

A Microscopic Investigation on the Effect of Hydrophobic Properties on Cell Adhesion on a PDMS Surface

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Abstract

In vitro cultivations of human mammary epithelial cells (HMEC), a human breast cancer cell line (MCF-7), and human brain cancer cells (U373MG) were performed in a PDMS chamber without any cell adhesion receptor or surface coating. The surfaces were treated by the exposure of air plasma to control the hydrophobic properties. The effects of the hydrophobic properties of PDMS on cell adhesion and growth were microscopically investigated, and their viability measured using a live and dead cell kit by laser confocal microscopy. The adhesion force difference between cells (HMEC and MCF-7) was investigated using shear stress induced by a micro-flow in the micro channel.

Keywords: HMEC, MCF-7, Hydrophobicity, PDMS, Adhesion

Introduction

As the main characteristics of cancer are a fast cell metastasis and unlimited proliferation, the importance of an early cancer diagnosis has emerged as cancer incidences increase worldwide¹. Cancer therapeutics can be simple and effective when early cancer diagnoses are developed with high sensitivity. A promising diagnosis for cancer comes about by direct-detecting of early cancer cells in the body using imaging technologies such as an MRI or PET; nevertheless, a few cancer cells are difficult to measure by imaging technologies even using contrast media^{2,3}. Currently, the best method to detect cancer cells is a direct investigation through a biopsy. The biopsy process is time consuming and labor intensive, and even requires a

highly-trained pathologist to diagnose suspected cancer in tissue extracted from the body. The pathologist confirms the presence of cancer cells using a microscopic investigation and sophisticated procedures⁴. False negative results can occur if the pathologist is unable to detect the cancer cells using microscopy when the tissue sample possesses only a few cancer cells. Therefore, a highly-sensitive detection method is required to confirm cancer in suspected tissue extracted from the body.

Detection technologies and isolation methods have been widely studied to improve detection sensitivity on cancer cells in a tissue. Biomarkers specific to cancer cells and fused with nano-technology have been intensively developed^{5,6}. Biomarkers utilize an immunological reaction between the outer membrane protein of cells and their specific antibodies bound to label materials such as nano particles and fluorescent dyes. These methods eventually require well-trained experts and sophisticated processes to measure cancer cells, as well as long sample preparation steps because cancer cells grow with different metastases, morphologies, and proliferations.

Because the detection sensitivity is increased when only cancer cells are isolated in a tissue, various methods of isolating cancer cells from the mixed cells supplied from a tissue have been developed using a micro-chip⁷⁻⁹. The physical properties of cells, such as their size, density, and charge can be used directly for cell isolation. One of the best methods to isolate cells is to utilize the their adhesion difference on a surface¹⁰⁻¹², as *in vitro* cell cultures have been intensively studied to immobilize cells and to control cell survival growth rate, and metabolism, as well as gene expressions.

The modification technologies of surface properties are key to growing most animal cells including cancer cells. The surface properties can be modified by the patterning nanostructure exposed to oxygen plasma and coating with organic molecules such as charged molecules, collagen, fibrinectin, and peptides¹³⁻¹⁹. These modification technologies are focused on changes of hydrophobicity, charge, and the specific biological properties of the surface on which the cells attach by using physical adsorption, chemical reaction, and biological affinity between cell adhesion receptor and ECM. The most commonly used technique is to coat ECM protein on a substrate non-specifically. This method is simplest for cell adhesion, and could

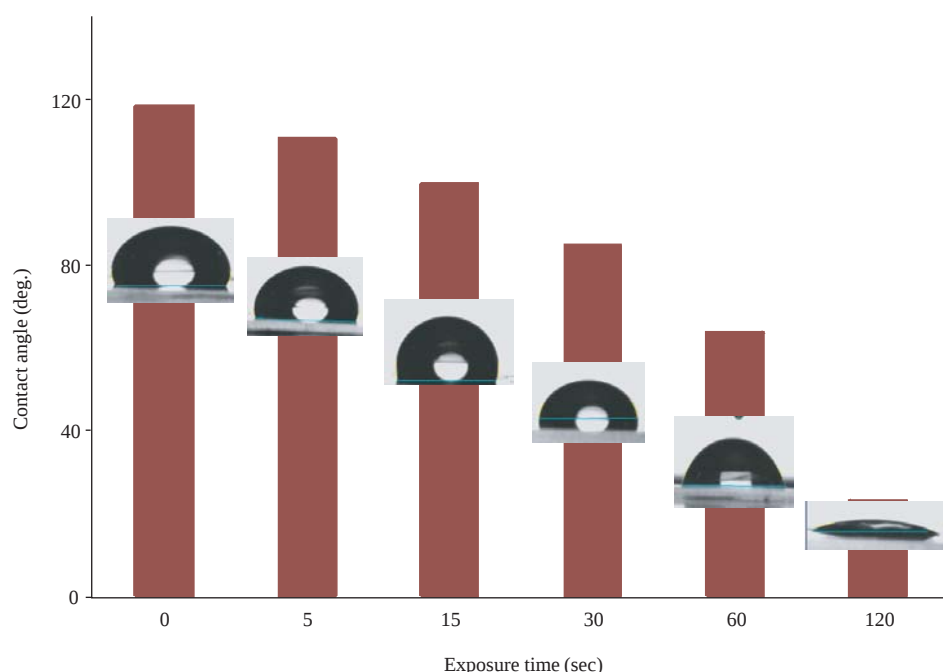


Figure 1. The hydrophobic property of PDMS as exposure time of air plasma.

be considered as an *in vivo*-like environment²⁰⁻²¹.

To isolate cells of interest from a mixed cell cluster, the surface properties need to be re-considered in order to control the adhesion of the cells, as most integrins in a variety of cell types are differently expressed for different cells to adhere to many different ECM molecules. On the other hand, cells of interest can be isolated from different cells by controlling the surface modifications. Cell separation was achieved using a nano-structured surface²². The results indicated that normal cells (MCF10A) showed a stronger adhesion on the surface than cancer cells (MCF-7). However, this result requires an additional process to fabricate a nanostructural pattern on the surface.

A microchip fabricated using MEMS technology is a very promising format to reduce analysis time and medium for a cell culture. PDMS (Polydimethylsiloxane) is the most popular material for fabrication of a micro pattern and structures by soft lithography because of this economic advantage. Cell adhesion on PDMS has been studied using an adhesion protein and nanostructure applied on a surface^{16,23}. However, this investigation did not show the effect of cell adhesion on the property of the PDMS surface itself.

In this study, we investigate the possibility of cell isolation by controlling the properties of a PDMS surface of which a cell would adhere upon, rather than using a nanostructure and adhesion protein. This will be worthy for separating a cell of interest from different cells, but not for mammalian cell cultures. A sur-

face activation method by air plasma is used to control the surface properties such as hydrophobicity and charge. The cell adhesion is investigated as air plasma exposure time using normal cells (HMEC) and cancer cells (MCF-7). The shear stress derived by a micro-flow in a micro channel is used as the driving force for the separation between a normal cell and cancer cell adhered on a PDMS surface. These results can provide basic information for purification and concentration of cancer cells from a mixed cell solution.

Results and Discussion

The contact angle of the PDMS surface was dramatically decreased as air plasma exposure time increased. As shown in Figure 1, the surface was activated by air plasma, and it showed a hydrophilic property due to a negative charge.

The HMEC and MCF-7 cells were incubated on each PDMS surface and treated by air plasma for 0, 5, 15, 30, 60, and 120 sec as shown in Figure 2. The cultivation conditions for all experiments were 37°C and 5% CO₂. As shown in Figure 2, both the MCF-7 and HMEC cells were well adhered onto the PDMS surface treated by air plasma for a relatively long exposure time. This shows that at least MCF-7 and HMEC cell lines can be incubated on a polymer substrate without any adhesion agents such as collagen, fibronectin, and peptide if the hydrophobicity of the

surface can be somewhat controlled below 100° , even though the growth rate of cancer cell, MCF-7, on the PDMS looked slower than that on a commercial culture dish. Actually, it is known that cancer and nor-

mal cells are difficult to adhere onto a hydrophilic surface²⁴. However, this result shows that a hydrophilic surface does not affect the adhesion of cells; even cells grew on a substrate fully treated by air plas-

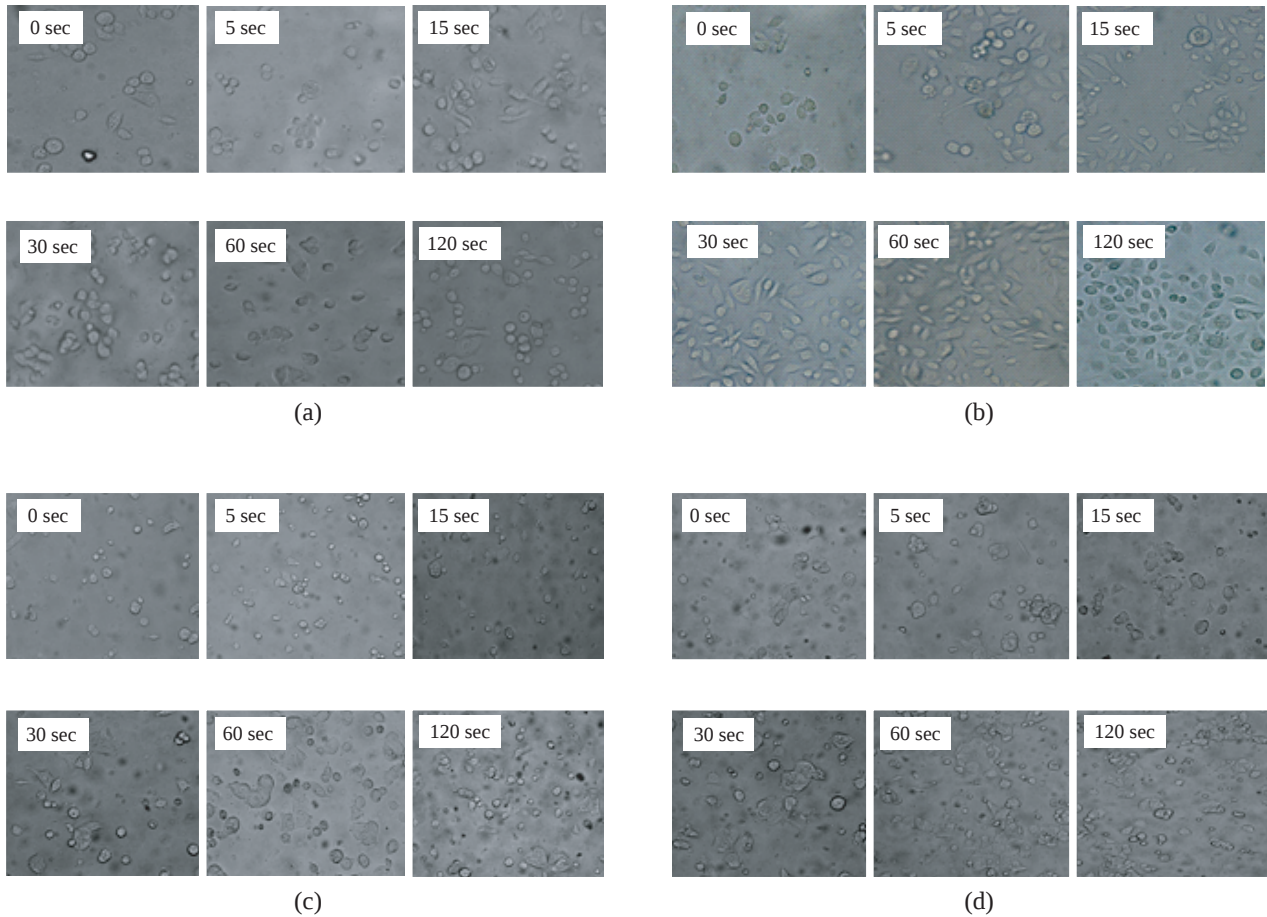


Figure 2. The microscopic results of cell cultivation on a PDMS surface: (a) HMEC after 6 hrs, (b) HMEC after 24 hrs, (c) MCF-7 after 6 hrs, and (d) MCF-7 after 24 hrs.

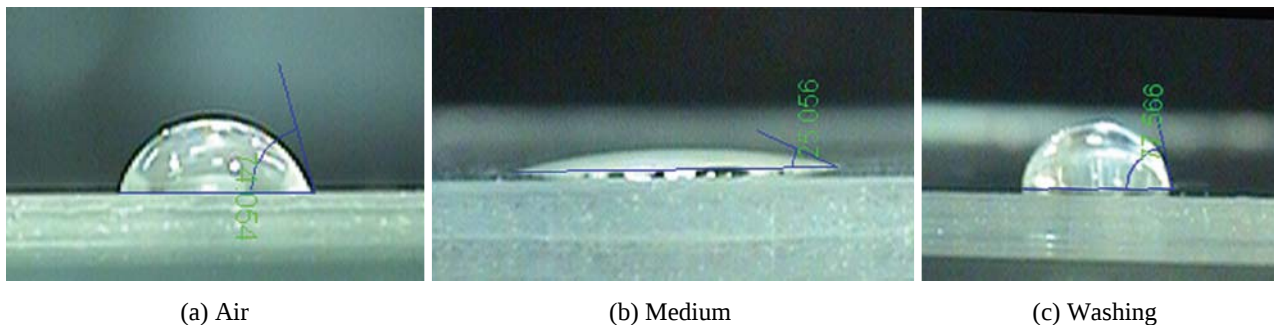


Figure 3. The contact angle measurement of PDMS (a) kept in air 24 hr after the exposure of air plasma, (b) kept in a cell medium 24 hr after the exposure of air plasma, and (c) kept in cell medium 24 hr after the exposure of air plasma and washed by D.I. water.

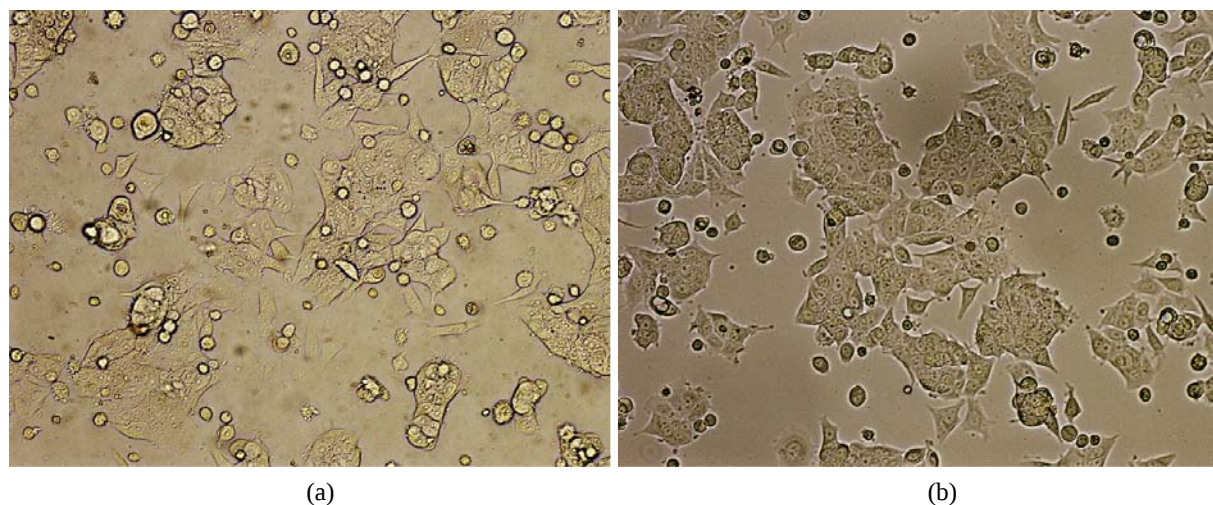


Figure 4. The microscopic results of MCF-7 cultivated for 72 hours on (a) a PDMS surface and (b) commercial culture dish.

ma better than on a surface with moderate hydrophobicity. The main reason for this seems to be that the hydrophobicity of a PDMS surface is recovered even it is immersed in solution, as in Figure 3(c). At the early stage (~6 hr), both cells were not fully adhered, as shown in Figure 2(a) and (b), but after 1 day, both cell lines, particularly HEMC cells, seem to be adhered firmly onto PDMS treated for 120 sec. It makes sense that mammalian cells are grown on a moderate hydrophobic surface (contact angle $50-90^\circ$)^{11,14,18,25}. The second reason is due to amino acid contained in the cell growth medium. The surface charge of the PDMS was negative after air plasma treatment. When the medium is loaded on a PDMS layer, various amino acids can be adsorbed easily on a negatively-charged surface. We realized that the contact angle of the PDMS surface was not changed after 24 hrs when the contact angle was measured without a heavy washing step, as shown in Figure 3(b). The surface possesses positive and negative charges, both, on the PDMS surface by various amino acids adsorption. This allows cells to adhere to the PDMS surface.

MCF-7 cultivated on the PDMS surface and commercial culture dish were compared after a 72 hr culturing as, shown in Figure 4. The morphology of MCF-7 adhered on the PDMS layer does not show a large difference with that on the commercial culture dish. Only some cells not adhered onto the surface were adhered on other cells instead of the PDMS surface. It is likely that the PDMS surface is not a preferred surface for cellular growth. In other words, some cells started to adhere to and grow on a relatively proper region of the PDMS, and other cells began to adhere onto the cells already occupying the preferred

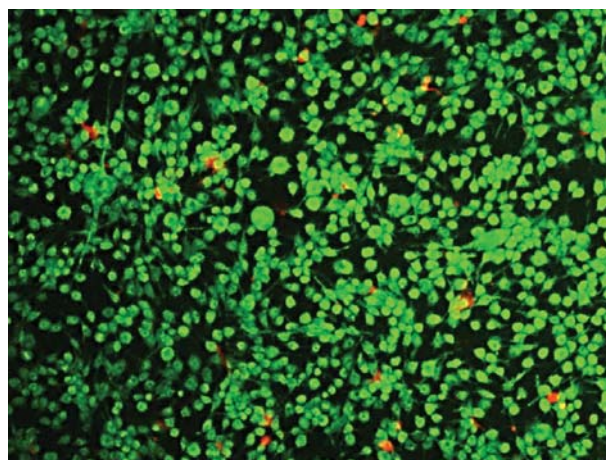


Figure 5. The fluorescent results of U373MG cultivated for 48 hours on a PDMS surface (green: viable, red: dead).

surface because no more proper surface region existed any more. Nevertheless, most cells introduced on the PDMS surface showed good on-surface adhesion, which implies that PDMS can be used for a simple cell cultivation process when its surface is fully activated by air plasma. This result can be also proved using confocal microscopic results for cell viability.

The viability of cells (U373MG) on a PDMS surface was investigated using a live and dead cell kit as shown in Figure 5. A green color indicates live cells and a red color is dead cells. As shown in Figure 5, most cells adhered onto the PDMS surface, and except a few cells, are alive after 72 hr cultivation. This result does not indicate whether the intrinsic properties of

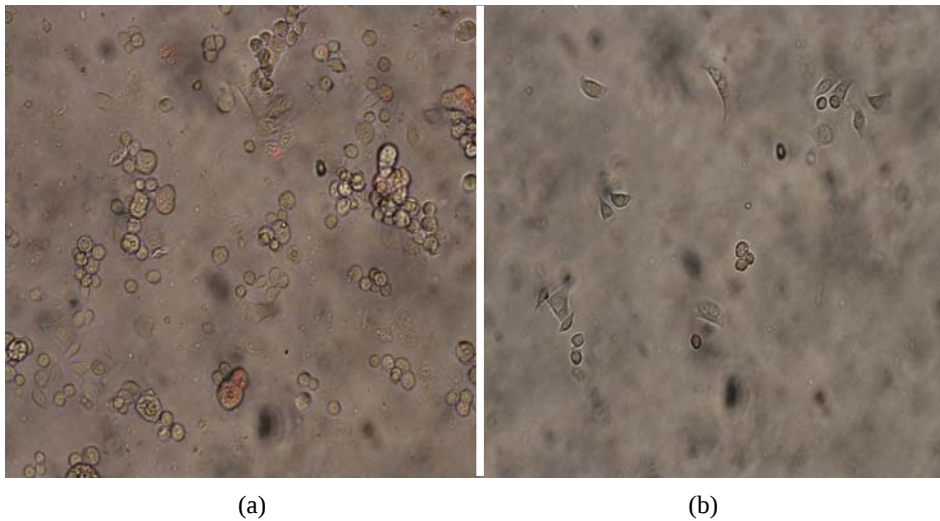


Figure 6. The microscopic results of a cell mixture (HMEC and MCF-7) for 6 hours with (a) no flow and (b) after an introduction of 15 mL/min.

cells grown and adhering onto the PDMS are being sustained for the cultivating time. However, it shows that mammalian cells grow well on PDMS for at least 72 hours under normal conditions (37°C, 5% of CO₂).

The separation of cells of interest from cell mixtures was performed using a PDMS microchip. The cell mixture (HMEC and MCF-7, 1 : 1) was introduced in the microchip and was incubated for 6 hours under same condition. Figure 6(a) showed cell mixture adhered onto a PDMS layers after 6 hours. The red color region indicates an MCF-7 cluster. After introduction of a medium flow of 15 μ L/min into the micro channel containing the cell mixture, the MCF-7 was desorbed from the PDMS layer by the shear stress of the hydraulic force. This result implies that the adhesion force of MCF-7 on a PDMS layer is weaker than that of HMEC. This result agrees well with the results published by Kwon *et al.*²². This implies that cancer cells can be isolated from normal cells by controlling only the surface properties.

Conclusion

PDMS is one of the popular materials in biological micro systems because of its great mechanical properties as well as its well-established soft lithographic technologies. These surface properties have already been studied intensively as the surface properties of the material are becoming more interesting in micro systems. An *in vitro* cultivation of mammalian cells in a micro system requires some more detailed investigation for the surface properties on which cells of interest adhere. In this paper, it is shown that the hydrophobic property of a surface is the most important one in a micro cell chamber. For the best *in vitro* cell

cultivation by providing *in vivo* like environments into a cell microchip, other surface properties of materials need to be investigated based on the results provided in this study.

Materials and Methods

Cell Cultivation

Human Mammary Epithelial Cells (HMEC) were purchased from Lonza, Ltd (Wakersville, MD, USA). A Mammary Epithelial Basal Medium (MEBM) was used as its specific medium, adding MEGM SingleQuots provided from Lonza, Ltd (Wakersville, MD, USA). MEGM SingleQuots, supplements, and growth factors consist of BPE, hydrocortisone, hEGF, insulin and gentamicin/amphotericin-B. A human breast cancer cell line (MCF-7) was purchased from ATCC (USA). It was cultivated using RPMI-1640 (PAA) containing 10% of FBS, 1% of penicillin-streptomycin (GIBCO), and 1% of GultaMAX (GIBCO, USA). U373MG (Human brain cancer cell) was also cultured using a DMEM high glucose (GIBCO) medium containing 10% FBS, 1% penicillin, and streptomycin. All of the cells were cultivated under the conditions of 37°C and 5% CO₂.

Cell Mixture

In the cell mixture, cell concentration was controlled to $\sim 10^6$ cells/mL of the medium with a 9 : 1 ratio of HMEC : MCF-7. RPMI-1640 containing 10% FBS was used for co-cultivation. A second sub-culture of HMEC was carried out using RPMI-1640 in order to avoid any shock by different medium prior to co-cultivation.

Surface Treatment

For a preliminary investigation of the effect of the surface properties of PDMS (Sylgard 184, Dow Corning, MI, USA) on cell growth, a PDMS layer (2 mm) was coated in a well 3.4 cm in diameter. The PDMS was baked at 80°C for an hour. The surface was treated by air plasma (Femto Science Cografe, 8W, Korea) for 0 sec, 5 sec, 15 sec, 30 sec, 60 sec, and 120 sec. The contact angles of each PDMS layer were measured using a contact angle meter (FTA200, VA, USA). DPBS (CIBCO) was added in the well containing PDMS just after measurement.

Cell Viability Test

Cells cultivated on a PDMS surface were stained to measure its viability using a live-dead cell staining Kit (Bio Vision, CA, USA), which provides an emitted fluorescence of 518 nm for live cells and 615 nm for dead cells.

Microscopic Investigation

All microscopy works were performed using a Nikon Eclipse, TE2000-U, Japan. Cells cultivated on the PDMS were investigated for 2 to 4 days.

Separation

The microfluidic channel format was introduced in order to measure cell adhesion force using shear stress induced by a micro-fluid. The PDMS micro-channel was fabricated by soft lithography and its dimension is 1 mm × 2 cm × 100 μm (width × length × depth). The flow was generated by micro syringe pump (Harvard '11' Plus, MA, USA) with a volumetric rate of 15 mL/min.

Acknowledgements

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