Journal of Adhesion Science and Technology

ISSN: 0169-4243 (Print) 1568-5616 (Online) Journal homepage: http://www.tandfonline.com/loi/tast20

Influence of incubation time and action of fluid flow on SPEV cell adhesion strength to a glass substrate

A. F. Todrin, O. V. Timofeyeva & T. P. Petrenko

To cite this article: A. F. Todrin, O. V. Timofeyeva & T. P. Petrenko (2018) Influence of incubation time and action of fluid flow on SPEV cell adhesion strength to a glass substrate, Journal of Adhesion Science and Technology, 32:16, 1838-1848, DOI: 10.1080/01694243.2018.1448500

To link to this article: https://doi.org/10.1080/01694243.2018.1448500

JOURNAL OF ADHESION SCIENCE AND TECHNOLOGY, 2018
VOL. 32, NO. 16, 1838–1848
https://doi.org/10.1080/01694243.2018.1448500

Taylor & Francis
Taylor & Francis Group

Influence of incubation time and action of fluid flow on SPEV cell adhesion strength to a glass substrate

A. F. Todrin[a], O. V. Timofeyeva[a] and T. P. Petrenko[b]

[a]Department of Low Temperature Preservation, Institute for Problems of Cryobiology and Cryomedicine NAS of Ukraine, Kharkiv, Ukraine; [b]Department of Cryomicrobiology, Institute for Problems of Cryobiology and Cryomedicine NAS of Ukraine, Kharkiv, Ukraine

ABSTRACT

The results of study of SPEV cell adhesion to the glass substrate that were obtained with a radial-flow chamber shown that in some cases the cells which were incubated on the substrate for a longer time were more strongly attached to it, and under certain conditions such cells detached easier than cells with a shorter incubation time. For example, when the channel height was decreased more of cells were detached from substrate with increasing incubation time. Besides an increase of flow action time on the cells increased an amount of detached cells, and an increase of initial shear stress resulted in decrease an amount of cells which detached from the surface regardless of incubation time. During each experiment more spread cells detached from the substrate earlier than the more spherical one. When both the flow rate and channel height were reduced in the radial-flow chamber, but the initial shear stress was the same as before the changes of these parameters, then the critical radius, the critical shear stress and the time of reach these critical values sharply decreased. In addition, in this case, the area of the projection of cells remaining on the substrate also decreased.

ARTICLE HISTORY
Received 11 August 2017
Revised 31 January 2018
Accepted 23 February 2018

KEYWORDS
SPEV cells; adhesion strength; incubation time; flow action time; initial shear stress; critical radius; critical shear stress; cell projection area

1. Introduction

Cell adhesion plays an important role in many biological processes such as cell growth, differentiation, mobility, apoptosis, formation and regeneration of tissues. Therefore, understanding basic principles of interaction force regulation of cell-cell and cell-substrate has great importance for medicine, biology and bioengineering.

To study adhesion strength of a large number of cells simultaneously one most commonly applies the methods of fluid flow action on the cells. Under the action of hydrodynamic force cells detach from the substrate and it is registered with a microscope during the experiment or after its completion The magnitude of this force can be easily regulated by changing the flow rate [1]. However, the application of hydrodynamic force does not allow directly to determine the strength of cell adhesion to the substrate. Therefore, as the determining

CONTACT A. F. Todrin altodrin@gmail.com, cryo@online.kharkov.ua

parameter, one as a rule applies a value of critical shear stress. At a smaller value of shear stress the cells do not detach from the surface of substrate with the fluid flow.

The hydrodynamic forces are applied to the cells in special flow chambers where a controllable flow of fluid is created in channels along the surface with the cells. Among flow chambers main types are parallel-plate flow chambers [2,3] and radial-flow chambers [4–8].

The magnitude of the critical shear stress depends on a cell size and physico-chemical properties of a substrate [1]. Increasing the cell size has two opposite effects. On the one hand, the total energy of the interaction of cells with the substrate increases with the cell contact area, and on the other hand, the hydrodynamic drag (forces) of the cell increases [1].

The contact area of the cell with the substrate in addition to the cell size depends on the time of cell attachment to the surface (the incubation time). The last goes from the fact unstimulated cells frequently take many hours to spread on the substrates even if the cell adheres at once in a few points [9]. And basing on theoretical conceptions, the force should increase together with the perimeter of spreading [9]. Thus, the incubation time of cells on the substrate can be one of the factors affecting the strength of cell adhesion.

The contradictory data are in the literature on this matter.

In various experiments different types of cells were incubated on the substrates during different time periods until measuring the adhesion strength. So, the study of rabbit's chondrocyte adhesion to the glass surface showed [10] that the average area projection of cells on the substrate increases from 161 μm^2 when the cells were incubated during 1 h up to 369 μm^2 after 6 h of incubation. At the same time the force required to detach these cells from the substrate grows from 231 Pa up to 1085 Pa, respectively.

Pendergrass C. et al. [11] investigated the immortalized human dermal fibroblasts. The cell projection area increased from 400 μm^2 after incubation for 1 h up to 700 μm^2 after incubation for 24 h. And the shear stress has increased from 8.49 Pa up to 12.5 Pa, respectively. After incubation for 96 h the average projection area increased significantly up to 2248.8 μm^2, but the shear stress significantly decreased – down to 7.7 Pa.

Truskey A. et al. [12,13] studied the adhesion of 3T3 fibroblasts to the glass surface in the plane-parallel flow chamber. After incubation for 30 min the critical shear stress was 1.72 Pa and after 120 min – 4.7 Pa. The fibroblast projection area in these cases was 526 μm^2 and 746 μm^2, respectively.

Christophis C. et al. [14] examined the adhesion of rat embryo fibroblasts (REF52) to the glass. The critical shear stress increased from 44.7 Pa after incubation during 3 h up to 83.2 Pa after 6 h of incubation.

Décavé E. et al. [1] studied the adhesion of Dictyostelium discoideum to a borosilicate glass plate with the radial-flow chamber. The observation of cells that remained on the substrate showed that their average projection area was much smaller at higher shear stresses. For example, when the shear stress was 4 Pa, the average projection area of attached cells was 95 μm^2 comparing to 110 μm^2 at the shear stress 1 Pa. It shows that cells with the large projection area are easier to detach from the substrate.

Thus, it is clear from the presented data that the increase of cell incubation time leads to the growth of cell projection area on the substrate and to the increase of the shear stress required for the detachment of cells from the substrate. However, in some cases the increase of cell incubation time and, as a result, the increase of projection area leads to the reducing of the shear stress. Moreover, in each individual experiment more spread cells are detached easier from the substrate [1].

In addition to the incubation time of cells the action time of shear flow also influences the critical shear stress. Thus, it was established that the number of detached cells was increasing in time and a certain maximum was attained at the current shear stress. The time of achievement of this maximum is shorter and the level of maximum is higher at a higher shear stress [1]. For example, when the shear stress was equal to 4 Pa the percentage of detached cells reached 65% after 7.5 min and then it did not change, and when the shear stress was increased up to 9.5 Pa after the flow action for 3.5 min the number of detached cells reached 90% and it did not increase further. Pendergrass C. et al. [11] shown that after 1 s of the flow action on the cells the detachment radius was equal to 0.36 cm at the critical shear stress of 14.47 Pa, and after 60 s the detachment radius was equal to 0.44 cm when the critical shear stress was equal to 11.84 Pa. Thus, at the increase of flow action time the detachment radius increases and the shear stress reduces. Truskey A. et al. [13] also demonstrated that when the shear stress was equal to 4.7 Pa the number of detached cells increased from 57% during the first 2 min of the flow action up to 77% after 120 min. Christophis C. et al. [14] presented that the increase of flow action duration resulted in the reduction of shear stress. Thus, the critical stress was equal to 64.6 Pa after 4.5 min of the flow action, and then decreased down to 43.8 Pa after 10 min and down to 24.4 Pa after the flow action for 18 min.

The aim of our study was to determine the influence on the critical shear stress of the initial shear stress, the flow action time on the cells, and the incubation time of SPEV cells on the glass substrate.

2. Materials and methods

2.1. Preparation of cell culture

In the experiments the SPEV cells (porcine embryo kidney cells) were used, which had been obtained from the company 'BIOLEK' (Kharkiv, Ukraine). To study the adhesion strength cells were seeded into culture Petri dishes 3.0 cm in diameter with microscope slides placed in a 199 medium (Austria, PAA Laboratories GmbH) with supplemented 10% fetal bovine serum (Austria, PAA Laboratories GmbH) at 37 °C in an atmosphere of air with 5% of CO_2 and 95% of humidity. Considering the number of attached SPEV cells to the glass becomes noticeable no sooner than in 4 h of incubation [15], the adhesive properties of cells were studied after 5, 24, and 168 h of incubation. The initial seeding concentration was $1 \div 8 \times 10^4$ cells cm^{-2} depending on the cell incubation time.

2.2. Preparation of the substrate

A glass plate (the microscope slide) with dimensions of 24.5 × 24.5 × 1 mm was cleaned thoroughly before it was used. For this purpose it was immersed into sulfochromic acid solution (4% v/v) for 1 h at a room temperature, rinsed three times with distilled water, and then dried in the air [16].

2.3. Cell detachment chamber and experiment parameters

The critical shear stress during the cell detachment was determined with a radial-flow chamber, which was constructed by us according to the diagram which is presented on the Figure 1.

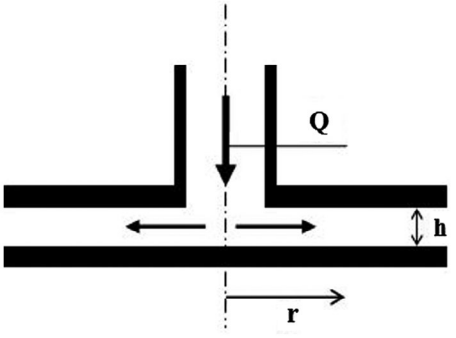

Figure 1. The diagram of the radial-flow chamber. *Q* – volumetric flow rate, *h* – the height of the channel, *r* – the current radius of the disc (lower disc is the substrate for cells).

The upper disc 25 mm in diameter was made of stainless steel with a central 2 mm diameter orifice. The substrate with cells is placed under this disk. A channel of required height is created between the disc and the substrate. The height of the channel can be changed by means of special inserts. The liquid is fed into the channel through the hole with a syringe pump. The liquid flows radially in the channel between the disc and the substrate creating the shear stress on both of them. Cells are detached from the substrate due to the action of this shear stress. Thus, on the substrate a cell-free region is formed having a circular shape the radius of which must be measured. Inside the cell-free region, there is often some amount of attached cells.

The detachment of cells from the substrate surface was carried out with the flow of 150 mM NaCl aqueous solution. The studies were performed at the temperature of t = (20 ± 1) °C.

During the experiment or after its completion the detachment radius (the radius of cell-free region) is measured with a microscope. (The radius of the cell-free region after the end of the experiment is termed the critical radius.) This gives an opportunity to calculate the value of a critical shear stress τ_c according to the equation [7]:

$$\tau_c = \frac{3\mu Q}{\pi r_c h^2}, \tag{1}$$

where μ is the viscosity of fluid, Q is the volumetric flow rate, r_c is the critical radius (or the detachment radius), h is the height of the channel between the top disc and the substrate.

From Equation (1) it can be seen the shear stress can be changed by changing the flow rate and/or by changing the height of the channel between the disc and the substrate using the same liquid. The application of Equation (1) is possible only at the laminar flow in the radial-flow chamber [8].

Since the main characteristic spatial value of this system is the height h of the channel, the initial shear stress at the wall in the zone of turn of the fluid flow after its exit from the orifice was determined [7]:

$$\tau_0 = \tau_{w(r=h)} = \frac{3\mu Q}{\pi h^3} \tag{2}$$

We used this value as the initial parameter of the experiment for association of the fluid volumetric flow rate Q and the channel height h, i.e. those meanings which directly influence the force of fluid flow action on the cells. The larger the value of initial shear stress τ_0 is the stronger the action of flow on the cells is. The critical shear stress was calculated using Equation (1) after measuring the critical radius value. As it was mentioned above, Equation (1) may be applied only in the case if the fluid flow in the chamber was laminar. This condition can be satisfied in the case when the Reynolds number satisfies the inequality $Re_h < 2000$ [5,8]. The Reynolds number Re_h is defined by the following equation [4,5]:

$$Re_h = Re_{(r=h)} = \frac{\rho Q}{\pi \mu h} \tag{3}$$

The Reynolds number Re_h is a convenient parameter, because it does not depend on the radial position.

The values of Reynolds numbers Re_h and initial shear stresses τ_0 for the parameters of our experiments are presented in Table 1. For 150 mM aqueous solution of NaCl at the temperature of 20 °C the dynamic viscosity $\mu = 1.0282\times10^{-3}$ Pa·s [17], and the density $\rho = 1004$ kg·m^{-3} [18].

In our experiments, the initial shear stress τ_0 and Reynolds number Re_h varied with changing the flow rate Q and the channel height h (see Table 1).

As the table data show we have the laminar flow in our chamber because the number Re_h does not exceed 2000 at all regimes.

2.4. Measurement of critical radius

The record of the experiment results was done with an inverted microscope LSM 510 Meta (Carl Zeiss, Germany) in a light regime. In a certain period of time after the beginning of the flow action on the cells the substrate was transferred into a Petri dish with saline. Then it was placed on the microscope stage and was photographed by stepwise from the flow stagnation point (the center of cell-free region) towards four sides of the glass with overlapping the microscopy areas in order to subsequent receive a cross-shaped panorama. Then the substrate was returned to the chamber and the experiment was continued.

Using four directions of the panorama we measured the radius of the cell-free region. The average value of this radius was taken as the detachment radius. When the detachment radius was not growing the experiment was over.

The interruption time of flow action on the cells did not exceed 7 min. In our opinion this fact must not significantly affect the additional attachment of cells to the substrate because as the above mentioned attachment of SPEV cells to the glass occurs during 4 h.

Table 1. Experiment parameters.

Q, ml c^{-1}	h, mm	τ_0, Pa	Re_h
0.65	0.5	5.11	404.3
0.96	0.5	7.54	597.1
1.92	0.5	15.1	1194.1
0.415	0.3	15.1	430.2
0.96	0.3	34.93	995.11
1.92	0.3	69.86	1990.2

2.5. Measurement of cell projection area

The measurement of cell projection area on the substrate was done on samples which were not subjected to the flow action or after it action, using the AxioVision software 40 V 4.7.2.0 (Carl Zeiss, Germany). We measured the projection areas of 120–150 cells for each incubation time before and after the flow action.

2.6. Statistical analysis

A statistic processing of experiment results were performed by the standard method using the Student's t-test. The significance level was set to $p < 0.05$.

3. Results and discussion

The critical radius r_c of cell-free region is a directly measured value in our experiments. Its value is directly associated with the critical shear stress τ_c (see Equation (1)) i.e. with the parameter that shows the cell adhesion power. The smaller the critical radius is under the same conditions, the stronger the cells are attached to the substrate.

We determined the values of detachment radii and calculated the shear stresses depending on the incubation time of SPEV cells, the initial shear stress, and the action time of the fluid flow on the cells (or the detachment time). In Figures 2 and 3 are presented the typical dependences of the detachment radius and the shear stress on detachment time for the experiment with the SPEV cells which were incubated on the glass substrate for 5 h. It is seen from the diagrams that the detachment radius increases and the shear stress reduces together with the increase of the detachment time but to a certain level. In this case the radius reaches its maximum value of 8.4 mm, and the shear stress – minimum value of 0.389 Pa after 36 min of flow action and then these values do not change. Namely this radius we call the critical radius, the corresponding shear stress – the critical shear stress and the time to achieve these values – the full detachment time. The results in Figures 2 and 3 agree with several works of other authors [1,11,13,14] which show that the number of the cells detached from the substrate also increases and the shear stress decreases at the increase of the action time of the fluid flow on the cells.

The parameters and the results for different experimental conditions are shown in Table 2.

It is seen from the Table 2 that under the action of the low initial shear stresses (5.11–15.1 Pa; h = 0.5 mm) the more the incubation time of the cells on the substrate is, the smaller the critical radius is, and the more the critical shear stress is. These data correspond to the data of articles [10,12–14], where the critical shear stress also grew at the increase of incubation time of cells on the substrate.

However, at the higher initial shear stresses (15.1–69.86 Pa; h = 0.3 mm) the critical radius grows and the critical shear stress falls at the increase of incubation time. These data correspond to the data of the article [11], according to which the critical shear stress decreases after a certain time of cell incubation.

As for the full detachment time of cells, it increases in all cases with the increase in the incubation time.

Moreover, the results from the Table 2 show that the more the value of initial shear stress is, the smaller the value the critical radius has and the larger the value of critical shear stress

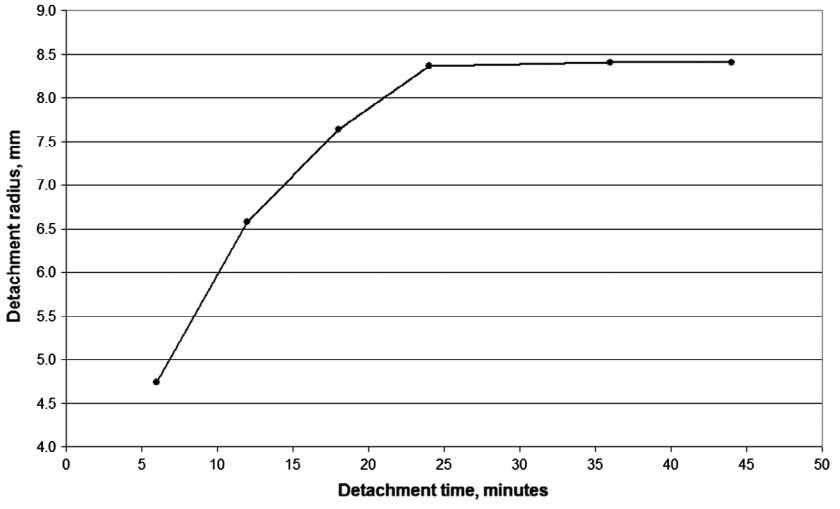

Figure 2. The dependence of the detachment radius on the detachment time. (the initial shear stress $\tau_0 = 7.54$ Pa).

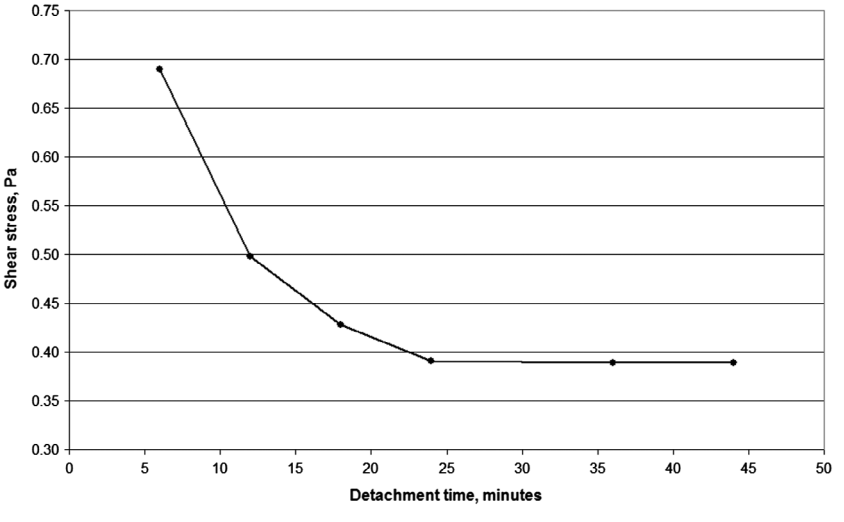

Figure 3. The dependence of the shear stress on the detachment time. (the initial shear stress $\tau_0 = 7.54$ Pa).

is. That is with a stronger flow action the cells are attached to the substrate more firmly. This corresponds to the results of the article [19], which state that the adhesive force may increase with the increase of action force value on the cells.

Another feature of these results is that when the initial shear stress was smaller or equal to 15.1 Pa (the height of channel h = 0.5 mm) the critical radius decreased and the full detachment time and the critical shear stress increased at the increase of initial shear stress. With a shear stress of 15.1 Pa but at different channel heights all the above values sharply decreased with decreasing the channel height. However, when the initial shear stress was greater than 15.1 Pa (the channel height $h = 0.3$ mm), the critical radius and full detachment

Table 2. Parameters and results of the experiments on the adhesion of SPEV cells to the glass substrate.

Incubation time, hours	τ_0, Pa	r_c, mm	τ_c, Pa	Full detachment time, minutes
5	5.11	8.46	0.299	30
	7.54	8.4	0.389	36
	15.1	7.99	0.944	48
	15.1	5.84	0.775	44
	34.93	5.34	1.961	26
	69.86	4.87	4.301	16
24	5.11	7.81	0.327	36
	7.54	7.7	0.49	42
	15.1	6.96	1.083	74
	15.1	5.82	0.778	70
	34.93	5.42	1.932	58
	69.86	5.04	4.156	50
168	5.11	7.54	0.339	116
	7.54	7.28	0.518	122
	15.1	6.78	1.112	130
	15.1	5.61	0.807	120
	34.93	5.52	1.897	82
	69.86	5.45	3.843	60

time decreased and the critical shear stress increased as compared the same values for smaller initial shear stresses. At the same time, the critical radius is smaller with the smaller value of channel height even though the initial shear stresses are significantly higher at the same flow rates.

It turns out that not only a magnitude of shear stress is important but also the way it is achieved. That is the values of the critical shear stress can only be used as a comparative parameter with constant parameters of flow chamber.

Above we presented the data from the works of other authors where the correlation between the cell projection area onto the substrate and the critical shear stress was considered. We measured the area of cell projection onto the substrate at different incubation time both before the flow action on the cells and after its action. The measurements were carried out on the edge of cell-free region, i.e. on the border area where cells remained after the end of experiment. The results are shown in Table 3.

From Table 3 it can be seen an abrupt decrease in the area of the projection of the cells with a decrease both of the channel height and the critical shear stress. In addition, an increase in the critical shear stress leads to a decrease in the projection area, regardless of the height of the channel.

Moreover, from these data it can be concluded that during the experiment the more spread cells (with the large projection area) were detached from the substrate whereas the less spread cells stayed attached to the substrate. Similar results were obtained Décavé E. et al. [1].

The images in Figure 4 illustrate this case.

These images were obtained from the same field of microscope, which was located on the edge of cell-free region when the experiment was finished. In the marked circle we can see the cells which remained inside of the cell-free region at a distance of 6.9 mm from the centre whereas the critical radius was equal to 7.54 mm after the end of experiment. Exactly these cells can be seen before the beginning of the experiment and during the entire time of experiment on the same place. These cells were slightly spreaded and had a nearly spherical shape before, during and after the experiment. Meantime the detachment radius was

Table 3. The projection area of the cells before and after the action of the flow.

	Cell projection area, μm²		
Incubation time, hours	Before flow action	After flow action	τ_0, Pa
5	225.3 ± 62.7	219.7 ± 35.4	5.11
		218.3 ± 30.6	7.54
		211.4 ± 40.4	15.1
		201.2 ± 35.1	15.1
		190.7 ± 37.2	34.93
		180.4 ± 39.9	69.86
24	340.0 ± 92.8	217.9 ± 41.4	5.11
		214.6 ± 34.7	7.54
		207.8 ± 42.0	15.1
		190.0 ± 39.9	15.1
		178.6 ± 38.7	34.93
		171.9 ± 43.5	69.86
168	380.1 ± 103.2	213.8 ± 41.3	5.11
		208.1 ± 40.8	7.54
		198.5 ± 42.7	15.1
		182.2 ± 43.9	15.1
		168.8 ± 38.9	34.93
		161.4 ± 39.6	69.86

varying during the experiment. So after 16 min of the flow action on the cells the detached radius was equal to 4.07 mm, after 30 min – 5.3 mm, after 56 min – 6.17 mm, and after 70 min – 6.55 mm. At the same time, it can be seen that after 16 min of flow action there were little spread cells, and after 30 min such kind of cells almost was not in this region. This confirms the conclusion that during the experiment the spread cells were detached first of all. Although spread cells having a large area of contact with the substrate should hold to the surface stronger like more flat having less drag [20].

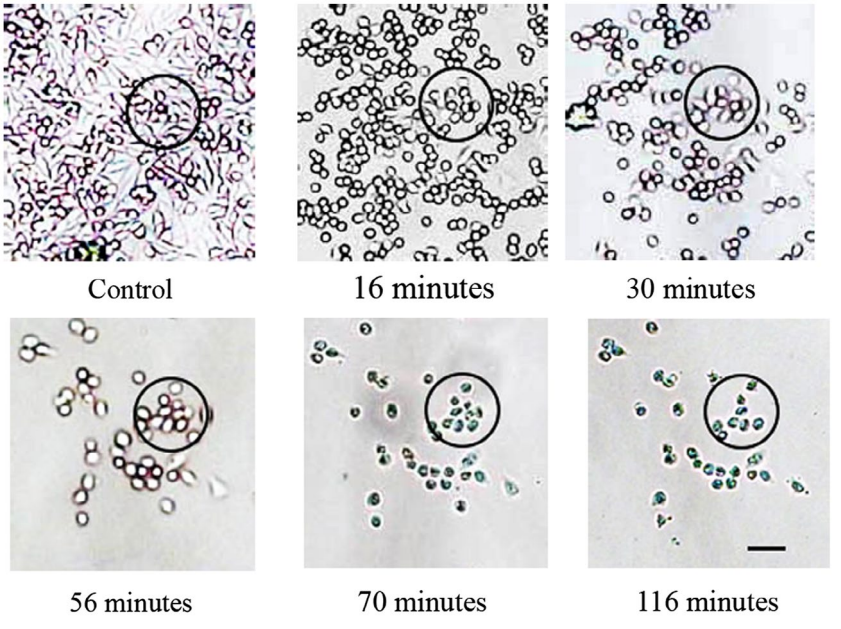

Figure 4. The images of cell-free region edge after different action time of flow on the cells. The cells were incubated for 168 h, τ_0 = 5.11 Pa. Bar 50 μm.

Comparing the data of Tables 2 and 3 it can be seen that more spread cells (after a long incubation time) were more strongly attached to the surface within the interval of initial shear stresses 5.11–15.1 Pa. But when the initial shear stress was equal to 69.86 Pa the more spread cells detached at the smaller values of critical shear stress, as it has already been noted above. It is consistent with data of the article [11].

Earlier detachment of more spread cells can be explained by the fact that the total area of focal adhesions of cells can reach 4% of the total area of the cell membrane [21]. The contact area of the cell with the substrate in more spread cells must be larger than in the less spread cells. Therefore, assuming that all cell adhesive receptors are collected in cells in the contact region with the substrate then the specific area of focal contacts (a ratio of focal contacts area to cell projection area) in the more spread cells will be smaller than in the less spread cells. This may result in less effort for the detachment of such cells from the substrate.

4. Conclusions

The SPEV cells with a long incubation period on the glass substrate can keep stronger on it in the range of low initial shear stresses, but at a high initial shear stress these cells detached from the substrate at lower values of critical shear stress.

The longer incubation time of cells on the substrate was before the experiment the longer flow action time on the cells was required to achieve the critical radius.

At high initial shear stresses after a long-term action of the flow on the cells the critical shear stress becomes smaller at the detachment of cells that were incubated on the substrate for a longer period of time.

At the increase of flow action time on the cells the amount of detached cells increases and the shear stress that is necessary for their detaching decreases.

The larger initial shear stress is the smaller number of cells detach from the substrate.

At the decrease of channel height in the radial-flow chamber the critical radius, the full detachment time and the critical shear stress decrease too.

The critical shear stress can only be a comparative parameter if studies are carried out in a chamber with the same channel height, and flow rate.

During each experiment the cells which have the large projection area are detached in the first place.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Décavé E, Garrivier D, Bréchet Y, et al. Shear flow-induced detachment kinetics of *Dictyostelium discoideum* cells from solid substrate. Biophys J. 2002;82:2383–2395.
[2] Barber KM, Pinero A. Truskey GA effects of recirculating flow on U-937 cell adhesion to human umbilical vein endothelial cells. Am J Physiol Heart Circ Physiol. 1998;275:591–599.
[3] Mikos AG, Peppas NA. Bioadhesive analysis of controlled-release systems. IV. An experimental method for testing the adhesion of microparticles with mucus. J Control Release. 1990;12:31–37.
[4] Cozens-Roberts C, Quinn JA, Lauffenburger DA. Receptor-mediated adhesion phenomena: model studies with the radial-flow detachment assay. Biophys J. 1990;58:107–125.

[5] Dickinson RB, Cooper SL. Analysis of shear-dependent bacterial adhesion kinetics to biomaterial surfaces. AZChE J. 1995;41:2160–2174.
[6] Goldstein AS, DiMilla PA. Application of fluid mechanic and kinetic models to characterize mammalian cell detachment in a radial-flow chamber. Biotechnol Bioeng. 1997;55:616–629.
[7] Goldstein AS, DiMilla PA. Comparison of converging and diverging radial flow for measuring cell adhesion. AIChE J. 1998;44:465–473.
[8] Detry JG, Deroanne C, Sindic M. Hydrodynamic systems for assessing surface fouling, soil adherence and cleaning in laboratory installations. Biotechnol Agron Soc Environ. 2009;13:427–439.
[9] Evans E. Physical action in biological adhesion. In: Lipowsky R, Sackmann E, editors. Handbook of biological physics. Vol. 1, Amsterdam: Elsevier; 1995. p. 723–753.
[10] Huang W, Anvari B, Torres JH, et al. Temporal effects of cell adhesion on mechanical characteristics of the single chondrocyte. J Orthop Res. 2003;21:88–95.
[11] Pendegrass CJ, Middleton CA, Gordon D, et al. Measuring the strength of dermal fibroblast attachment to functionalized titanium alloys *in vitro*. J Biomed Mater Res. 2010;92A:1028–1037.
[12] Truskey GA, Proulx TL. Relationship between 3T3 cell spreading and the strength of adhesion on glass and silane surfaces. Biomaterials. 1993;14:243–254.
[13] Truskey GA, Pirone JS. The effect of fluid shear stress upon cell adhesion to fibronectin-treated surfaces. J Biomed Mater Res. 1990;24:1333–1353.
[14] Christophis C, Grunzeab M, Rosenhahn A. Quantification of the adhesion strength of fibroblast cells on ethylene glycol terminated self-assembled monolayers by a microfluidic shear force assay. Phys Chem Chem Phys. 2010;12:4498–4504.
[15] Pavlovich EV, Volkova NA, Stepanuk LV, et al. Adhesion and proliferation processes in SPEV cell line under the influence of europium oxide nanoparticles. Biotechnol acta. 2013;6:119–124.
[16] Mercier-Bonin M, Ouazzani K, Schmitz P, et al. Study of bioadhesion on a flat plate with a yeast/glass model system. J Colloid Interf Sci. 2004;271:342–350.
[17] Todrin AF, Popivnenko LI, Kovalenko SY. Thermophysical properties of cryoprotectants. II. Dynamic viscosity of some cryoprotectants, their solutions and mixtures. Probl Cryobiol Cryomed. 2010;20:266–281.
[18] Todrin AF, Popivnenko LI. Thermophysical properties of cryoprotective agents. III. Density, kinematic viscosity and surface tension of some cryoprotective agents, their solutions and mixtures. Probl Cryobiol Cryomed. 2010;20:416–435.
[19] Mogilner A. Mathematics of cell motility: have we got its number? J Math Biol. 2009;58:105–134.
[20] Brooks SB, Tozeren A. Flow past an array of cells that are adherent to the bottom plate of a flow channel. Comput Fluid. 1996;25:741–757.
[21] Biton YY, Safran SA. Theory of the mechanical response of focal adhesions to shear flow. J Phys Condens Matter. 2010;22:194111(1–8).