated onto COOH-SAM. Upon plating of cells (Fig. 2a), fluorescence was hardly observed in a TIRFM image because almost all of the cells are still suspended in the medium and the fluorophores in the cell membrane are out of the evanescent field. The cells then start to adhere and spread on the SAM surface, and bright spots can be observed by TIRFM (Fig. 2b). When cells attach and spread onto the surface (Fig. 2c, d), the area of the bright spots observed by TIRFM becomes larger with time. Thus, TIRFM is a useful tool to obtain information about cell adhesion behaviors at solid/liquid interface from changes in images of fluorescently labeled cell membranes.

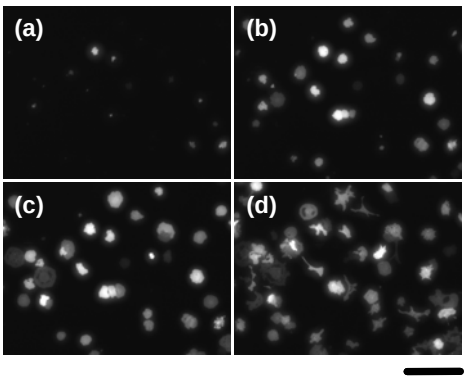


Fig. 2 Adhesion behavior of HUVECs on COOH-SAM observed by TIRFM at (a) 10 min, (b) 30 min, (c) 60 min, and (d) 180 min of incubation. Scale bar: 200 μm.

Figure 3 summarizes the time-course of fraction of adherent cell and adhesion area per cell for the SAMs presenting different functional groups. On COOH- and $NH_2$-SAMs, the fraction of adherent cells increased with incubation time from 5 min after plating cells and leveled off at 60 min, while adhesion area increased up to 180 min. On the OH-SAM, the fraction of adherent cells and adhesion area was smaller than those on COOH- and $NH_2$-SAMs. Although the fraction of adherent cells on $CH_3$-SAMs reached that of OH-SAMs after long-term incubation, its initial increase was slower in comparison. The adhesion area on the $CH_3$-SAM was much smaller than compared to the other three SAMs. These results clearly demonstrate that surface functional groups affect cell adhesion behaviors on SAMs.

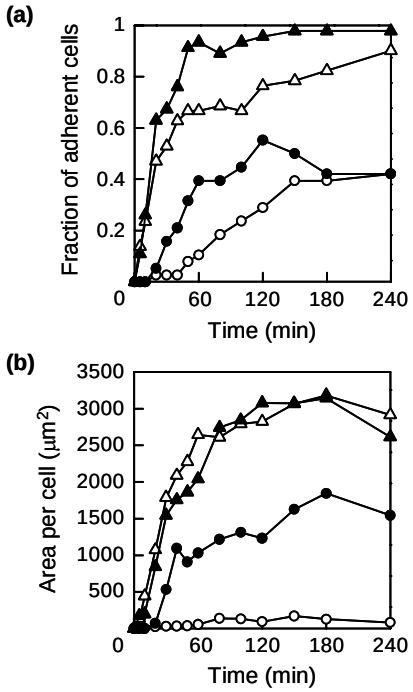


Fig. 3 (a) Fraction of adherent cell and (b) adhesion area per cell on $CH_3$- (open circle), OH- (filled circle), COOH- (open triangle) and $NH_2$-SAMs (filled triangle) determined from TIRFM images acquired during a cell adhesion process of HUVECs.

## 4. Conclusions

We examined the effect of surface functional groups on initial cell adhesion using well-defined SAMs of alkanethiols. Combination of SPR and TIRFM allow for real-time monitoring of protein adsorption and subsequent cell adhesion including cell approach, attachment, and spreading. Cell adhesion was greatly affected by surface functional groups: HUVECs adhered well to COOH- and $NH_2$-SAMs, whereas poorly to $CH_3$- and OH-SAMs. These findings provide insight into the mechanisms of cell adhesion including protein adsorption, leading to the control of cell-material interactions.


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