JBACHS
Journal of Basic and Clinical Health Sciences

J Basic Clin Health Sci 2019; 3:73-77
https://doi.org/10.30621/jbachs.2019.601

Original investigation

Optimization of Different Surface Modifications for Binding of Tumor Cells in a Microfluidic Systems

Hanife Ecenur Meco[1], Aslıhan Karadag[2], Sevde Omeroglu[3], Gizem Aydemir[4], Gizem Calibasi Kocal[5], Muhammed Enes Oruc[6], Huseyin Uvet[7], Yasemin Basbinar[8]

[1]Institute of Health Sciences, Translational Oncology Department, Izmir, Turkey
[2]Institute of Health Sciences, Translational Oncology Department, Izmir, Turkey
[3]Institute of Science and Technology, Chemical Engineering, Kocaeli, Turkey
[4]Faculty of Mechanical Engineering, Mechatronics Department, İstanbul, Turkey
[5]Dokuz Eylul University, Institute of Oncology, Department of Translational Oncology, Izmir, Turkey
[6]Institute of Science and Technology, Chemical Engineering, Kocaeli, Turkey
[7]Faculty of Mechanical Engineering, Mechatronics Department, İstanbul, Turkey
[8]Institute of Oncology, Department of Translational Oncology, Izmir, Turkey

Address for Correspondence: Hanife Ecenur Meco, **E-mail:** mecoecenur@gmail.com
Received: 02.04.2019; **Accepted:** 11.04.2019; **Available Online Date:** 28.05.2019
©Copyright 2018 by Dokuz Eylül University, Institute of Health Sciences - Available online at www.jbachs.org

Cite this article as: Meco HE, Karadag A, Omeroglu S, Aydemir G, Calibasi Kocal G, Oruc ME, Uvet H, Basbinar Y. Optimization of Different Surface Modifications for Binding of Tumor Cells in a Microfluidic Systems J Basic Clin Health Sci 2019; 3:73-77. https://doi.org/10.30621/jbachs.2019.601

Abstract

Objectives: Microfluidic technology is a fast-growing area and provide high-efficient MEMS (Micro-Electro-Mechanical-Systems) sensor integration platform that helps to advance healthcare systems. Due to proper the chemical and mechanical properties of polymers, PDMS (Polydimethylsiloxane) (6) and PMMA (Poly-methyl-methacrylate), they became on the best candidate for health care studies in microfluidic studies (7). Besides, they perform great optical properties for observation of living cell experiments. To increase their performance, surface interactions works with cells, modification techniques are widely used in microfluidic chips. In this paper, our primary purpose is to modify such polymers and glass with matrigel, PDA and APTES so as to increase cell-surface interaction.

Patients and Methods: Cells were seeded into the micro-channels that is modified with 1%, 3% and 5% matrigel. The cell culture were observed 48-hours and images were taken. In the next stage, static culture experiments were performed on glass surfaces modified with PDA (polydopamine) and APTES (3-Aminopropyl triethoxysilane). 3-hour images were received.

Results: The area filled by the cells was calculated using the ImageJ software ver.1.149. In the Matrigel modified chips, at the end of the 48th hour, the surface area of the cells reached to 22.58%-29.14- 26.97% for the matrigel sample rates 1%, 3% and 5%, respectively. In PDA, APTES modified surfaces (dissolved in ethanol) and APTES modified surface (dissolved in water), the cells overlaid on surface after 3rd hour 5,32% 8,08%; and 3,33%, respectively.

Conclusion: The tendency of the cells to attach and colonize on increasing matrigel concentrations and on PDA, APTES modified surfaces was higher than the unmodified surface.

Keywords: Microfluidic Chips, Surface Modification, Cell Culture

INTRODUCTION

Microfluidic chips are systems that enable the control and movement of microliters and smaller volumes of fluids within micro-scale channels. Since the early 1990 s, many researchers have been working on systems that can be done with smaller volumes of analysis done by traditional methods in laboratories. By the growing needs of miniaturizations of chemical and biological test kits, these fluidic systems have taken great interest in recent years (1), and the developed systems have contributed to the-state-of-the-art lab-on-a-chip, cell-on-a-chip and organ-on-a-chip technologies. These fluidic systems have tiny in size internal volume devices so that tiny volume of samples are enough to measurement (2). The technologies used in these systems also allow to keep the flow rate under control, which in turn has led to a shorter diffusion distance and a reduced mixing time. The controlled flow also provides heat control and process control of the chemical reaction (3). These developed systems have a large share in the studies in the fields of analytical chemistry, medicine, food industry and health (4). Today, although the diagnostic and treatment methods used in the field of health are improved, there is a need for technological developments that can give fast and

reliable answers. Sensors and imaging techniques integrated in the chip systems can provide the enable development in this field. By combining chip technologies with microfluidic technologies, further analyzes can be achieved especially on cancer and other diseases. Microfluidic technologies have been readily implemented in cancer researches such as large-scale screening and diagnostic studies (such as chip-based mutation scans), advanced tumor biology studies (such as migration, metastasis, proliferation studies) and circulating tumor cells (5).

There are different imaging techniques for investigation of living cells. The applicability of these imaging techniques varies with the materials used in microfluidic chip fabrication. Especially, two polymers as PDMS (Polydimethylsiloxane) (6) and PMMA (Poly (methyl methacrylate) (7) show great transparency for visible light spectrum, as well as they are bio-compatible future which perfectly suits medical applications. For the use of different imaging techniques, various materials can be utilized in fabrication phase. However, the material properties must be carefully selected in order to increase interaction between a surface and a cell. Thus, there is a prevalent demand on surface modification studies in vitro models (8).

In this study, a glass coverslip is chosen due to its high optical transparency, well-known surface chemistry and fabrication technologies. As a glass surface was preferred many microfluidic applications, in our experiments, the glass surface was treated by MatriGel, PDA and APTES. Cancer and glass surface interaction results were reported to show success ratio of treatment processes. Cell adhesion force highly depends on matrix proteins, fibronectin and type 1 collagen according to previous efforts by Park et al., (9). For this reason, MatriGel was selected as an extracellular matrix protein for our work (10). Moreover, surface modifications with Polydopamine (PDA) have been carried out since 2007 (11) because PDA contains both catechol and amine groups. In *in vitro* modeling, because the superhydrophobic structure of the surface will increase cell surface interaction, it is advantageous to coat the surface with PDA (12). Likewise, APTES [(3-Aminopropyl) triethoxysilane], which is a group of aminosilanes, has been also utilized as modifying agents. Since APTES facilitates the formation of siloxane bonds by surface silanols and it has the advantage of catalytic activity by the amine group (13–14).

MATERIAL and METHOD

Cell culture

The ONCO DG-1 (ovary adenocarcinoma) (DSMZ: ACC 507) cell line was provided by DSMZ. Dulbecco's Modified Eagle Medium (DMEM) cell culture medium, supplemented with 10% fetal bovine serum (FBS, Biotech GmbH, Stadtallendorf, Germany) and 1% penicillin/streptomycin (BiochromGbmH, Berlin, Germany), under normoxic conditions (at 37° C), 5% CO_2) was cultured.

Modification with Matrigel on Micro Channels

First of all, Microfluidic chips were fabricated by using 1.5 mm thick polymethyl methacrylate (PMMA) and 50-μm thick double-sided adhesive film (DSA film). The microfluidic chip layers were bonded each other under pre-conditioned safety class II cabinet and sterile conditions. In the beginning, all the components of the microfluidic chip were cleaned via 70% ethyl alcohol and after that assembled parts were sterilized under UV. Prior to the biological cells in MatriGel (Corning, New York) were cultured in the microfluidic chip channel, it was completely sealed and leaking-free closure was maintained.

A glass surface which is bonded with PMMA layers to create micro-channels was treated by 1%, 3% and 5% MatriGel solution. The cells incubated under normoxic conditions were homogenously plated into the channels of the chips. After 12 hours, cell culture medium was removed by means of a micro-pipette and FBS-containing cell culture and FBS-free cell culture were added to the wells. The method steps performed are shown in Figure 1.6, 24 and 48 hour images that were taken by JuLI Br, live cell movie analyzer (NanoEnTek, Korea).

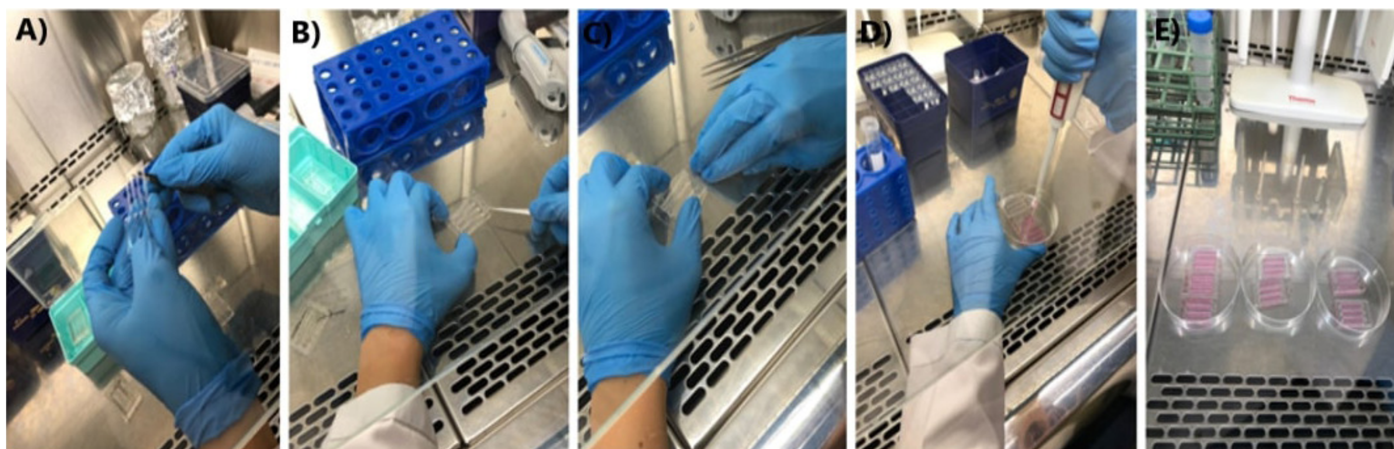

Figure 1. 50-μm thick double-sided adhesive film (DSA film) **(a)**. Combining 1.5 mm thick polymethyl methacrylate (PMMA) and 50-μm DSA film **(b)**. Combining the microfluidic chip components **(c)**. Addition of MatriGel to PMMA channels **(d)**. Addition of cell solution to PMMA channels and incubation **(e)**.

Figure 2. Modified glass surface **(a)**. Addition of cell solution to modified surfaces **(b)**. Incubation **(c)**.

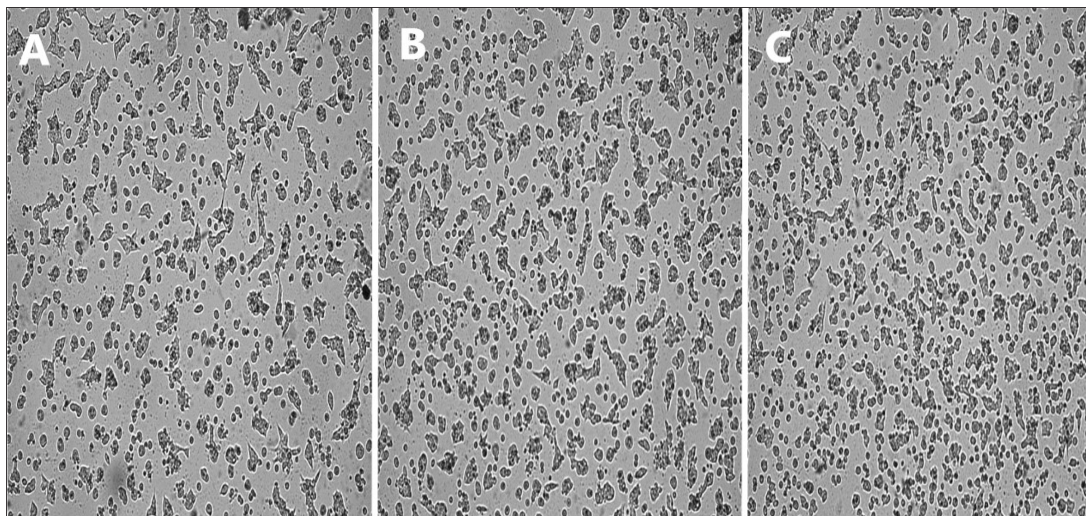

Figure 3. 48th hour microscopic images of cells on glass surface modified with 1% MatriGel **(a)**. 48th hour microscopic images of cells on glass surface modified with 3% MatriGel **(b)**. 48th hour microscopic images of cells on glass surface modified with 5% MatriGel **(c)**.

Chemical Modification on Surfaces

Static culture experiments were performed on a glass surface that was modified by polydopamine (PDA) (Sigma-Aldrich) and 3-Aminopropyl triethoxysilane (APTES) (Sigma-Aldrich). The surfaces were treated by different samples as APTES dissolved in ethanol, APTES dissolved in water, and PDA surface. Homogeneous cell solution was added to the modified surfaces in a sterile environment. After 3 hours, the modified surface was washed with cell culture medium and all non-adherent cells were removed. The method steps performed are shown in Figure 2. Surfaces images were taken by the inverted microscope (Carl Zeiss Suzhou Co., Ltd, Axio Vert. A1).

Cell Count

Using the cell images acquired by the inverted microscope (Carl Zeiss Suzhou Co., Ltd, Axio Vert. A1), the total area occupied by cells on the modified surfaces was calculated by using Image J software ver. 1.149 image processing analysis.

RESULTS

Modification with MatriGel-Cell Attachment to Modified Surface

The cell morphology on modified surface with MatriGel was observed and as a consequence of its propagation, the area that were occupied by cells, was calculated at the end of the 48th hour in the static culture medium. The results shown that for the surface modification of% 1 matrix, the cells were grouped around the region of interest by the ratio of 22.58%, and for the surface modification of% 3% matrix, the cells were grouped around the region of interest by the ratio of 26.97% and for the surface modification of 5% matrix, the cells were grouped around the region of interest by the ratio of 29.14%. After 48 hours, the images of the cells on the modified glass surfaces are shown in Figure 3. Calculations shows that increasing matrigel concentrations increase the area filled by cells on modified surfaces (Figure 3).

Chemical Modification-Cell Attachment to Modified Surfaces

Firstly, APTES was dissolved in ethanol and then, dissolved in

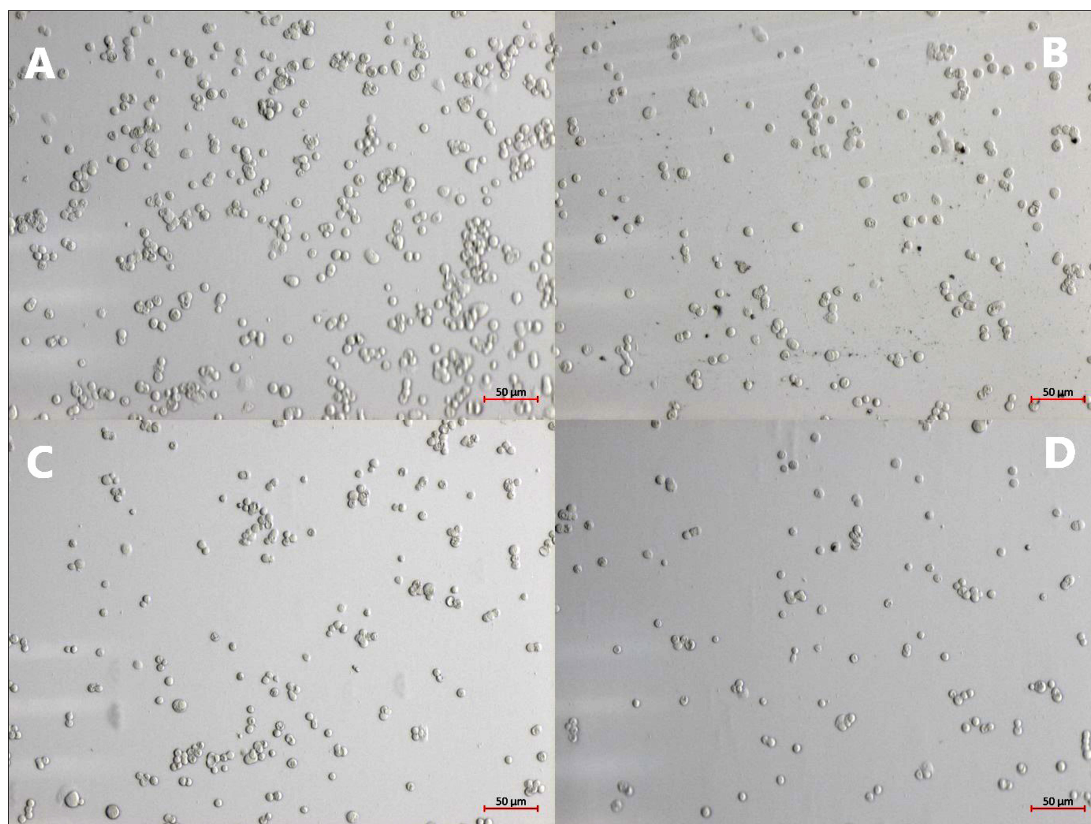

Figure 4. 3rd hour microscopic images of cells on glass surface modified with APTES (dissolved in ethanol) **(a)**. 3rd hour microscopic images of cells on glass surface modified with PDA **(b)**. 3rd hour microscopic images of cells on glass surface modified with APTES (dissolved in water) **(c)**. 3rd hour microscopic images of cells on non-modified glass surface **(d)**.

water. Afterwards, glass surface was treated by PDA and modified successfully. For the purpose of a comparison, untreated glass surfaces were also tested for the same cell culture. At the end of the 3rd hour in static culture, the total area occupied by cells on the modified surfaces was measured with Image J. The results exhibit that the occupied area by the cells are 8.08%, 3.33%, 5.32%, and 2.47% respectively. The experiments were repeated for the surfaces modified by APTES in ethanol, APTES in water, PDA and non-modified surfaces in the same order. After 3 hours, the images of the cells on the modified glass surfaces can be observed in Figure 4. Calculations and Figure 4 show that cell-surface interaction on all surfaces with different modifications is greater than the unmodified surface. Modification with APTES dissolved in ethanol increased cell surface interactions more than other modifications. It is followed by PDA based modification and APTES based modification dissolved in water, respectively.

DISCUSSION

Research studies have also brought new technological advances. In particular, new technological advances in the field of micro-technology have led to the need for new imaging and image processing systems. With inherent advantages such as small sample volume, high sensitivity and fast processing time, microfluidics is well-positioned to serve as a promising platform for applications in oncology (15). Microfluidics; and that labor intensive and time-consuming steps, such as sample preparation, purification, mixing, reactions, separations, and detection, can be carried out in a single monolithic microfabric device and all can be made using nanoliter volumes (16). Microfluidic-based separation techniques possess several advantages including small sample volume, high throughput, sensitivity, and low fabrication cost. An increasing number of microfluidic devices have been applied to study the responses of cancer cells against different drugs and various dosages. For example, Siyan et al. developed a microfluidic gradient generation system to study the drug resistance of human lung cancer cells (17).

The miniaturization, combined with the integration of multiple functionalities that benefit from unique micro-scale events, has led to microfluidic systems that have better performance than macro-scale systems, reduce labor input and have low-cost serial production potential. Since then, the field of microfluidics has opened and is now preparing to influence various fields ranging from chemical synthesis, biological analysis, optics, information technology, forensics and environmental monitoring (16).

This work has covered surface modification techniques for tailoring glass' s surface properties in order to render the material more useful for microfluidic applications. In the modification with MatriGel, cell-glass surface interaction increased as the MatriGel mimicked extracellular matrix proteins. In this way, the adherent cancer cells, which are already adherent to the surface of the glass, showed a confluent behavior. However, it was observed that the area occupied by the cells on the modified surface increased in proportion to the increasing MatriGel

concentrations. Polydopamine (PDA) coatings promote a variety of reactions with organic species for the creation of functional organic ad-layers (12). Compared with the hydrophilic surfaces, the hydrophobic surfaces exhibit stronger adhesion with the PDA coatings (18). this hydrophobic surface is available for cell adhere. PDA used in chemical modification has increased cell-glass surface interaction due to chemical groups. APTES is widely used in surface modification. There are NH_2 groups at the end of spontaneous surface which modified with APTES. This NH_2 groups let the cells attach on the surface.

These surface modifications and patterning approaches have proven to be ideal for applications such as biomolecules separations, immunoassays and cell culture studies. They are also very promising for a paradigm shift in the immobilization of biomolecules in micro-channels for the capture/release of proteins, antifouling and cell cultures. Finally, the relatively new application of emulsion formation within surface-modified microfluidic devices is also receiving considerable attention.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - HU; Design - MEO; Supervision - GCK; Fundings - YB, HU; Materials - GA, SO; Data Collection and/or Processing - GA; Analysis and/or Interpretation - HEM, AK; Literature Search - EO; Writing Manuscript - HEM, AK; Critical Review - GCK

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This work was supported by the TUBITAK 1003 Primary Subject R&D Funding Program. This work was performed by 116E866 project numbered which is sub-project of 15062016 project numbered of large scale project.

REFERENCES

1. Zhang X, Jones P, SHaswell S. Attachment and detachment of living cells on modified microchannel surfaces in a microfluidic-based lab-on-a-chip system. Chem Eng J 2008;135:S82-S88. [CrossRef]
2. Sakamoto C, Yamaguchi N. Yamada M, Nagase H, Seki M, Nasu M. Rapid quantification of bacterial cells in potable water using a simplified microfluidic device. J Microbiol Methods 2007;68:643–647. [CrossRef]
3. Timur S. Protein Analitiği. Bölüm: Protein Chip'ler. Telefoncu A, Kılınç A, editörler. İzmir, Bornova: Ege Üniversitesi Basımevi; 2010.
4. Manz A, Becker H, editors. Microsystem Technology in Chemistry and Life Sciences. Berlin: Springer Verlag; 1998.
5. Zhang Z, Nagrath S. Microfluidics and cancer: are we there yet? Biomed Microdevices 201315:595–609. [CrossRef]
6. Fujii T. PDMS-based microfluidic devices for biomedical applications. Microelectron Eng 2002;61-62:907–914. [CrossRef]
7. Mas Haris MRH, Kathiresan S, Mohan S. FT-IR and FT-Raman spectra and normal coordinate analysis of polymethylmethacrylate. Der Pharma Chem 2010;2:316–323.
8. Prakash S, Long TM, Selby JC, Moore JS, Shannon MA. "Click" Modification of Silica Surfaces and Glass Microfluidic Channels. Anal Chem 2007;79:1661–1667. [CrossRef]
9. Park S, Joo YK, Chen Y. Dynamic adhesion characterization of cancer cells under blood flowmimetic conditions: effects of cell shape and orientation on drag force. Microfluidics Nanofluidics 2018;22:108. [CrossRef]
10. Kleinman HK, Martin GR. Matrigel: basement membrane matrix with biological activity. Semin Cancer Biol 2005;15:378–386. [CrossRef]
11. Lee H, Dellatore SM, Miller WM, Messersmith PB. Mussel-inspired surface chemistry for multifunctional coatings. Science 2007;318:426–430. [CrossRef]
12. Ding YH, Floren M, Tan W. Mussel-inspired polydopamine for bio-surface functionalization. Biosurf Biotribol 2016;2:121–136. [CrossRef]
13. Vansant EF, van Der Voort P, Vrancken KC. Characterization and Chemical Modication of the Silica Surface, Chapter 9. New York: Elsevier; 1995.
14. Blitz JP, Shreedhara Murthy RS, Leyden DE. The role of amine structure on catalytic activity for silylation reactions with Cab-O-Sil. J Colloid Interface Sci 1988;126:387–392. [CrossRef]
15. Chaudhuri PK, Warkiani ME, Jing T, Kenry K, Lim CT. Microfluidics for research and applications in oncology. Analyst 2016;141:504–524. [CrossRef]
16. Chen J, Li J, Sun Y. Microfluidic approaches for cancer cell detection, characterization, and separation. Lab Chip 2012;12:1753–1767. [CrossRef]
17. Siyan W, Feng Y, Lichuan Z, et al. Application of microfluidic gradient chip in the analysis of lung cancer chemotherapy resistance. J Pharm Biomed Anal 2009;49:806–810. [CrossRef]
18. Zhang C, Gong L, Xiang L, et al. Deposition and Adhesion of Polydopamine on the Surfaces of Varying Wettability. ACS Appl Mater Interfaces 2017;9:30943–30950. [CrossRef]