Culturing of Rat Intestinal Epithelial Cells-18 on Plasma Polymerized Ethylenediamine Films Deposited by Plasma Enhanced Chemical Vapor Deposition

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Many researchers studied cell culturing on surfaces with chemical functional groups. Previously, we reported surface properties of plasma polymerized ethylenediamine (PPEDA) films deposited by plasma enhanced chemical vapor deposition with various plasma conditions. Surface properties of PPEDA films can be controlled by plasma power during deposition. In this work, to analyze correlation of cell adherence/proliferation with surface property, we cultured rat intestinal epithelial cells-18 on the PPEDA films deposited with various plasma powers. It was shown that as plasma power was decreased, density of cells cultured on the PPEDA film surface was increased. Our findings indicate that plasma power changed the amine density of the PPEDA film surface, resulting in density change of cells cultured on the PPEDA film surface.

Key Words: Cell culturing, Rat intestinal epithelial cells-18, Plasma polymerized ethylenediamine film, Plasma enhanced chemical vapor deposition

Introduction

Many results about cell adsorption on surfaces were presented. Clear mechanisms of cell adsorption, however, are yet to be elucidated. Chemical functional group and surface energy on the surface can be among main factors of cell adsorption. Generally, a hydrophilic surface, which has a high surface energy, induces cell adsorption, and a hydrophobic surface, which has low surface energy, restrains cell adsorption.[1,2] Functional groups, such as hydroxyl, carboxyl and amine groups, on the surface also have strong effects on the cell adsorption.[3,4] Amine functional groups (NH_2-) on the surface are often used for cell adsorption.[5] Various techniques were introduced to make amine functional groups on the surface, such as self-assembled monolayer,[6] photolithography[7] and deposition of plasma polymerized films using PECVD system.[8]

Stomach and intestines are involved in the digestive organ system. These digestive organs are essential to absorb a variety of nutrients such as digested biomolecules and water. These organs are also associated with other biological processes like nutrient transport, exo- or endocrine secretions, and their regulations. Intestinal epithelium is an important tissue for regulating the above described biological processes. Anatomically, intestinal epithelia locate in the innermost intestinal layer. Thus, they directly expose to inside lumen and play roles in various relevant functions as well as protecting from external environment. Due to their direct exposure to lumens, epithelia easily and frequently contact with pathogens originated from external environment, leading to a broad spectrum of diseases, for instance, diarrhea and intestinal cancer. According to accumulated data for intestinal epithelial cells, various intestinal diseases are caused by epithelial malfunctions. However, detailed mechanisms for epithelial malfunctions remain little known. To better understand intestinal epithelial pathophysiology and physiology, a wide variety of well-established culture plates for growing epithelial cells have to be developed and utilized.

In our previous reports, plasma polymerized ethylenediamine (PPEDA) films deposited on glass substrates by plasma enhanced chemical vapor deposition (PECVD) using the ethylenediamine precursor with various plasma powers showed various surface amine densities on the film surface. In this work, we cultured rat intestinal epithelial cell-18 (IEC-18) on PPEDA film surfaces to study the correlation of PPEDA surface properties with cell adhesion and proliferation.

Experiments

We deposited thin films onto the glass slide by the PECVD system using ethylenediamine as a precursor. Detailed description of the PECVD system is presented previously.[8] Glass slides were sonicated in trichloroethylene, acetone, and methanol each 5 minutes, respectively. Cleaned glass slides were rinsed in deionized water, and remained water was blown off by nitrogen gas. Glass slides were put into the deposition chamber, and chamber was pumped down to ~10^{-5} Torr with a turbo molecular pump. Highly purified argon gas was used as a carrier gas. The bubbler, which contained the ethylenediamine precursor, was maintained at 45 °C. Inductively coupled plasma (ICP) and substrate bias (SB) plasma were generated by two different 13.56 MHz radio frequency

generators. When Argon gas of 15 sccm was flowed into the bubbler, Ar gas and vaporized precursor molecules were mixed in the bubbler, and then were flowed into the deposition chamber through the shower ring. During PPEDA film deposition for 2 minutes, the slide temperature was maintained at room temperature, and chamber pressure was kept at 30 mTorr. The SB power was kept at 3 W while ICP power was varied from 3 W to 70 W. Surface roughness of the PPEDA film was measured by atomic force microscopy. Intestinal epithelial cells-18 (IEC-18) were grown at 37 °C, 5% CO_2 in the growth media (DMEM (Wel GENE Inc., Korea)) containing 10% fetal bovine serum (Wel GENE Inc., Korea), 0.5% streptomycine/penicillin, and 0.0005% insuline on the PPEDA film surfaces or a conventional culture dish (polystyrene Cell BIND surface manufactured by Corning).

Results and Discussion

Figure 1 show optical images of cells cultured for 12 hrs on the PPEDA films deposited with various ICP powers and on a conventional culture dish. Cells of highest density were cultured on the PPEDA film deposited with 3 W ICP power (hereafter referred to as PPEDA (3 W)), which has the highest density of amine functional groups. On PPEDA films deposited with higher ICP powers, which have lower amine functional group than PPEDA (3 W), cells of less density were cultured. In addition, cells of higher density were cultured on the PPEDA films deposited with ICP power ≤ 10 W than on the conventional culture dish. Figure 2 numerically summarizes the results of Fig. 1. When ICP power was increased from 3 W to 70 W, cultured cell density decreased from 326 cells/mm^2 to 134 cells/mm.^{2} Moreover, density of cells cultured on PPEDA (3W) was about 1.7 times higher than on the conventional culture dish. Figure 3 shows density of cells cultured for various time periods on PPEDA films deposited with various ICP powers. The general trend is that density of cells was higher as cells were cultured for a longer time period on PPEDA deposited with a less ICP power.

For adherent cells like epithelial cells, it is important to figure out which types of interactions between cell surface and extracellular matrix mainly contributed to cell adhesion. For mechanism of cell adhesion on a solid substrate, three-step process is suggested in Ref. 9. Initial contact between the cell and the substrate occurs, and then bonds between receptors on cell surface and ligands on the substrate are formed. Finally, the cytoskeleton is reorganized by progressive spreading of the cell on the substrate for increased attachment strength[9]. To elaborate those interactions, we must develop well-established matrixes like surfaces of culture plates. Accordingly, to investigate correlation of PPEDA surface properties with cell growth, we measured surface roughness of PPEDA films by using atomic force microscopy (AFM) versus cell growth. Surface roughness of PPEDA films deposited with various ICP powers was randomly distributed in the range of 0.32 Å - 0.42 Å, not showing any notable trend. It is considered that surface roughness did not have significant effects on cell culture. Previously, we determined that amine density on the PPEDA film surface was decreased from 5.4 amine groups/nm^2

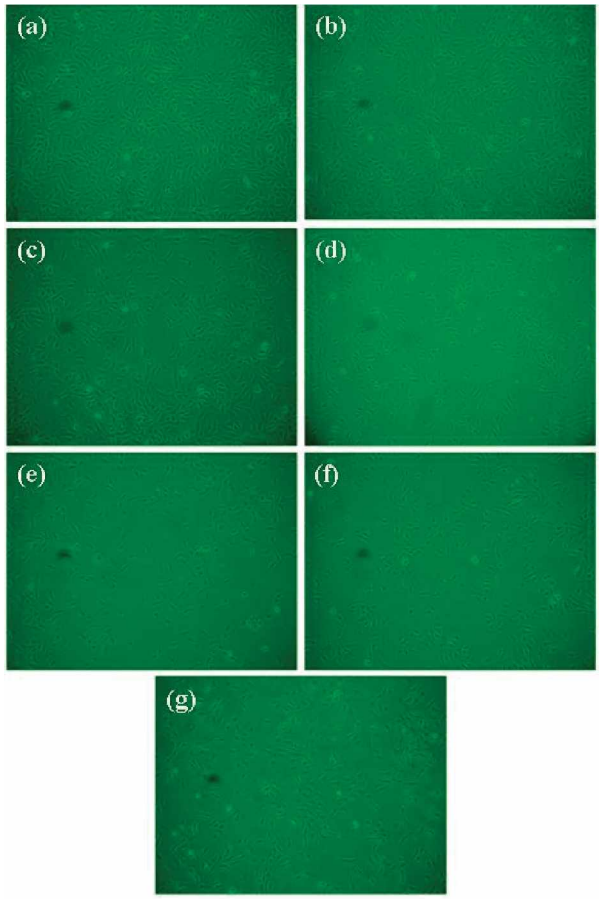

Figure 1. Optical images of cells cultured for 12 hrs on PPEDA films deposited with ICP power of 3 W(a), 5 W(b), 10 W(c), 30 W(d), 50 W(e), 70W(f), and on a conventional culture dish (g).

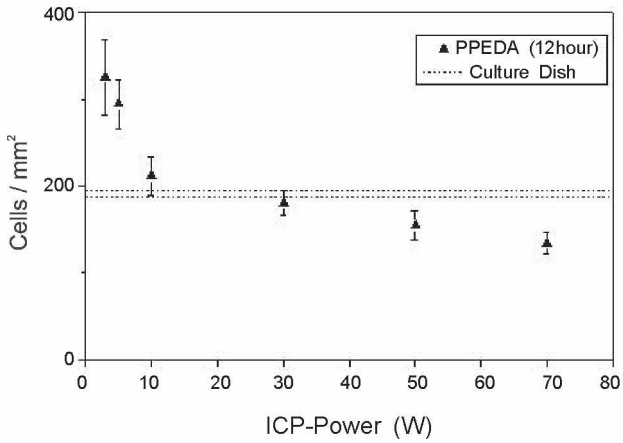

Figure 2. Density of cells cultured for 12 hrs on PPEDA films deposited with various ICP-powers. Dashed line is density of cells cultured on a convectional culture dish for 12 hrs.

to 2.7 amine groups/nm^2 as ICP power was increased from 3 W to 70 W.[10,11] In addition to the results of Refs. 10 and 11, we could also correlate the surface amine density with water contact angle. Oh *et al.* reported that, as amine density is increased, water contact angle was decreased.[12] We measured water contact angle of PPEDA thin films by sessile drop

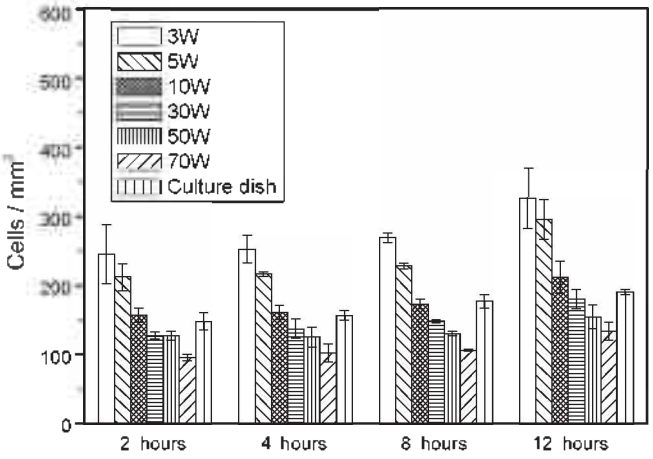

Figure 3. Density of cells cultured for various time periods on PPEDA films.

method. Water contact angle of PPEDA films were increased from 25.2° to 35° when ICP power was increased from 3 W to 70 W, indicating that amine density is decreased as ICP power was increased. In our other previous report, when immunoglobulin G (IgG) protein was immobilized onto the PPEDA surfaces deposited with various ICP powers, a larger amount of immobilized protein was achieved on a PPEDA surface deposited with a lower ICP power.[13] The previous results and this work address, that surface amine functional groups play an important role in cell culturing. It seems that more cells adhere and/or proliferate on surfaces with higher amine density. Further work remains studied for better understanding of cell adhesion/proliferation on surfaces.

Conclusions

Rat intestinal epithelial cells-18 were cultured on surfaces of PPEDA films deposited on glass slides by PECVD using the ethylenediamine precursor to study the correlation of PPEDA surface properties with cell adsorption/growth. Density of cells was higher as cells were cultured for a longer time period on the surface of PPEDA deposited with a less ICP power. When ICP power was increased from 3 W to 70 W, density of cells cultured for 12 hrs decreased from 326 cells/mm^2 to 134 cells/mm.^{2} Density of cells cultured on the PPEDA film deposited with 3 W ICP power was about 1.7 times higher than on a conventional culture dish. It seems that cells of higher density were cultured on surfaces with higher amine density, implying that surface amine functional groups play an important role in cell adherence and/or proliferation on surfaces.

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