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Cell Attachment and Detachment of PaTu 8988t and PaTu 8988s Cell Lines Using Thermoresponsive Homopolymer Layers

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Abstract

Polymer brushes such as poly (diethylene glycol) methyl ether methacrylate) (PDEGMA) are known to prevent protein adsorption and cell attachment to surfaces treated with initiators such as ω-mercaptoundecyl bromoisobutyrate (MUBiB). They have been found out to display thermoresponsive behavior, which can be tuned by changing the thickness of the polymer brush or altering its grafting density. PDEGMA brushes with a dry ellipsometric thickness of 5.00 ± 1.00 nm change from cell adherent at 37°C to cell non-adherent at 22°C. On the other hand, the irreversible adsorption of fibronectin to the gold surfaces is also dependent on temperature changes, which in turn affects the adhesion properties of the cells even more. PDEGMA brushes swell below its lower critical solution temperature (LCST) due to the absence of paxillin and action containing cellular structures which are responsible for the adhesion of cells. These characteristics of thin homopolymer PDEGMA brushes make them an interesting constituent for enzyme-free culture of cells such as PaTu 8988t and PaTu 8988s.

Keywords: PDEGMA; Thermoresponsive; Cell detachment; PaTu cells; Polymer brushes

Introduction

Polymer brushes have become increasingly significant in surface modification and the nano- and microfabrication of functional biointerfaces. These polymers exhibit unique physicochemical properties, particularly their ability to respond to external stimuli. Such responsiveness enables rapid and reversible alterations in their structure, morphology, surface characteristics, and solubility in response to environmental triggers such as temperature, pH, or ionic strength [1]. This versatility makes polymer brush coatings highly suitable for biomedical applications, including the control of protein adsorption and cell attachment on surfaces. Stimuli-responsive polymer coatings are especially useful for the design of functional biomaterials, medical implants, and disposable biomedical devices [2]. Polymer brushes are thin layers of polymer chains that are covalently anchored to a solid substrate. These can be synthesized using either the "grafting-to" or "grafting-from" approach. In the grafting-to technique, preformed polymers with reactive end groups are attached to functionalized surfaces [3]. However, steric hindrance often limits the achievable grafting density. In contrast, the grafting-from method involves immobilizing initiator groups on the substrate and subsequently growing polymer chains directly from the surface. This approach allows higher chain density and improved uniformity, making it more suitable for applications requiring well-controlled surface properties [4]. Self-assembled monolayers (SAMs) are organized molecular films formed spontaneously on a substrate

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from a surfactant solution. They consist of headgroups with strong affinity for the substrate and tail groups that determine the surface chemistry and functionality. SAMs play a crucial role in facilitating the controlled growth of polymer brushes. In this study, ω-mercaptoundecyl bromoisobutyrate (MUBiB) was employed as the initiator molecule. The thiol group of MUBiB binds strongly to gold surfaces, while the bromoisobutyrate group serves as an initiation site for polymerization [5]. This configuration enables the uniform growth of polymer brushes such as poly (di (ethylene glycol) methyl ether methacrylate) (PDEGMA). Poly (di (ethylene glycol) methyl ether methacrylate) (PDEGMA) is a thermoresponsive polymer known for its reversible conformational transitions near its lower critical solution temperature (LCST), typically around 26°C. Below the LCST, PDEGMA chains collapse, forming a compact hydrophobic layer that discourages protein adsorption and cell attachment [6]. Above the LCST, the polymer becomes hydrated and swollen, promoting cell adhesion. This temperature-dependent switch between hydrophilic and hydrophobic states provides an effective mechanism for controlling cell attachment and detachment, making PDEGMA-coated surfaces attractive for tissue engineering and cell culture applications [7]. The human pancreatic adenocarcinoma cell lines PaTu 8988t and PaTu 8988s are widely used as model systems for studying tumor cell behavior and metastasis. Although both cell lines originate from the same tumor source, they exhibit distinct phenotypic characteristics. PaTu 8988s cells display a more epithelial-like morphology with limited invasive potential, while PaTu 8988t cells are more aggressive and tumorigenic, capable of forming tumors in nude mice. The differential behavior of these two cell lines provides a valuable comparative system for investigating cell–substrate interactions on thermoresponsive polymer layers.

Materials and Methods

Di (ethylene glycol) methyl ether methacrylate (DEGMA), fibronectin, 2,2-bipyridyl, copper (II) sulfate pentahydrate, and sodium bromide were obtained from Sigma-Aldrich. Phosphate-buffered saline (PBS) for cell culture was purchased from Lonza (Belgium). Gold (99.99%, granules) was obtained from Allgemeine Gold und Silberscheideanstalt AG (Pforzheim, Germany). Chloroform, methanol, ethanol, and isopropanol were procured from J.T. Baker and Fisher Scientific. Aluminum oxide (neutral, for column chromatography) was purchased from Macherey-Nagel. Vybrant™ DiO and DiD cell-labelling solutions were obtained from Thermo Fisher Scientific. Milli-Q ultrapure water (18.0 MΩ·cm, Millipore Direct Q8 system, Germany) was used in all aqueous preparations. The initiator, ω-mercaptoundecyl bromoisobutyrate (MUBiB), was synthesized following [8].

Preparation of Self-Assembled Monolayers (SAMs) of MUBiB

Gold substrates were prepared by electron-beam evaporation of a 2 nm titanium adhesion layer followed by 25–80 nm of gold on Schott D263 borosilicate glass (3 cm × 1.5 cm or 2.5 cm × 2.5 cm, thickness 1 mm) under high vacuum (Edwards E306 coating system, UK). The substrates were cleaned sequentially with chloroform, ethanol, and water, then dried under nitrogen. Further cleaning was performed by UV/ozone treatment for 30 min. SAMs were formed by immersing the cleaned gold substrates overnight in a 1 mM MUBiB solution in absolute ethanol at room temperature, followed by rinsing with ethanol and drying under nitrogen [9].

Synthesis of PDEGMA Polymer Brushes

PDEGMA polymer brushes were synthesized via surface-initiated atom transfer radical polymerization (SI-ATRP). Purified DEGMA monomer (3.08 g, 16.7 mmol) was passed through an alumina column to remove inhibitors. In a 100 mL round-bottom flask, 3 mL of Milli-Q water and 12 mL of methanol were mixed with bipyridine (312 mg, 2.0 mmol) and stirred under argon for 45 min. CuBr (143 mg, 1.0 mmol) was then added, and the mixture was bubbled with argon for 15 min to remove oxygen. The resulting catalyst solution was transferred via a metal cannula to the polymerization flask containing the MUBiB-modified gold substrates under an argon atmosphere at 23°C [9,10]. After the desired polymerization time, the reaction was quenched by transferring the mixture into Milli-Q water. The substrates were rinsed thoroughly with Milli-Q water and ethanol and dried with nitrogen.

Ellipsometry

Ellipsometric measurements were performed using an alpha-SE ellipsometer (J.A. Woollam Co., USA) at incidence angles of 65°, 70°, and 75° with wavelengths between 380–900 nm. The layer thickness was determined using a two-layer Cauchy model, assuming a refractive index of 1.52 for the polymer layer at 632.8 nm [11]. Each sample was measured at three different locations, and the mean thickness was recorded.

Contact Angle Measurements

Static water contact angles were determined at room temperature (22°C) using an OCA-15 contact angle goniometer (Dataphysics, Germany) by the sessile drop method. A 2 µL droplet of Milli-Q water was placed on the surface for each measurement [12]. The mean and standard deviation were calculated from three measurements per sample.

X-Ray Photoelectron Spectroscopy (XPS)

XPS analyses were conducted using an SSX-100 S-probe

Akther K, et al., Arch Vet Sci Med 2026
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spectrometer (Surface Science Instruments, USA) with Al Kα X-ray radiation (200 W). Survey spectra (0–1200 eV) were recorded with a 1.0 eV resolution, while high-resolution spectra were collected at 0.1 eV resolution. The C1s peak at 285.0 eV was used for charge referencing. Data was processed with CasaXPS software (v2.3.16) [13].

Effect of Polymerization Time on PDEGMA Brush Thickness

Polymerization times were varied (5, 10, 15, 20, 30, 45, and 60 min) under identical reaction conditions to study their effect on brush thickness. At each point, samples were removed, washed, and dried before ellipsometric thickness measurement [14].

Cell Culture

Human pancreatic adenocarcinoma cell lines PaTu 8988t and PaTu 8988s were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% fetal bovine serum, 5% horse serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine (Thermo Fisher Scientific). Cells were maintained at 37°C in a humidified incubator with 5% CO_2 [15].

Temperature-Responsive Cell Attachment and Detachment

Gold substrates with SAM and PDEGMA coatings were sterilized in 70% ethanol for 1 h and coated with fibronectin (10 µg/mL in PBS) for 1 h before cell seeding. PaTu 8988t and PaTu 8988s cells were seeded at densities of 1×10^5 cells/cm² (and additionally 5×10^4 cells/cm² for PaTu 8988s) on both PDEGMA-coated substrates and control tissue culture polystyrene (TCPS) surfaces. After incubation at 37°C, cells were cooled to 22°C for 30 min to induce detachment. Cell morphology was observed using a Zeiss Primovert light microscope equipped with an AxioCam ERc5s camera. Detached cells were collected, reseeded onto TCPS dishes, and cultured for an additional 24 h for viability assessment. Images were analyzed using Zeiss Zen software [16].

MTT Cell Viability Assay

Cell viability was assessed using the Vybrant™ MTT Cell Proliferation Assay Kit (Life Technologies) following the manufacturer's protocol. After 4 h incubation with MTT, formazan crystals were solubilized with sodium dodecyl sulfate and incubated for 18 h. Absorbance was measured at 570 nm using a Varian Cary 50 BioSpectrometer (Agilent, USA). Green fluorescence indicated viable cells, while red fluorescence indicated non-viable cells [16].

Quantitative Analysis of Cell Number and Size

Cell numbers were quantified using a Neubauer Improved counting chamber after detachment with trypsin. For temperature-dependent morphology studies, images of defined 1 cm² regions were analyzed using Zen software to measure average cell area before and after cooling to 22°C. The mean values from multiple regions were calculated to assess cell size changes.

Results and Discussion

Characterization of MUBiB and PDEGMA Layers

XPS Analysis of MUBiB and PDEGMA Brushes

The XPS spectra of the gold with MUBiB and 5.00 ± 1.00 nm PDEGMA was measured as described in Section 2.6.

The formation of the PDEGMA brushes on gold was verified by XPS. The XPS survey spectra (Figure 1) showed the presence of the C1s and O1s signals at 286 and 533 eV, which agreed well with data from literature [17]. The high-resolution scan of the C1s region shows C1s signals that can be attributed to the carbon of the following groups: O-C=O 289.2 eV and C-O 287.7 eV.

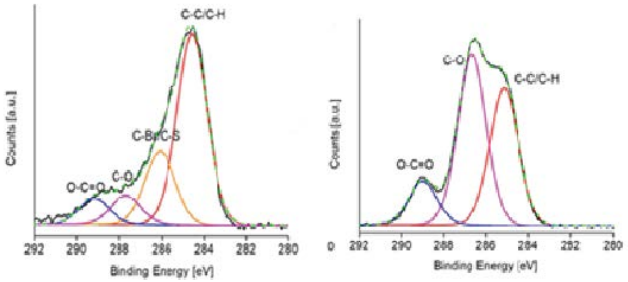

Figure 1: XPS high resolution C1s spectra of: a) MUBiB layer, and, (b) 5 nm PDEGMA brush (peaks are assigned at O-C=O (289.2 eV, blue), C-O (287.7 eV, pink), C-Br/C-S (286.1 eV, orange), and C-C/C-H (285.0 eV, red); dashed green line indicates the fitted peaks).

Thickness Determination of MUBiB layer and PDEGMA Brushes

SAMs of MUBiB were prepared on precleaned gold substrates as described in the Experimental Section. The dry ellipsometric thickness of the MUBiB layer on gold substrate was measured using the ellipsometer, which was approximately 1.00 ± 0.02 nm. This result agrees well with data in the literature [18]. PDEGMA brushes were synthesized on MUBiB modified gold substrates and the polymerization times were varied accordingly, that is, 5 min, 10 min, 15 min, 20 min, 30 min, 45 min and 60 min. The dry ellipsometric thicknesses of PDEGMA brushes were measured and the results were plotted as shown in Figure 4. The error bars represent the standard deviation of each reading.

As shown in Figure 2, the dry thickness of PDEGMA layers increases linearly with polymerization time. This is in line with the previous work of Tranchida et al [19]. The longer the gold substrate is treated with the polymer solution,

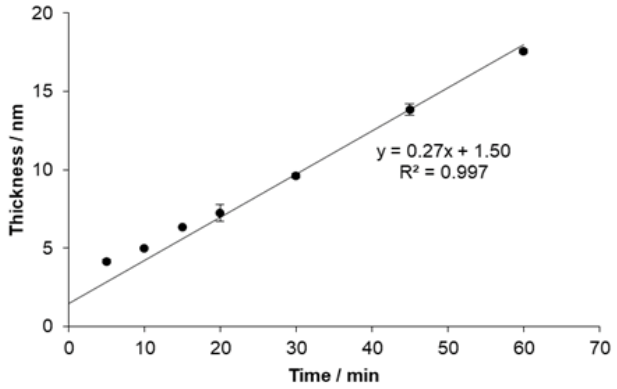

Figure 2: Ellipsometric dry thickness of PDEGMA layers versus polymerization time.

the higher is the extent of polymerization reaction between them and this the thickness of the polymer layer increases with time. According to Jiang et. al, the dry ellipsometric thickness of the PDEGMA layer was confirmed to be around 5.00 ± 1.00 nm, which was previously determined from experiments where the apparent transition temperature for the adsorption of the protein bovine serum albumin (BSA) was found out to be around 32°C [20]. So, the cell cultures were carried out at an optimum temperature of 35°C. From the graph above, the polymerization time for a thickness of 5 nm was estimated to be approximately 10 min. Thus, the polymerization of PDEGMA on the substrates to be later used for cell culture were carried out for 10 min.

Contact Angle Measurements

The static water contact angles of MUBiB and 5.00 ± 1.00 nm PDEGMA on the gold substrates were measured. Three different positions on each sample were measured.

Table 1: Contact Angles of MUBiB and PDEGMA on gold

	Contact angle of respective layer on gold						
	1	SD	2	SD	3	SD	Average
MUBiB	81.5°	0.2	81.5°	0.2	83.2°	0.37	82.1°
PDEGMA	50.0°	0.13	49.1°	0.17	49.8°	0.07	49.6°

As can be seen in Table 1, it is observed that the presence of the initiator layer increases the contact angle of the gold surface with water and decreases its wettability. This is because the thiol group present in the MUBiB molecule is highly hydrophobic. However, the presence of PDEGMA layer on the MUBiB modified gold substrate decreases the contact angle significantly, thus increasing the wettability of the gold surface. The PDEGMA layer is more hydrophilic than MUBiB due to the presence of the ester bonds in the structure of the polymer. The change in contact angles proves the reaction that takes place between the MUBiB and the PDEGMA layer.

Cell Cultures on PDEGMA Brushes

PaTu 8988t were seeded at 100,000 cells/cm^3 medium and for PaTu 8988s cells, 100,000 cells/cm^3 and 50,000 cells/cm^3 medium were seeded on PDEGMA brushes with dry ellipsometric thickness of 5.00 ± 1.00 nm and on TCPS for 24 h. The cell morphology of the PaTu 8988t and PaTu 8988s cells were investigated after 24 h cell cultivation at standard culture conditions (37°C, 5% CO_2). As in the light microscopy images (Figure 3), PaTu 8988t cells were attached and spread on both PDEGMA and TCPS (as a reference culture condition).

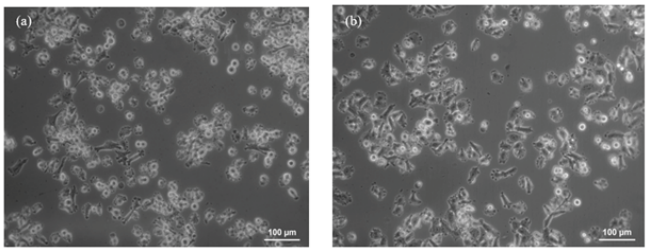

Figure 3: Light microscopy images of PaTu 8988t cells cultured on (a) PDEGMA layer and (b) TCPS after 24 h cell incubation at standard culture conditions (37°C, 5% CO2).

Figure 3 shows typical light microscopy images obtained from PaTu 8988t cells on PDEGMA and TCPS after 24 h. Analysis of few images using the software Zen shows that approximately three times more cells were attached on TCPS than on PDEGMA. This shows that PaTu 8988t cells show considerable spreading on PDEGMA at this seeding number and thickness of PDEGMA, whereas the cells showed more adherence on TCPS than on PDEGMA. This is because TCPS is treated with plasma. This replaces the hydrophobic phenyl groups in untreated polystyrene with hydrophilic carbonyl, hydroxyl or amine containing functional groups that make it more suitable for cell adhesion [21]. So, TCPS is used as a control to compare cell adherence with other polymer brushes.

Detachment of PaTu 8988t Cells on PDEGMA via Temperature Reduction

Cell detachment of PaTu 8988t cells was observed on PDEGMA by light microscopy (Figure 4) after 30 min incubation at 22°C.

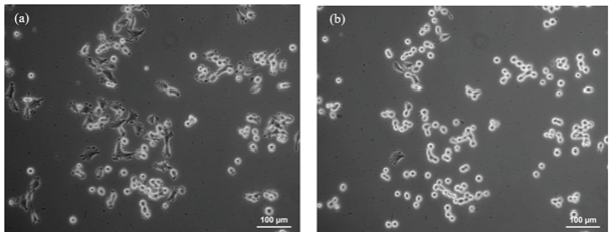

Figure 4: Light microscopy images of PaTu 8988t cells cultured on PDEGMA after 24 h cell incubation at standard culture conditions (37°C, 5% CO2) at: (a) 37°C, and (b) 22°C.

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As can be seen in Figure 4, the cells adhere, spread and proliferate on the PDEGMA layer at 37°C after 24 h incubation at standard conditions and after 30 min at 22°C, more than 90% of the cells get rounded up as shown above. However, there are several rounded-up cells in Figure 6 (a). A possible explanation for this can be the overlapping of other cells on the spread cells, which causes them to appear rounded-up in the light microscope. This is because at higher temperatures, the protein fibronectin causes the gold surface to become more cell adherent. The polymer brushes are in a collapsed state, which increases their grafting density and causes the cells to get adhered to their surfaces, whereas at lower temperatures, especially below the LCST, the polymer brushes extend to an elongated state. This change in polymer conformation and reduced functionalization of fibronectin cause the adhered cells to get released from the brushes after rinsing with cell culture medium, thus resulting in the rounded-up appearance.

Detachment of PaTu 8988t Cells on TCPS via Temperature Reduction

PaTu 8988t cell attachment on TCPS was observed by light microscopy at 37°C and 22°C (Figure 5).

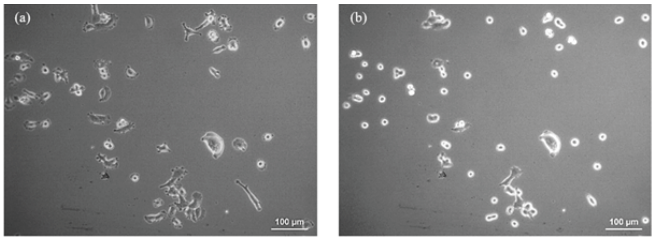

Figure 5: Light microscopy images of PaTu 8988t cells cultured on TCPS after 24 h cell incubation at standard culture conditions (37°C, 5% CO2) at: (a) 37°C, and (b) 22°C

As can be seen in Figure 5, the cells adhere, spread and proliferate on the TCPS at 37°C after 24 h incubation at standard conditions and after 30 min at 22°C, more than 90% of the cells get rounded up as shown above. This contrasts to the results obtained from the PDEGMA brushes above in Figure 3 because of the absence of the PDEGMA polymer brush layer. On TCPS, similarly, fibronectin causes more adsorption at higher temperatures, whereas at lower temperatures, it releases the cells, so the cells are detached and result in the rounded-up appearance as shown above.

PaTu 8988t Cell Detachment on PDEGMA After Washing

The PaTu 8988t cells were liberated from the polymer brushes by mild washing after 30 min incubation at 22°C, the supernatant was collected and reseeded on a new TCPS dish. The remaining cells and released cells were imaged with a light microscope after 24 h incubation at standard conditions and the images were shown in Figure 6.

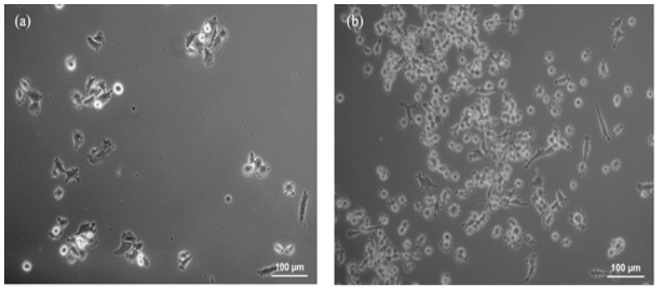

Figure 6: Light microscopy images of PaTu 8988t cells after cultured on PDEGMA after 24 h cell incubation at standard culture conditions (37°C, 5% CO2): (a) remaining on PDEGMA, and (b) in supernatant washed from PDEGMA, after washing.

Figure 6(a) shows that, most of the cells were released from PDEGMAafter rinsing step at 22°C. As a result, after washing, only a few remaining cells were attached to the PDEGMA after 24 h. On the other hand, most of the detached cells can be observed attached to the new TCPS dish (Figure 6 (b)). The rounded-up appearance of the numerous cells in this image proves that due to presence of too many cells in the medium, some of them overlap the adhered cell and appear to be rounded-up under the microscope.

PaTu 8988t Cell Detachment on TCPS After Washing

The PaTu 8988t cells were detached from the TCPS after mild rinsing step at 22°C, the supernatant was collected and reseeded on a new TCPS dish and then incubated for 24 h. The resultant images are shown in Figure 7.

In Figure 7(a), most of the cells remained on the TCPS after rinsing step. The remaining cells were adhered and spread on TCPS and the area captured had PaTu 8988t cells growing again on the culture dish after 24 h. The cells prefer to remain attached to TCPS because of the TCPS characteristics described earlier. On the other hand, the supernatant from this dish showed almost no attachment to the new TCPS dish after 24 h (cf. Figure 7(b)). This further proves that negligible number of cells were released during the washing step from TCPS.

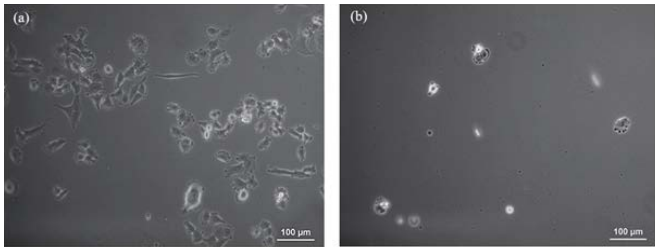

Figure 7: Light microscopy images of PaTu 8988t cells after cultured on TCPS after 24 h cell incubation at standard culture conditions (37°C, 5% CO_2): (a) remaining on TCPS, and (b) in supernatant washed from TCPS, after washing.

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Akther K, et al., Arch Vet Sci Med 2026
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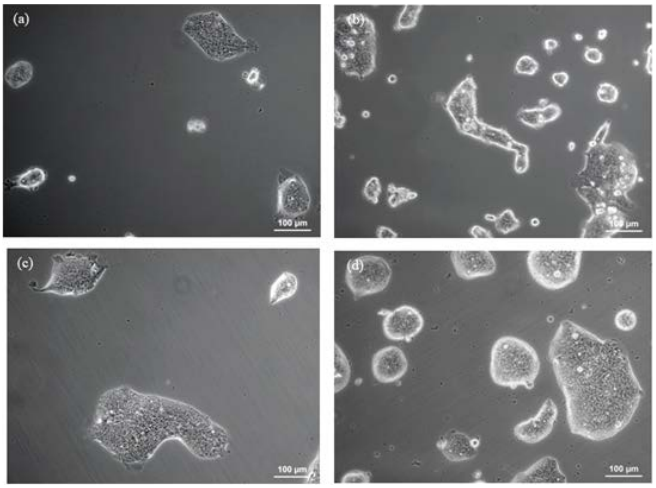

Figure 8: Light microscopy images of PaTu 8988s cells seeded at 50,000 cells/cm3 after 24 h cell incubation at standard culture conditions (37°C, 5% CO2) on: (a) PDEGMA after 24 h, (b) PDEGMA after 48 h, (c) TCPS after 24 h, and (d) TCPS after 48 h.

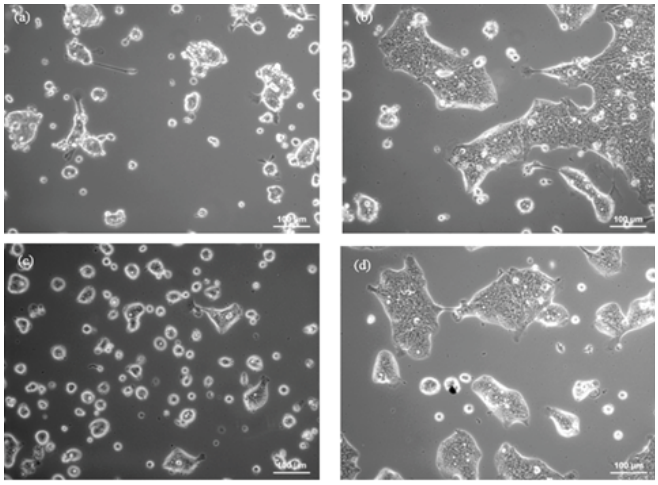

Figure 9: Light microscopy images of PaTu 8988s cells seeded at 100,000 cells/cm3 after 24 h cell incubation at standard culture conditions (37°C, 5% CO2) on: (a) PDEGMA after 24 h, (b) PDEGMA after 48 h, (c) TCPS after 24 h, and (d) TCPS after 48 h.

PaTu 8988s Cell Culture on TCPS and PDEGMA Brushes

In a similar way as PaTu 8988t cells, the PaTu 8988s cells were cultured on the PDEGMA and TCPS surfaces (seeding number of 50,000 cells/cm^3 and 100,000 cells/cm^3 medium) were photographed after 24 h and 48 h incubation at 37°C using the light microscope. The number of cells adhered to PDEGMA and TCPS were observed from the photos above of this specific area. The average areas of the cells in a specific area of the pictures were analyzed using the software ZEN Lite from ZEISS Microscopy. The pictures below show the results obtained.

PaTu 8988s cells were imaged with a light microscope after 24 h and 48 h at standard conditions. Figure 8(a) shows few cells getting attached to the PDEGMA layer. The cell-cell interaction on the surface shows that PaTu 8988s cells grow as colonies on surfaces. The cells interact with each other and adhere to the polymer brush and proliferate on it. However, the density of these colonies was very low and only a couple of attached colonies of cells were observed at a seeding number of 50,000 cells/cm^3 medium after 24 h. So, the incubation was continued for another 24 h and the dishes were observed again for changes. Figure 8(b) shows the cells after 48 h and as expected, the cells proliferate more but the cells continue to adhere to each other and cluster but this time in larger sizes. The morphology of PaTu 8988s cells was similar on TCPS and 5 nm PDEGMA. From the software, it was calculated that the average areas of the colonies on TCPS were more than three times that on PDEGMA. After 48 h, the number of cell colonies on TCPS becomes double but the size of the colonies remains approximately the same. This is because the cells proliferate and spread more on TCPS, but the cells do not form any more clusters.

The images above obtained for a seeding number of 100,000 cells/cm3 of PaTu 8988s cells show that at a higher seeding density, the cells are extensively clustered together. However, qualitative analysis states that after 48 h, the growth of these cells rapidly increases and almost 70% of the area of the culture dish is covered by these cells, as analyzed using the software. This proves that the cell culture of PaTu 8988s cells are suitable for both higher and lower seeding numbers. Additionally, they grow and proliferate more slowly than PaTu 8988t cells because PaTu 8988t grow as single cells but PaTu 8988s grow as colonies so they require more time growth. As a result, the analysis is more feasible after culturing them for 48 h.

Detachment of PaTu 8988ts Cells on PDEGMA and TCPS via Temperature Reduction

The temperature-triggered cell detachment was also observed for PaTu 8988s cells on PDEGMA at seeding numbers of 50,000 cells/cm^3 and 100,000 cells/cm^3 medium by light microscopy at 37°C immediately after taking out from the incubator and after 30 min until the temperature reaches 22°C. The morphology of the PaTu 8988s cell on PDEGMA were observed at 37°C and 22°C under the light microscope (Figure 12). The surface areas of the cell colonies were analysed using the software and the readings were recorded.

The images above show that in contrast to PaTu 8988t cells, the morphology and detachment of colonies of PaTu 8988s cells are not triggered by changes in temperature. The analysis of the average areas of the cells in a specific area shows that the area of the cell clusters adhered to the PDEGMA surface decreases by approximately 10 ± 2% for both seeding densities. This means that after 48 h, the cells

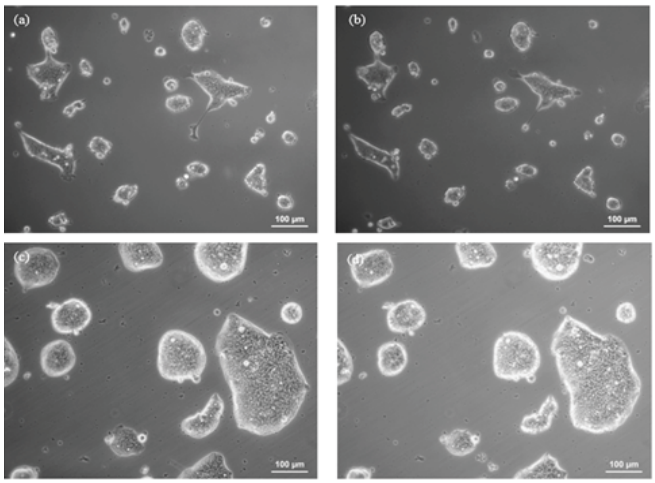

Figure10: Light microscopy images of PaTu 8988s cells seeded at 50,000 cells/cm3 after 24 h cell incubation at standard culture conditions (37°C, 5% CO2) on: (a) PDEGMA at 37°C, (b) PDEGMA at 22°C, (c) TCPS at 37°C, and, (d) TCPS at 22°C, after 48 h

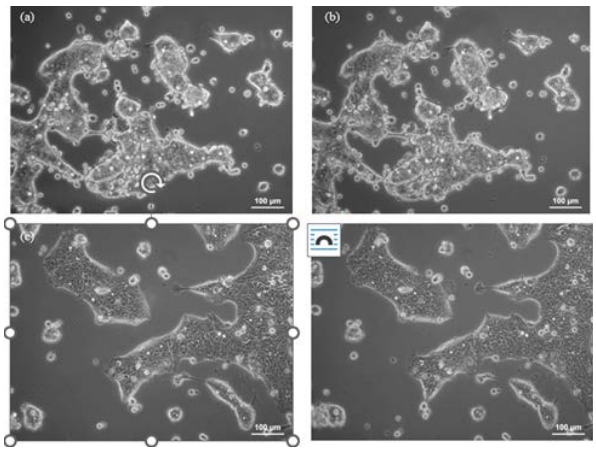

Figure 11: Light microscopy images of PaTu 8988s cells seeded at 100,000 cells/cm3 after 24 h cell incubation at standard culture conditions (37°C, 5% CO2) on: (a) PDEGMA at 37°C, (b) PDEGMA at 22°C, (c) TCPS at 37°C, and (d) TCPS at 22°C, after 48 h.

are in the process of getting released from the surface and that PaTu 8988s cells, when adhered to PDEGMA polymer brushes, show temperature dependence to a small extent and thus start shrinking.

However, on TCPS, the change in area of the cell colonies was negligible, they prefer to remain attached to the TCPS surface because of the characteristics of TCPS described earlier. As shown in Figure 11, it is clear that at a seeding density of 100,000 cells/cm^3 medium, the cell colonies are too closely packed together, which means that with incubation, the cell colonies grow and interact with adjacent colonies.

PaTu 8988s Cell Detachment on PDEGMA After Washing

The released PaTu 8988s cells from the polymer brushes at both seeding densities after 30 min incubation at 22°C were obtained by mild washing with cell medium; the supernatant was collected and reseeded on a new TCPS dish. Both dishes were incubated at standard culture conditions for 24 h, then photographed under the light microscope and images were captured as shown in Figure 12.

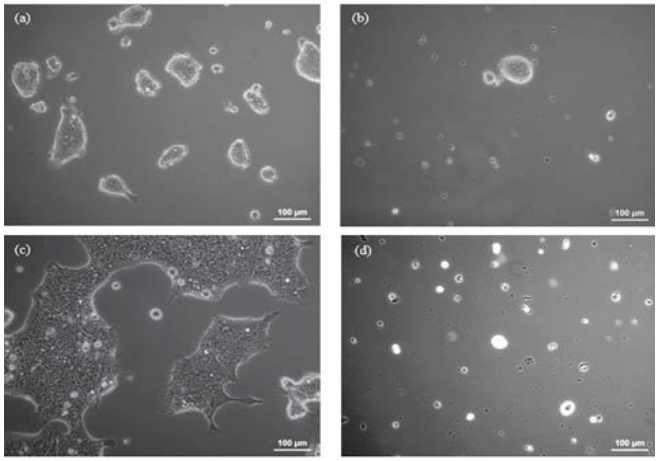

Figure 12: Light microscopy images of PaTu 8988s cells after 24 h with a seeding number 50,000 cells/cm^3 medium: (a) remaining on PDEGMA, (b) in supernatant washed from PDEGMA, and, with a seeding number 100,000 cells/cm^3 medium, (c) remaining on PDEGMA, and (d) in supernatant washed from PDEGMA, after washing.

Figure 12(a) shows that after cooling to 22°C, cell detachment on PDEGMA was not significant. Thus, Figure 12(b) shows almost no released cells on the fresh TCPS dish after 24 h and most of the cells were still remaining on the PDEGMA layer after rinsing step. It is possible that most of the detached cells were floating in the supernatant (Figure 12(b)) but they were not visible under the microscope. The results were similar at a higher seeding number, where the cell clusters were observed to interact with each other more (Figure 12(a)). After 24h of cell reseeding, the detached cells floating in the in the TCPS dish (Figure 12(b)) prove that the cells which were detached and remain floating in the medium and do not adhere to the TCPS after 24 h and none of the cells grew on the TCPS dish. A possible explanation for this result is that the cells may be dead after washing and do not adhere to the TCPS dish anymore because after 72 h, the nutrients in the medium may not have been enough to sustain the cells.

PaTu 8988s Cell Detachment on TCPS After Washing

The released PaTu 8988s cells from the TCPS at both seeding densities after 30 min incubation at 22°C were obtained by mild washing with cell medium; the supernatant was collected and reseeded on a new TCPS dish. Both dishes were incubated at standard culture conditions for 24 h, then photographed under the light microscope and images were captured as shown in Figure 13.

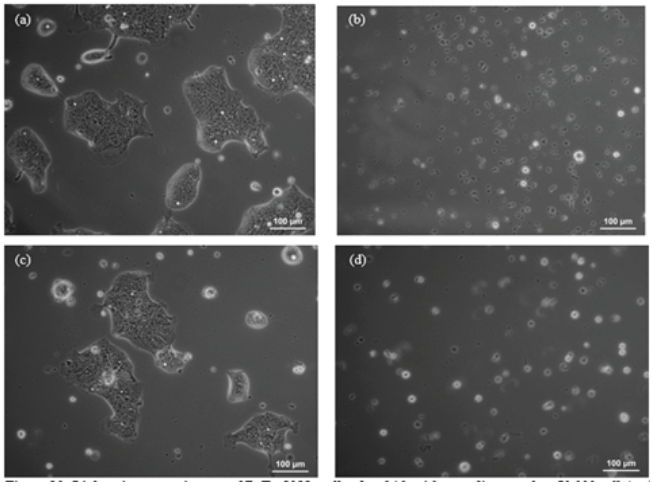

Figure 13: Light microscopy images of PaTu 8988s cells after 24 h with a seeding number 50,000 cells/cm3 medium: (a) remaining on TCPS, (b) in supernatant washed from TCPS, and, with a seeding number 100,000 cells/cm3 medium, (c) remaining on TCPS, and (d) in supernatant washed from TCPS, after washing.

As shown in Figure 13(a) and 13(c), most of the cells were still remaining on TCPS after rinsing with cell medium. The cells prefer to remain attached to TCPS as explained earlier. On the other hand, the number of attached cells on fresh TCPS was negligible after cell reseeding and 24h incubation at (Figure 13(b) and 13(d)).

Viability Test of PaTu 8988t Cells

The PaTu 8988t cells cultured were stained according to the method described in the Experimental Section 2.9 and observed under the fluorescence microscope. The pictures below show the results right after taking the culture dishes out of the incubator, at 37°C, and after cooling to 22°C, where green indicates living cells and red indicates dead cells. The same experiment was repeated for the cells grown on TCPS.

The pictures above show that after a 24 h culture, most of the cells on PDEGMA at 37°C are alive because almost 70% of the detected cells show green colour. However, there are quite a reasonable number of red spots which indicate that few cells may have died due to lack of nutrition and suitable environmental conditions or inability to attach to the polymer brushes. Figure 14(b) shows that at 22°C, most of the cells had died already, so more than 80% of the cells showed red colour. Thus, a temperature of 37°C and controlled oxygen content in the atmosphere was required for the viability of PaTu 8988t cells.

Figure 15 shows the live and dead cells which were remaining on PDEGMA after the washing step. The picture on the left side shows that after washing, mostly dead cells remained attached to the PDEGMA layer, which proves that most of the cells had been released away during the washing step. Only dead cells remained back on the polymer brushes, thus the several red spots. Figure 15(b) shows that the medium that was washed away from the PDEGMA contained all the living cells, so several faint green spots were visible from this TCPS dish. So, it can be concluded that PaTu 8988t cells get released when the temperature is reduced to 22°C or below room temperature.

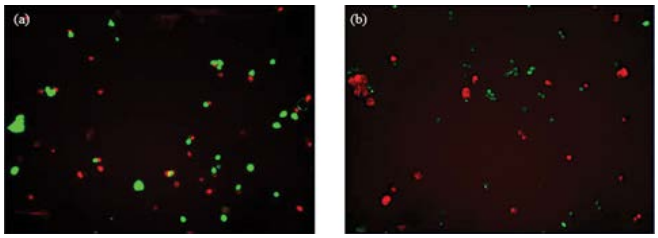

Figure 14: Live-dead staining of PaTu 8988t cells on PDEGMA at: (a) 37°C, and (b) 22°C.

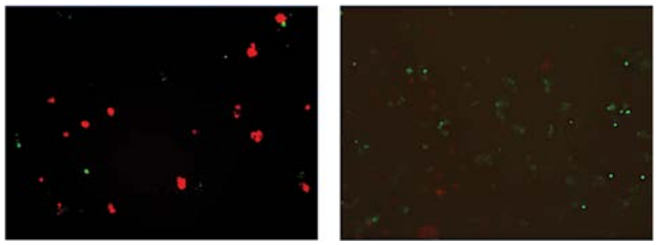

Figure 15: Live-dead staining of PaTu 8988t cells: (a) remaining on PDEGMA, and (b) in supernatant washed from PDEGMA, after washing

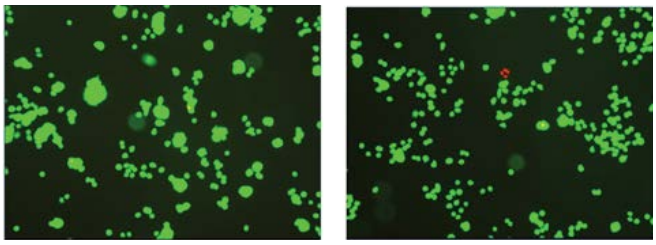

Figure 16: Live-dead staining of PaTu 8988t cells on TCPS at: (a) 37°C, and (b) 22°C.

For comparison, the staining was carried out on the TCPS cultures as well. Figure 16 shows that most of the cells preferred to remain attached to TCPS at 37°C as well as at 22°C. As a result, almost 99% of the spots observed in the pictures above are green. So, it can be concluded that the cells prefer to stay live and attached to TCPS layer and get released only when the temperature reduces to below the LCST of polystyrene.

The pictures above show that when PaTu 8988t cells are washed from TCPS, only dead cells stay attached to the TCPS (Figure 17(b)). This was because most of the live cells got washed away with the supernatant. As a result, several green spots were observed in the supernatant medium (Figure 17(b)).

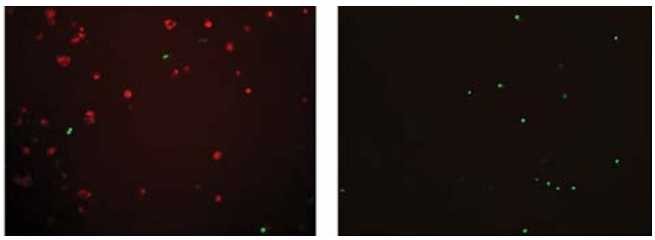

Figure 17: Live-dead staining of PaTu 8988t cells: (a) remaining on TCPS, and (b) in supernatant washed from TCPS, after washing.

Conclusion

The experiments concluded that PDEGMA brushes with a dry ellipsometric thickness of 5.00 ± 1.00 nm showed optimum cell adhesion and displayed thermoresponsive behaviour from cell-adherent at 37°C to cell non-adherent at 22°C. This is consistent with the temperature-dependent irreversible adsorption of fibronectin from PBS and other proteins present in the cell culture medium. At 37°C, PaTu 8988t cells show pronounced filaments and proliferation on substrates treated only with fibronectin. These filaments are due to cellular features such as paxillin and actin causing adsorption on polymer brushes and TCPS. The cell viability was also feasible for the cell cultures on PDEGMA brushes because the extent of dead cells was negligible. So, PDEGMA brushes can be used as an alternative thermoresponsive layer for the long-lived cell culture or enzyme-free cell culture systems.

References

1. Ayres N. Polymer brushes: Applications in biomaterials and nanotechnology, Polym. Chem 1 (2010): 769-777.
2. Kumar, A.; Srivastava, A.; Galaev, I. Y.; Mattiasson, B. Smart polymers: Physical forms and bioengineering applications, Prog. Polym. Sci 32 (2007): 1205-1237.
3. Zapotoczny S. Stimuli responsive polymers for nanoengineering of biointerfaces. Nanotechnology in Regenerative Medicine: Methods and Protocols 811 (2011): 51-78.
4. Kim P, Kim DH, Kim B, et al. Fabrication of nanostructures of polyethylene glycol for applications to protein adsorptionand cell adhesion. Nanotechnology 16 (2005): 2420.
5. Lilge I. Introduction to Polymer Brushes. Polymer Brush Films with Varied Grafting and Cross-Linking Density via SI-ATRP; Springer Fachmedien Wiesbaden: Wiesbaden (2017): 27-36.
6. Großhans S, Lilge I, Schönherr H. Detailed Analysis of Pancreatic Tumor Cell Attachment on Gradient PDEGMA Brushes. Macromol. Biosci 18 (2018): 1700317.
7. Wischerhoff E, Uhlig K, Lankenau A, et al. Controlled cell adhesion on PEG-based switchable surfaces. ANGEWANDTE CHEMIE-INTERNATIONAL EDITION IN ENGLISH-.47 (2008): 5666.
8. Magnusson JP, Khan A, Pasparakis G, et al. Ion-sensitive "isothermal" responsive polymers prepared in water. Journal of the American Chemical Society 130 (2008): 10852-10853.
9. Elsässer H P, Lehr U, Agricola B, et al. Establishment and Characterisation of Two Cell Lines with Different Grade of Differentiation Derived from One Primary Human Pancreatic Adenocarcinoma. Virchows Archiv B Cell Pathol 61 (1992): 295-306.
10. Jellison Jr, G E. Encyclopedia of Spectroscopy and Spectrometry (1999).
11. Dimitrov A S, Kralchevsky P A, Nikolov A D, et al. Contact Angle Measurements with Sessile Drops and Bubbles. Journal of Colloid and Interface Science 145 (1991): 279-282.
12. Engelhard M H, Droubay T C, Du Y. X-Ray Photoelectron Spectroscopy Applications. In Encyclopedia of Spectroscopy and Spectrometry; Elsevier (2017): 716-724.
13. Mather RR. Surface modification of textiles by plasma treatments. InSurface modification of textiles (2009): 296-317.
14. Gunning, P. A. Light Microscopy: Principles and Applications to Food Microstructures. In Food Microstructures; Elsevier (2013): 62-95.
15. Ogundele O M, Adeshina A, Adeniyi P, et al. Basic Principles of Fluorescence Microscopy (2013).
16. Lilge I, Steuber M, Tranchida D, et al. Tailored (Bio) interfaces via surface initiated polymerization: Control of grafting density and new responsive diblock copolymer brushes. InMacromolecular Symposia 328 (2013): 64-72.
17. Voß Y, Wassel E, Jiang S, et al. Thin Poly(Di(Ethylene Glycol) Methyl Ether Methacrylate) Homopolymer Brushes Allow Controlled Adsorption and Desorption of PaTu 8988t Cells. Macromol. Biosci 17 (2017): 1600337.
18. Canepa M, Maidecchi G, Toccafondi C, et al. Spectroscopic Ellipsometry of Self Assembled Monolayers: Interface Effects. The Case of Phenyl Selenide SAMs on Gold. Phys. Chem. Chem. Phys 15 (2013): 11559.
19. Tranchida D, Sperotto E, Staedler T, et al. Nanomechanical properties of oligo(ethylene glycol methacrylate) polymer brush-based biointerfaces, Adv. Eng. Mater 13 (2011): B369-B376.
20. Jiang S, Müller M, Schönherr H. Toward Label-Free Selective Cell Separation of Different Eukaryotic Cell Lines Using Thermoresponsive Homopolymer Layers. ACS Appl. Bio Mater 2 (2019): 2557-2566.
21. Lerman M J, Lembong J, Muramoto S, et al. The Evolution of Polystyrene as a Cell Culture Material. Tissue Engineering Part B: Reviews 24 (2018): 359-372.

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