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Effect of surface potential on epithelial cell adhesion, proliferation and morphology

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ABSTRACT

Cell adhesion is the basis of individual cell survival, division and motility. Hence, understanding the effects that the surface properties have on cell adhesion, proliferation and morphology are crucial. In particular, surface charge/potential has been identified as an important factor that affects cell behavior. However, how cells respond to incremental changes in surface potential remains unclear. By using binary self-assembled monolayer (SAM) modified Au surfaces that are similar in mechanical/chemical properties and provide a series of surface potentials, the effect of surface potential on the behavior of cells can be studied. In this work, the effect of surface potential on epithelial cells, including human embryonic kidney (HEK293T) and human hepatocellular carcinoma (HepG2), were examined. The results showed that the adhesion density of epithelial cells increased with increasing surface potential, which is similar to but varied more significantly compared with fibroblasts. The proliferation rate is found to be independent of surface potential in both cell types. Furthermore, epithelial cells show no morphological change with respect to surface potential, whereas the morphology of the fibroblasts clearly changed with the surface potential. These differences between the cell types were rationalized by considering the difference in extracellular matrix composition. Laminin-dominant epithelial cells showed higher adhesion density and less morphological change than did fibronectin-dominant fibroblasts because the more significant adsorption of positively charged laminin on the surface enhanced the adhesion of epithelial cells. In contrast, due to the dominance of negatively charged fibronectin that adsorbed weakly on the surface, fibroblasts had to change their morphology to fit the inhomogeneous fibronectin-adsorbed area.

1. Introduction

Cell behavior on material surfaces has garnered attention for the development of biological applications, such as cell sorting, biosensors and biochips. Cell adhesion is a physiological process that allows cells to attach to a surface by forming cell–cell and cell–matrix junctions that determine the cell's shape, density, arrangement and eventually tissue structure. Adhesion is also the key to the regulation of cell survival, division and motility on surfaces. Through the focal adhesion, living cells adapt to the environment by interacting with the extracellular matrix (ECM) or the substrate surface to regulate the signaling pathway [1–4]. Therefore, understanding the cellular interaction with surfaces is important for the development of biomaterials and bioassay devices.

Protein-coated polystyrene (PS), polymer thin films, hydrogels, thermoresponsive polymers and self-assembly monolayers (SAMs) have generally been used to modify the substrate surface [5–8]. Among these, SAM-modified surfaces have been a popular platform for tailoring surfaces due to its ease of synthesis and preparation. SAMs consist of a headgroup that anchors to the surface, an alkane chain as a backbone to promote molecular alignment and a functional group that controls the surface properties [9]. SAMs have been widely used to modify the surface for specific requirements, such as stiffness, roughness, surface potential, wettability, smoothness, and molecular distribution [10–12]. The common SAM systems are amines and alcohols on Pt substrates, fatty acids on oxides of metals, siloxane on glass substrates, thiols on Au substrates and polymer films [13–18]. Of these systems, SAMs on

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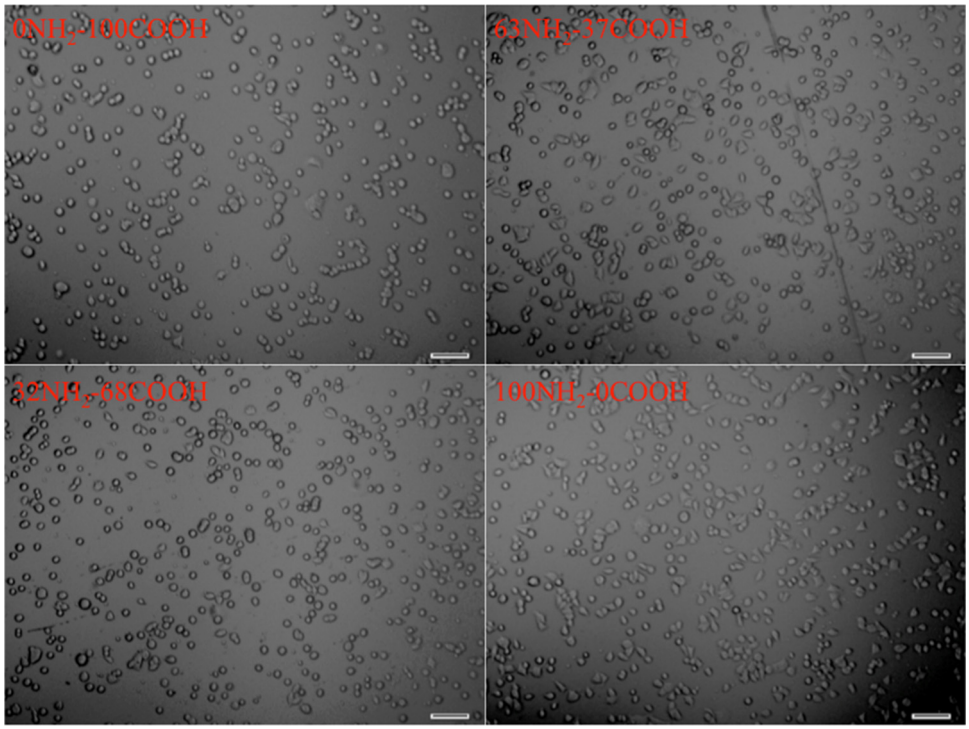

Fig. 1. DIC images of HEK293T cells after 4 h of incubation on binary SAM-modified Au surfaces. The seeding density was 2.5×10^4 cell/cm^2, and the scale bar is 90 μm.

Au substrates provide a wider selection of compatible functional groups with thiol molecules [19,20]. Moreover, thiol-based SAMs hold a powerful capability to investigate the effect of one surface property by selecting an appropriate functional group [21].

SAM-modified Au and Si substrates with different surface properties have been used to study cell–surface interaction in terms of adhesion and proliferation. It has been known that decreasing the contact angle (increasing surface wettability) of $-CF_3$, $-CO_2Me$ and $-CH_2OH$ functional group increases the adhesion and proliferation of canine endothelial cells [22]. Later work also revealed that when using —OH, —PEG, $-CH_3$ and $-CF_3$ functional groups, the adhesion of human osteosarcoma (MG-63) and mouse embryo fibroblast (NIH3T3) decreased with decreasing surface wettability [23]. Moreover, surfaces with moderate wettability (such as $-NH_2$ and —COOH) are found to be better for human fibroblast adhesion and spreading [11], and SAMs with moderate wettability are suitable for the adhesion of human umbilical vein endothelial cells (HUVEC) and cervical cancer cells (HeLa) [21]. In addition to wettability, it has been found that increasing surface potential enhances the adhesion and proliferation of human dermal fibroblasts on siloxane-SAM-modified glass substrates [24]. The adhesion and spreading of bovine aortic endothelial cells (BAEC) are found to be better on a SAM-modified surface of $-NH_2$ groups than on that of —COOH groups [25]. Furthermore, similar results were observed with neuronal cells on binary SAM-modified Au surfaces with mixed amine and carboxylic acid groups [26]. However, binary SAMs of $-SO_3H$/$-N^+(CH_3)_3$ and $-PO_3H_2$/$-N^+(CH_3)_3$ were found to have little or no effect on platelet adhesion [27]. This may be due to the strong interactions between ionic ammonium and acids in the deposition solution, which resulted in no significant change in the actual surface chemical composition or zeta potential. Therefore, the choice of functional groups in the binary SAM is important for fine-tuning the surface properties.

In previous work, homogeneously mixed binary SAMs, with functional groups that possess opposite properties, on Au substrates have been established for the further precision tuning of the surface properties of Au thin-films [28] and Au nanoparticles [29]. Using this concept, 16-mercaptohexadecanoic acid and 8-amino-1-octanethiol were used to fine-tune the surface potential (isoelectric point) and effective surface dipole moment [30]. Because of the opposite charge of an acid and a base, a series of ratios of these two functional groups yielded a series of zeta potentials and work functions. In addition, because the surface chemical composition changed in a much smaller increment than did those in which a single functional group was used to tailor the surface, the chemical properties of the binary SAM remain similar. With these zeta potential-tunable surfaces, the effects of surface potential on the adsorption of plasmid deoxyribonucleic acid [31] and its molecular delivery abilities were studied [32]. It was also found that the binary-SAM modified gold nanoparticles had low cytotoxicity to living cells [32] hence the binary SAM on gold has high biocompatibility. However, whereas the different lengths of backbones lead to a minor difference in SAM deposition rate and this difference in height may lead to an unnecessary topographical effect, the binary SAM system is further improved to be the homogeneous mixing of 6-mercaptohexanoic acid and 6-amino-1-hexanethiol [33]. Atomic force microscope (AFM) revealed that the surface morphology remains identical (root mean square, RMS roughness <2 nm) before and after the SAM deposition, though a series of surface potentials are obtained [34]. This system has been used to study the effect of surface potential on the adsorption of ECM proteins [33] and on NIH3T3 fibroblast cell behavior, including adhesion, proliferation and morphology [34].

It was found that surface potential significantly affected the initial adhesion density of NIH3T3 cells, i.e., with an increase in surface potential on surfaces, more cells were adhered and the cell shape changed from bipolar to well-spreading. In this work, to further investigate whether surface potential has a similar effect on other cell types and holds the potential to control cell behavior, epithelial cells, including human hepatocellular carcinoma (HepG2) and human embryonic kidney (HEK293T) cells, were seeded on binary SAM-modified Au surfaces to examine the effect of surface potential on their adhesion density, proliferation rate and morphology.