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Short Communication

THE SHAPE OF CELLS ADHERING TO SULFONATED COPOLYMER SURFACES

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Abstract: We studied the shape of L1210 leukaemia cells adhering in a protein-free medium to sulfonated (styrene/methyl methacrylate) copolymer surfaces of two sulfonic group densities, and thus of differing wettability. The use of our image analysis method and the mathematical procedure [1] allowed us to calculate the values of the so-called shape parameter, which quantitatively determines the three-dimensional cell shape. Here, we show that the values of the shape parameter of the adhering cells and the F-actin concentration, in the region near the cell-substratum interface, depend on the density of sulfonic groups present on the substratum surface.

Key Words: Cell-substratum Adhesion, 3D Cell Shape, Substratum Sulfonic Groups

INTRODUCTION

The adhesive ability and the shape changes of tumour cells are important in cell dissemination and metastasis. During these processes, which are initiated when tumour cells migrate and invade the surrounding tissue, a large number of adhesive interactions and simultaneous changes in the cell shape and in the actin cytoskeleton organization may take place [2]. Therefore, it seems important to study the mechanism controlling cell behaviour, in particular the relationship between the shape of cancer cells and their capability for adhesion and migration. It has been shown that the shape and locomotion of tumour cells can be affected by the surrounding or underlying normal cells [3]. In that study, rat sarcoma cells were found to be guided by underlying aligned fibroblasts; this results in the faster locomotion of the tumour cells.

In studies of the behaviour of tumour cells, substrata of defined composition (e.g. modified polymeric surfaces) are used since it was found that the

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physicochemical features of the substratum surface, such as charge and hydrophilicity, influence the adhesion and shape of cells [4, 5]. It has been shown that surface sulfonic groups of styrene/methyl methacrylate (S/MMA) copolymers influence the behaviour of cancer cells (L1210 leukaemia) and normal cells (3T3 fibroblasts) in a similar way. A functional relation was found between the number of adhering L1210 cells and the surface density of sulfonic groups [6, 7]. These groups also affect the F-actin and α -actinin distribution in the cell region near the substratum surface in cells adhering to the S/MMA surfaces in the medium containing serum [8]. In the case of 3T3 fibroblasts, in the early phase of adhesive interaction, the number of adhering cells correlates with the surface density of sulfonic groups. Furthermore, the shape of 3T3 cells and the pattern of distribution of cytoskeletal proteins considerably depend on this density [9]. By contrast, in the case of *Amoeba proteus*, the sulfonic groups present on S/MMA surfaces affect neither the cell shape nor the rate of locomotion [10].

In our recent work [1], we showed that, in the absence of serum in the medium, the sulfonic groups present on the polystyrene surface affect the shape of adhering cells. The quantitative analysis of the three-dimensional shape of the cells revealed that these groups cause a significant increase in the degree of cell flattening, as compared with the shape of cells adhering to nonsulfonated surfaces.

This stimulated us to undertake examinations of cell shape in connection with F-actin distribution for cells adhering to surfaces of sulfonic group densities lower than those used recently. The main goal of this study was to evaluate and quantitatively compare the shape of the cells adhering in a protein-free medium to S/MMA surfaces differing in their sulfonic group density.

MATERIALS AND METHODS

Cells

Lymphoid leukaemia L1210 cells grown in an ascitic fluid in the intraperitoneal cavity of DBA/2 mice were used [11, 12]. After washing (for details see [1]), the cells were suspended in Eagle minimal essential medium (Gibco); the cell concentration used was approximately 5×10^6 cells/ml. The survival of cells during the experiments, as tested by cell morphology and Trypan Blue staining, was at least 95%.

Preparation of styrene/methyl methacrylate (S/MMA) copolymer films

Copolymerization of styrene (S) and methyl methacrylate (MMA) was performed according to the method described previously [7]. The obtained solutions of two S/MMA copolymers of a molar percentage of styrene, c_s , equal to 10 mol% and 50 mol% were deposited on glass microscope cover slips. The surfaces of the obtained films were sulfonated with SO_3 (details in [7]). As a result of S/MMA surface sulfonation, the sulfonic groups ($-\text{SO}_3\text{H}$) are bound

covalently to the carbon of the styrene rings. Since only the rings of styrene mers are sulfonated on the surface [13], the number of $-\text{SO}_3\text{H}$ groups, and so the wettability of the surface, can be changed by altering the c_s value in the bulk of the copolymer. For surfaces of $c_s = 10$ mol%, the contact angles with water are respectively equal to 70° and 53° for the nonsulfonated and sulfonated surfaces, whereas for surfaces of $c_s = 50$ mol%, these angles are respectively equal to 80° and 12° [7].

Cell image analysis and quantitative description of cell shape and statistics

Cells interacted under static conditions (for details see [1, 6]) with the copolymer surfaces within a time t_{int} (defined below), during which the cell adhesion took place. All the examined cells were in stable adhesion with copolymer surfaces [see 14]. The cell-substratum interaction time t_{int} is defined as the time interval from the moment the first sedimented cells become sharp in the focus plane on the bottom of the chamber, until the moment the fixing procedure of the adhered cells is started. The fixed cells were stained with FITC-Phalloidin according to the standard procedure [8]. All the chemicals were purchased from Sigma.

A computer-based image analysing system described earlier [1] was used to obtain both the distribution of the F-actin and the three-dimensional (3D) shape of the cells. The analysis was made for free and adherent fluorescent-stained cells, which were normal in morphology and so separated from each other that interactions between the cells could not occur. A quantitative 3D presentation of cell shape was accomplished by analysing the distribution of F-actin in sequences of fluorescent images of the cell optical slices and by composing contours of 10 cell slices (the images were digitized and processed with the Bitanal program prepared by one of us (M. Inkielman). The method of the computer image analysis [15] and the mathematical procedure applied have already been described earlier [1], but for the readers' convenience, we will now repeat it in an abbreviated form¹.

For all statistical evaluations, the program STATISTICA was used.

¹We express the mean radius R of the contour of every given slice as a function of the distance z between this slice and the slice closest to the substratum surface (i.e. the 1st optical slice). An approximation close to reality is obtained when the function $R(z)$ is a two-degree polynomial, i.e.

$$R(z) = a(z - z_1)(z - z_2), \quad z_1 < z_2, \quad (1)$$

where a , z_1 and z_2 are parameters calculated for each cell individually, to minimize the approximation error.

The shape of the cell can quantitatively be described by means of a quantity SP, called the cell shape parameter, defined as follows:

$$\text{SP} = R(0) / R(z_2/2) = 4z_1 / (2z_1 - z_2), \quad (2)$$

where $R(0)$ is the mean radius of the slice closest to the substratum surface and $R(z_2/2)$ is that of the slice crossing the midpoint of cell height.

RESULTS

In our recent work, it was demonstrated that polystyrene surfaces, which have a sulfonic group density equal to 4×10^{14} groups/cm² influence the shape of adhering L1210 cells [1]. In this study, L1210 cells interacted with sulfonated S/MMA surfaces with either 4×10^{13} (low density) or 2×10^{14} (high density) sulfonic groups/cm². This study is divided into two parts. In the first part, the shape of cells adhering to S/MMA surfaces is quantitatively described. In the second part, the F-actin organization in the adhering cells is examined.

Shape of cells adhering to S/MMA copolymer surfaces

The shape of cells adhering to the nonsulfonated and sulfonated surfaces within t_{int} equal to 3 min and 8 min, and also the shape of free cells, were analysed and quantitatively compared. The values of both the cell shape parameter (SP) and the 1st optical slice area were calculated. These results are presented in Tab. 1.

Tab. 1. Shape parameter (SP) and area of the first optical slice of L1210 cells adhering to S/MMA copolymer surfaces. Cell-substratum interaction time $t_{int} = 3$ min and $t_{int} = 8$ min

Copolymer surfaces	SP	Standard deviations	Area of 1 st cell slice (μm ²)	Standard deviations	Number of cells analysed
$c_s = 10$ mol%					
Nonsulfonated					
$t_{int} = 3$ min	0.71	0.114	40.2	12.70	222
$t_{int} = 8$ min	0.73	0.127	40.3	13.14	574
Sulfonated					
$t_{int} = 3$ min	0.72	0.137	40.0	14.56	232
$t_{int} = 8$ min	0.74	0.153	39.5	12.90	830
$c_s = 50$ mol%					
Nonsulfonated					
$t_{int} = 3$ min	0.73	0.110	42.0	11.58	426
$t_{int} = 8$ min	0.72	0.117	39.6	12.13	614
Sulfonated					
$t_{int} = 3$ min	0.74	0.146	42.9	14.73	529
$t_{int} = 8$ min	0.83	0.189	47.3	17.08	385

The mean values of the SP, and, independently, of the 1st slice area, were compared for cells adhering to surfaces in the following cases: high density of sulfonic groups vs low density of sulfonic groups, high density of sulfonic groups vs nonsulfonated, with $t_{int} = 8$ min in both cases, and high density of sulfonic groups with $t_{int} = 3$ min vs the same density of these groups with $t_{int} = 8$ min. All the differences between the compared mean values are statistically significant at $p < 0.001$.

The mean values of SP and the 1st slice area for free cells were equal to 0.45 ± 0.128 and $17.8 \pm 9.07 \mu\text{m}^2$, respectively. These values were low in comparison with those for cells adhering to the copolymer surfaces (the latter values are given in Tab. 1; differences are statistically significant at $p < 0.0001$). These results concur with previously obtained ones (cf. [1, 16]).

For cells adhering to nonsulfonated surfaces, both the cell-substratum interaction time (in the interval of $3 \text{ min} \leq t_{int} \leq 8 \text{ min}$) and the surface wettability (i.e. the styrene content c_s) have no significant effect on the values of SP and of the 1st slice area. What is worth noting is that the cell adhesion to nonsulfonated surfaces also does not depend on the surface wettability, and is only slightly influenced by the cell-substratum interaction time [6].

For cells adhering to surfaces of low sulfonic group density (i.e. to surfaces with a low hydrophilicity), the cell-substratum interaction time (in the interval of $3 \text{ min} \leq t_{int} \leq 8 \text{ min}$) has no significant effect on the mean values of the shape parameter or the 1st slice area. These values are similar to those for cells adhering to nonsulfonated surfaces.

For cells adhering to surfaces of high sulfonic group density (i.e. to the highly hydrophilic surfaces), the cell-substratum interaction time (in the interval of $3 \text{ min} \leq t_{int} \leq 8 \text{ min}$) significantly influences the values of both the shape parameter and the 1st slice area; the longer the t_{int} , the higher these values (i.e. the higher the degree of cell flattening).

F-actin distribution in cells adhering to S/MMA copolymer surfaces

The pattern of F-actin distribution near the substratum surface (1st and 2nd optical slices) in cells adhering (within $t_{int} = 8 \text{ min}$) to the surfaces of low and high sulfonic group density, and, for comparison, in cells adhering to nonsulfonated surfaces was examined (see Fig. 1).

The distribution of F-actin in cells adhering to nonsulfonated surfaces of $c_s = 10 \text{ mol\%}$ and of $c_s = 50 \text{ mol\%}$ was similar (Fig. 1 A and C, respectively). The F-actin was slightly concentrated close to the cell periphery and located in the microvilli; only a small portion of this protein occurred in a scattered form. This pattern of F-actin distribution was representative for approximately 95% of the entire population of adhering cells. We may conclude that the distribution of F-actin in the adhering cells is not visibly affected by the surface wettability (i.e. the styrene content c_s).

In cells adhering to surfaces of low sulfonic group density, the F-actin was mostly concentrated close to the cell periphery and was also located in the microvilli (Fig. 1B). This pattern of F-actin distribution was representative of 94% of the entire population of adhering cells. Let us note that though the shape of cells adhering to nonsulfonated surfaces and to surfaces of low density of sulfonic groups is similar within the margin of experimental error, the pattern of F-actin distribution differs for cells on these two types of surface.

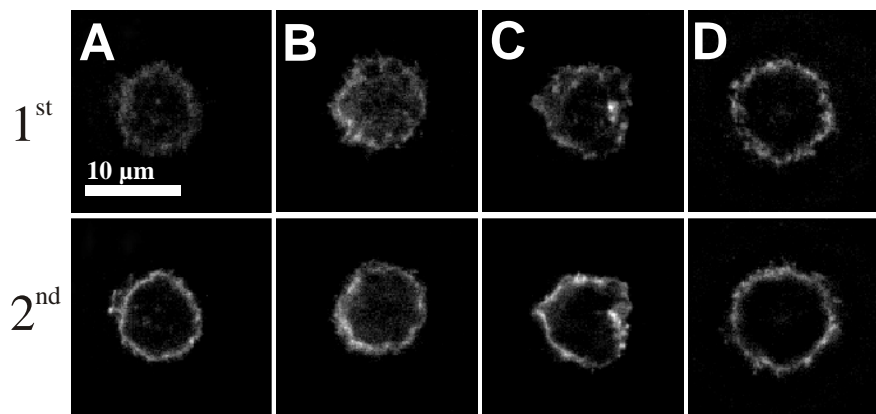


Fig. 1. F-actin distribution in L1210 cells adhering within $t_{int} = 8$ min to S/MMA copolymer surfaces; in the cells adhering to surfaces of $c_s = 10$ mol% nonsulfonated (A) and sulfonated (B), and to surfaces of $c_s = 50$ mol% nonsulfonated (C) and sulfonated (D). Cells were stained with FITC-Phalloidin. The images of the consecutive optical slices (1st and 2nd) are collected at 2.0 μm intervals; the first optical slice is at the cell-substratum interface.

In the case of cells adhering to surfaces of high sulfonic group density, F-actin filaments were concentrated at the periphery and in the central part of the cell (Fig. 1D). This pattern of distribution and the relatively strong distinctness² of F-actin concentration were representative of 86% of the entire population of adhering cells. Thus for cells adhering to the surfaces with a high density of sulfonic groups, the distinctness of F-actin concentration near the cell-substratum interface is stronger than that of cells adhering to the surfaces with a low density of these groups.

DISCUSSION

This study shows for the first time that the density of sulfonic groups present on the S/MMA copolymer surfaces affects the shape of L1210 cells and the F-actin distribution in the early phase of the adhesive interaction with these surfaces. The mean value of the shape parameter (SP) for the cells adhering to the surfaces with a high density of sulfonic groups is respectively 1.1, 1.1, and 1.8 times higher than the mean value for the cells adhering to the surfaces with a low density of these groups, for the cells adhering to nonsulfonated surfaces, and for the free cells. Like the relations between the mean values of SP described above,

²The term “distinctness” means a relative and discernible contrast between the F-actin structures (which have diffused fluorescent boundaries) and the cell interior [8]. We qualitatively graded the distinctness (discernibleness) from strong to weak. The structures of strong (or weak) distinctness emitted a fluorescent signal that was (or was not) easily distinguishable (discernible) in the image.

the mean value of the 1st slice area for the cells adhering to the surfaces with a high density of sulfonic groups is approximately 1.2 times higher than that for the cells adhering to the surfaces with a low density of these groups and to nonsulfonated surfaces, and 2.7 times higher than that of the free cells. Moreover, our results show that in the case of adhesion to the surfaces with a high density of sulfonic groups, the shape change depends on t_{int} . Note that we have not found such a dependence for the cells adhering to both the nonsulfonated surfaces and to surfaces with a low density of sulfonic groups. We may conclude that the dependence of the cell shape on the surface sulfonic group density should occur on the sulfonated S/MMA surfaces of c_s in the interval mainly from about 50 to 100 mol%, taking into account our recent study [1], where sulfonated polystyrene surfaces (i.e. of very high sulfonic group density – 4×10^{14} groups/cm²) were used and where analogous phenomena occurred. In that study, we obtained the following relations: (i) the mean value of SP approximately 1.4 times higher than that for the cells adhering to the nonsulfonated polystyrene surfaces and approximately 2.5 times higher than that for the free cells; and (ii) the mean value of the 1st slice area approximately 1.7 times higher than that of the cells adhering to the nonsulfonated surfaces and approximately 4.4 times higher than that of the free cells. Note that these quantities are considerably larger than the corresponding ones obtained in this study. This is consistent with the fact that the highest sulfonic group density used in this study (2×10^{14} groups/cm²) was two times lower than that used in [1].

The high degree of flattening in the case of L1210 cells adhering to the S/MMA surfaces with a high density of sulfonic groups (i.e. to the highly hydrophilic surfaces) may tentatively be explained in terms of the higher energy of interaction between these surfaces and the tips of cell microvilli, as compared with the case of surfaces with a low density of these groups. This supposition is supported by the duration of the Brownian motion of cells sedimented on the surfaces of high sulfonic group density, which is approximately three times longer than the duration on surfaces of low density [6]. Moreover, computer simulations of the interaction between a short polypeptide chain (chosen as a convenient substitute of a cell surface protein) and S/MMA surfaces have shown that the stabilization energy calculated for this polypeptide interacting with the surfaces of high sulfonic group density is twice as high as in the case of low density. It is interesting that in the initial phase of cell adhesion, the number of L1210 cells adhering (at the shearing force $F = 3.9 \times 10^{-9}$ N) to the S/MMA surfaces with a high density of sulfonic groups is almost 1.8 times larger than that for cells adhering to surfaces of low density [6, 14].

Our results also show the effect of the surface sulfonic group density on the distinctness of F-actin concentration in the adhering cells in the region near the substratum surface. Evidently, the changes in the cell shape correlate with the changes in the F-actin concentration, as a cell response to the contact with the sulfonated copolymer surfaces differing in their sulfonic group densities, i.e. in

their surface wettability. These results concur with those found for cells adhering to sulfonated polystyrene surfaces [1]. It is interesting that the pattern of F-actin distribution in the cells, both L1210 cancer cells and normal 3T3 fibroblasts, adhering to the S/MMA surfaces in the presence of serum in the medium also depends on the surface sulfonic group density [8, 9]. The changes in the shape of cells (in our case in the cell flattening) are found to be a time-dependent process. This concurs with the fact that the organization of the F-actin structures, which changes simultaneously with changes in the cell shape, is also a time-dependent process.

In conclusion, this study shows that: (i) the values of the shape parameter, which determine the degree of cell flattening, increase with the surface density of sulfonic groups; and (ii) the pattern of F-actin distribution in the adhering cells depends on the surface sulfonic group density – the distinctness of the F-actin concentration in the region near the cell-substratum interface grows with the surface density of these groups.

A quantitative description of the 3D shape of cells interacting with substrata (highly hydrophilic in our case) allows us to estimate the ability of living spherical cells for deformation. The strong tendency of cancer cells to deformation may be responsible for their facility in migrating and invading the surrounding tissues during cell dissemination.

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