FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 49, No. 4, 2011
pp. 706–718

ORIGINAL STUDY

VIA MEDICA

Interaction of PC-3 cells with fibronectin adsorbed on sulfonated polystyrene surfaces

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Abstract: The ability of cancer cells to invade neighboring tissues is crucial for cell dissemination and tumor metastasis. It is generally assumed that cell adhesion to extracellular matrix proteins is an important stage of cancer progression. Hence, adhesion of cancer cells under *in vitro* conditions to proteins adsorbed on a substratum surface has been studied to provide a better understanding of cell-protein interaction mechanisms. A protein, adsorbed in an appropriate conformation on a substratum surface, creates a biologically active layer that regulates such cell functions as adhesion, spreading, proliferation and migration. In our study, we examined the interaction of PC-3 cells under *in vitro* conditions with fibronectin adsorbed on sulfonated polystyrene surfaces of a defined chemical composition and topography. We investigated cell adhesion to fibronectin and cell spreading. Using automatic, sequential microscopic image registration, we are the first to present observations of the dynamics of PC-3 cell spreading and the cell shape during this process. Our results show that cell adhesion and the shape of spreading cells strongly depend on the time interaction with fibronectin. The analysis of images of cytoskeletal protein distribution in the cell region near the cell-substratum interface revealed that induction of a signal cascade took place, which led to the reorganization of the cytoskeletal proteins and the activation of focal adhesion kinase (FAK). (*Folia Histochemica et Cytobiologica 2011; Vol. 49, No. 4, pp. 706–718*)

Key words: PC-3 cell line, fibronectin, cell adhesion and spreading, cytoskeleton, sulfonated polystyrene

Introduction

Various tissue cells are in constant communication with the surrounding extracellular matrix (ECM) which contains, among other components, high molecular and multidomain glycoprotein fibronectin (FN), found on cell surfaces and in the ECM in a insoluble (oligo- or polymeric) form [1].

The main fragment of the fibronectin molecule active in cell adhesion, referred to as a central cell-binding domain (CCBD), is composed of the first 11 type III modules and represents the largest functional FN domain. It is responsible for the interaction with integrins on cell surfaces and features higher susceptibility to degradation compared to other FN domains [2, 3]. The CCBD domain includes the main Arg-Gly-Asp (RGD) amino-acid sequence in module III_{10}, and the Pro-His-Ser-Arg-Asn (PHSRN) synergy sequence found in module III_9. The integrins binding to the RGD fragment of FN have been found to be significantly enhanced by the PHSRN sequence [4–8].

Protein (e.g. FN) adsorption and conformation on nonbiological surfaces such as metal or synthetic polymers depends on the substratum chemical composition, wettability and structure, surface topography (e.g. surface roughness), and on the environmental conditions (e.g. pH value, ionic strength and temperature) [9–15]. As a result of adsorption, FN conformation can become compact or extended, depending on physicochemical conditions.

It has been observed that adhesion of normal and cancer cells to substrate surfaces containing chemical groups with a negative charge ($–SO_3H$, –OH, –COOH) or a positive charge ($–NH_2$) depends on surface wettability and the conformation of the protein molecule on the surface [9, 11–19].

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Fibronectin adsorbed on strongly hydrophilic polystyrene surfaces, in contrast to the adsorption on hydrophobic surfaces, is characterized by extended, biologically active conformation with the unfolded cell-binding domain (CCBD), which stimulates not only the early phase of adhesion, but also the cell spreading process [13, 14, 20, 21]. The polystyrene surfaces used in the present work have been modified according to the original method developed by Kowalczyńska and Kamiński [22], which unlike other methods of surface modification [16, 23–25], is mild and highly efficacious, making it possible to eliminate the harmful hydrolyzing effect of sulfuric acid.

On the basis of a large amount of experimental data, it has become clear that depending on the chemical composition of the substratum surface, the following events may occur: (1) nonspecific adhesion due to the effect of physical forces between cell microvilli and the substratum surface (cell adhesion to 'bare' polymer surface); or (2) specific adhesion to the adsorbed protein by receptors as evidenced in the case of adhesion to FN adsorbed on the hydrophilic polymer surfaces [9, 13, 17, 26, 27]. Specific adhesion constitutes an essential early stage in the cell-substratum interaction, which affects, among other things, cell functions such as spreading, migration and proliferation [28].

This is why, among the criteria to determine the type of adhesion, it is the signal cascade that leads to the reorganization of the cells' cytoskeleton proteins and a change in the cell shape and migration, as a result of the specific response of the cell to the signal from the substratum [29, 30].

There have been only a few reports on the interaction of PC-3 cells with single proteins adsorbed on planar surfaces of a defined chemical composition and surface structure. Zheng et al. [30] and Cooper et al. [31] investigated the interaction of PC-3 cells with fibronectin, laminin and collagen. However, they did not report any detailed information on the chemical composition of the surface, i.e. the factor affecting protein conformation, which regulates how the cell and the protein interact. Moreover, the above mentioned authors did not differentiate between the early stage of the cell-protein interaction, i.e. adhesion, and the cell spreading process.

Studies of the interaction between PC-3 cells and the ECM proteins have been, and are still being, carried out using matrigel membranes [32–35]. Investigations of this kind cannot provide any data on the mechanism of the interaction of cells with respective proteins. What is more, the three-dimensional structure of matrigels may have an effect on the change in protein conformation and could result in the partial blocking of the fragment of the fibronectin central cell-binding domain (CCBD). This is why the results concerning the mechanism of the cell-fibronectin interaction obtained in two-dimensional and three-dimensional systems can hardly be compared.

The relations between the physicochemical properties of surfaces (especially polarity) and the conformation of the adsorbed protein play an essential role in understanding the process of cell-to-ECM adhesion, and, consequently, elucidate the mechanisms that regulate cell functions.

In this work, we were the first (to the best of our knowledge) to investigate the interaction of prostate cancer PC-3 cells with fibronectin adsorbed on sulfonated (i.e. hydrophilic) polystyrene surfaces of a defined chemical composition and topography. Fibronectin conformation adsorbed on sulfonated surfaces was quite similar to that which occurs in *in vivo* conditions. Since the invasiveness of cancer cells depends on the mechanism of the interaction between these cells and the proteins of the extracellular matrix, our investigations focused on processes associated with cancer progression such as cell adhesion and spreading. The response of PC-3 cells to the signal from fibronectin was determined by qualitatively and semi-quantitatively estimating cell adhesion and spreading.

The main aims of the present study were, firstly, to measure cell adhesion and the dynamics of cell spreading, and secondly, to estimate the organization of cytoskeleton proteins in the adhering cells.

Material and methods

Cell culture. The epithelial human prostate cancer PC-3 cell line derived from bone metastases [36] was used in our experiments. Medium RPMI-Glutamax was supplemented with 1% antibiotic PSN (Penicillin-Streptomycin-Neomycin) solution and heat-inactivated 10% fetal bovine serum (FBS). The cells were cultured at 37°C in a humidified 5% CO_2 atmosphere. For experiments, PC-3 cells were grown for three days, until the cell culture attained 70–80% confluence. For plating, cells were treated with a 0.25% solution of trypsin-EDTA. Cell survival was tested by cell morphology and 0.4% Trypan Blue solution (Sigma-Aldrich): a score of at least 95% was obtained. The cell concentration was approximately 0.5×10^6 cells/ml. The cells were suspended in a medium supplemented with 10% FBS, and incubated for 1 h at 37°C. Subsequently, the cells were suspended in a medium containing 1% BSA (Jackson ImmunoResearch) diluted in PBS (pH 7.4, Sigma-Aldrich). All other chemicals used in cell culture were from Gibco.

Preparation of polystyrene films. Polymerization of styrene (Merck) was performed by the solvent method in toluene (POCH), using benzoyl peroxide (Fluka) as a radical initiator. The polystyrene solution after dilution with toluene to 5% concentration was deposited onto specially cleaned glass

microscope cover slips. The transparent polymer films thus obtained were dried for 48 hrs at 37°C.

Sulfonation of polystyrene surfaces. The surfaces of polystyrene films were sulfonated according to the original method previously described [22, 37]. The sulfonation reaction was carried out in a water-free atmosphere at 18°C with sulfur trioxide, a highly active sulfonating agent at the gas/polymer interface. The chemical analysis of polystyrene surfaces by X-ray photoelectron spectroscopy has shown that sulfur was found to occur only in $-SO_3H$ groups bonded covalently to carbon atoms [38]. As a result of this reaction, the $-SO_3H$ groups were introduced onto the surface, and the surface wettability significantly increased. The characteristic properties of polystyrene surfaces were as follows: contact angle with water ≤ 12°, interfacial surface tension ≤ 71.4 erg/cm, sulfonic group density equal to 4×10^{14}/cm^2 [39] and surface roughness equal to 0.25 nm [14].

Fibronectin isolation. Fibronectin was isolated and purified from fresh human plasma following the method described by Poulouin et al. [40], and stored at 8°C in a 50 mM Tris/HCl buffer containing 1 mM EDTA (pH 7.4, Sigma-Aldrich). The samples were highly pure (Figure 1) and chemically stable up to 19 days.

The concentration of FN in PBS, c_{FN}, used in our examinations was in the range of 1, 3, 5, 10, and 20 μg/ml; the respective fibronectin surface density σ^{FN} was in the range of 25, 75, 120, 250, and 500 ng/cm^2 (see Figure 2) [13]. In the routine experiments, c_{FN} was equal to 20 μg/ml (σ^{FN} = 500 ng/cm^2).

Experimental conditions. The measurements of cell adhesion and observations of cell spreading were carried out under static conditions at a stable temperature of 37 ± 0.5°C, with an inverted Nikon Diaphot 300 microscope. A chamber (540 μm height) consisting of a microscope slide with a hole and two microscope cover slips was used to measure cell adhesion and spreading. The chamber bottom consisted of a cover slip coated with a polymer film. In the experiments with the shortest cell-substratum interaction time (t_{int} = 30 and 60 s), another type of chamber (120 μm height) was used, as previously described in detail [41]. The cell concentration was 0.5×10^6/ml.

FN adsorption was performed for 1 h at 37°C. After the adsorption, the surfaces were rinsed 3 times with PBS. Then, nonspecific binding sites of the surface were blocked for 30 min by 1% BSA diluted in PBS. The conductivity of the water used in these experiments was below 1 μS/cm.

Cells were routinely examined with phase contrast optics and an objective of × 20. Approximately 70 cells were counted per area of 300 μm^2 for each microscopic field.

Measurements of cell adhesion. Cells were introduced into the chamber and interacted with adsorbed fibronectin for a time t_{int} (definition below) equal to 30 s, 1, 3, 4 or 12 min,

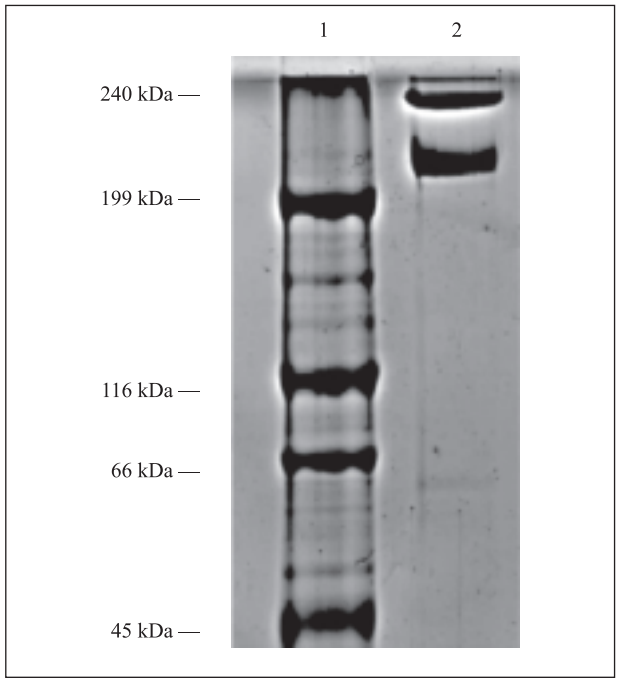

Figure 1. SDS-PAGE analysis of FN purity. Line 1 — a standard of molecular weights; Line 2 — fibronectin fraction eluted using heparin affinity chromatography

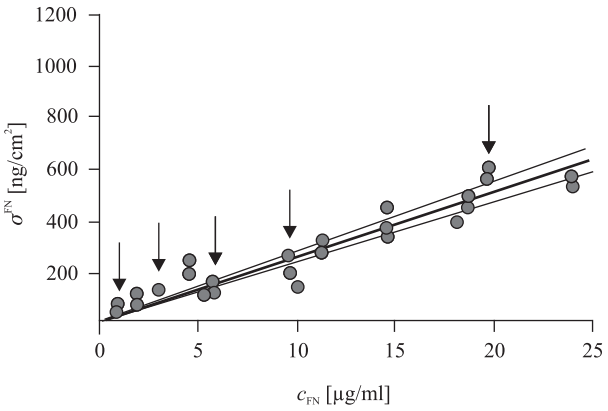

Figure 2. Radioisotope-derived (^{125}I-FN) isotherms for FN adsorption on sulfonated polystyrene surfaces. Linear fitting formula is $\sigma^{FN} = 24.8\, c_{FN}$. σ^{FN} denotes the total FN surface density [15]; c^{FN} denotes FN concentration in solutions. Arrows show σ^{FN} used in the present study

during which the cell adhesion took place. Next, the chamber was gently inverted, and the number of adhering cells was recorded in each microscopic field.

The cell-substratum interaction time t_{int} is defined as the time interval from the moment when the first sedimented cells become sharp in the focus plane at the bottom of the chamber, until the moment when the chamber is inverted (when the adhesion is measured) or until the moment when the observation (e.g. of the spreading) is performed.

The measure of cell adhesion is a dimensionless quantity a defined as the ratio of the number of cells adhered to

the substratum (within t_{int}, under static conditions) to the total number of sedimented cells. The adherent cells are those remaining on the surface when the gravitational force acts in the direction opposite to that of the adhesion forces.

Dynamics of cell spreading. The dynamics of cell spreading and also the cell shape during this process were examined using phase contrast microscopy. Cells interacted with fibronectin when $0 \le t_{int} \le 24$ h. Automatic sequential microscopic image registration and registration in selected time intervals were carried out with the use of a Lucia program. Sequences of the images of cells interacting with protein within 15 min time intervals were registered in the microscopic field. The number of cells spread within various cell-substratum interaction times was measured.

The adhered cells were observed as their projections on the substratum plane. Based on the observations of the form of cell projections on the plane and in comparison with the circle, the adhered cells were divided arbitrarily into two qualitative classes as follows: non-spread (and thus being still quasi-spherical) cells, and spread (and thus non-spherical) cells. Next, the number of quasi-spherical and non-spherical cells within the time interaction with fibronectin $0 \text{ h} \le t_{int} \le 24$ hrs was counted.

The quantity *QS* is defined as the ratio of the number of quasi-spherical cells to the total number of adhered cells in the microscopic field.

The quantity *NS* is defined as the ratio of the number of non-spherical cells to the total number of adhered cells in the microscopic field. Cells which belonged to the class of non-spherical cells were grouped into three subclasses (discoidal, quasi-triangle and quasi-rhomboidal, and elongated). (Morphometric analysis of the cell shape is in preparation.)

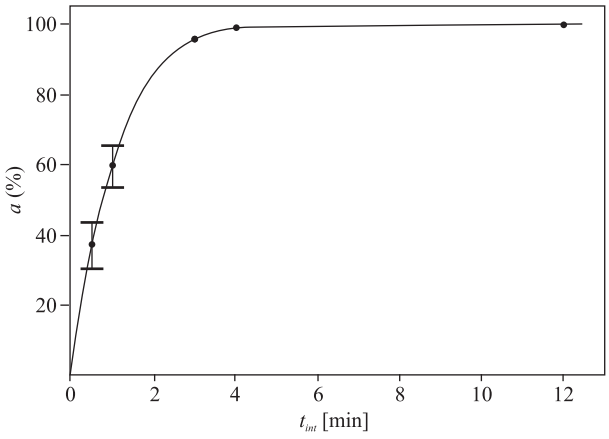

Figure 3. Adhesion of cells to FN adsorbed on sulfonated polystyrene surfaces as a function of the cell-substratum interaction time t_{int}. The measure of adhesion *a* is expressed here in percentages (n = 28). FN surface density $\sigma^{FN} = 500$ ng/cm^2. Error bars (corresponding to standard deviations) are not shown when they are smaller than the circles

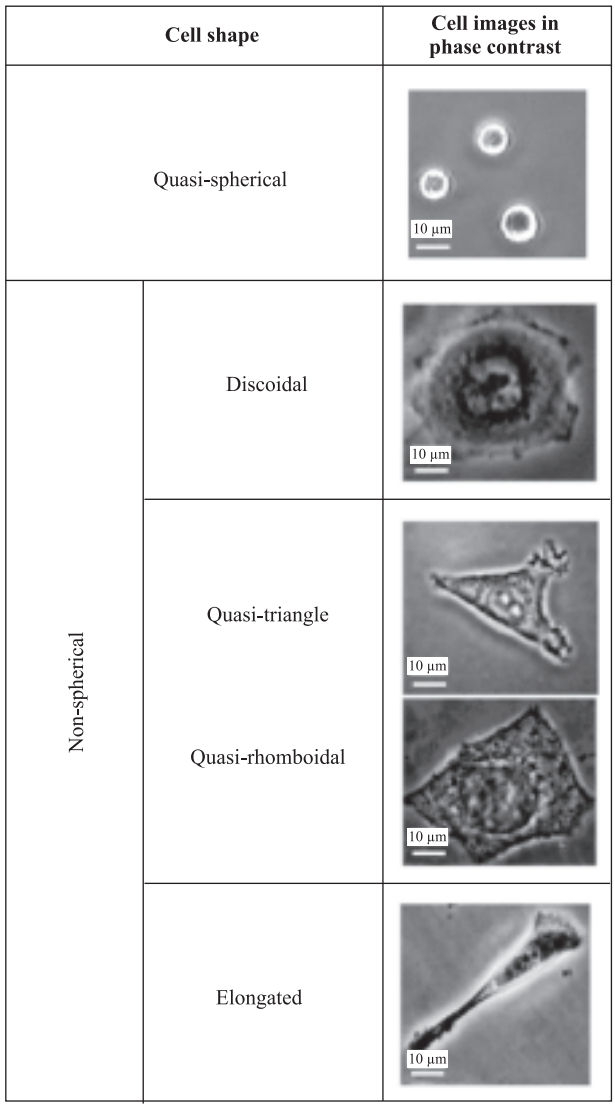

Figure 4. Classes and subclasses of cells interacting with FN adsorbed on sulfonated polystyrene surfaces. Examples of cell images obtained using phase contrast microscopy. The cell-substratum interaction time t_{int} was equal to 4 h; FN surface density $\sigma^{FN} = 500$ ng/cm^2

Staining of cytoskeletal proteins. Following the time of cell interaction with protein, the cells were washed with PBS, fixed in 4% paraformaldehyde (Sigma-Aldrich) in PBS for 15 min and next washed with PBS. Fixed cells were permeabilized with 0.2% Triton X-100 (Sigma-Aldrich) in PBS for 10 min and washed with PBS again.

For F-actin staining, the cells were incubated in a 1 μg/ml FITC-Phalloidin solution (Sigma-Aldrich) in PBS, for 1 h.

For α-actinin, tubulin, vinculin and FAK kinase staining, the cells were incubated with anti-proper cytoskeletal proteins and FAK kinase monoclonal antibodies (Sigma-Aldrich) for 1 h. Ig dilutions were: 1:40 (α-tubulin staining); 1:200 (α-actinin staining); 1:50 (vinculin staining) and 1:400 (kinase FAK staining). Then, cells were washed with PBS and incubated with the IgG secondary antibody (Sigma-Aldrich) conjugated to TRITC (α-actinin, tubulin, vin-

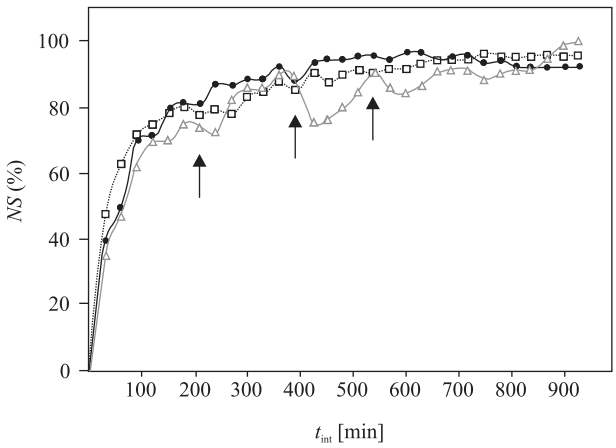

Figure 5. Spreading dynamics of cells interacting with FN adsorbed on sulfonated polystyrene surfaces. The relative number of non-spherical cells *NS* is expressed here in percentages (n = 3). FN surface density σ^{FN} = 500 ng/cm^2; t_{int} denotes the cell-substratum interaction time. Arrows show decrease in the number of non-spherical cells. Individual curves represent results obtained in three independent experiments

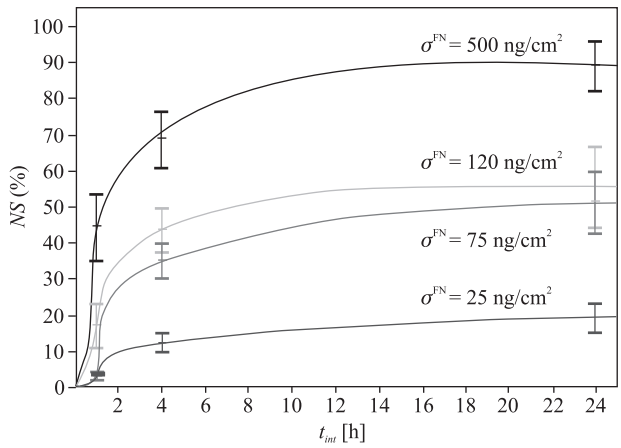

Figure 7. Spreading of cells interacting with FN adsorbed on sulfonated polystyrene surfaces as a function of the cell interaction time t_{int} and the fibronectin surface density σ^{FN}. The relative number of non-spherical cells *NS* is expressed here in percentages. Measurements were carried out for FN surface density σ^{FN} = 25, 75, 120 and 500 ng/cm^2 for t_{int} = 1, 4, and 24 hrs. For each t_{int} the number of measurements n was equal to 20

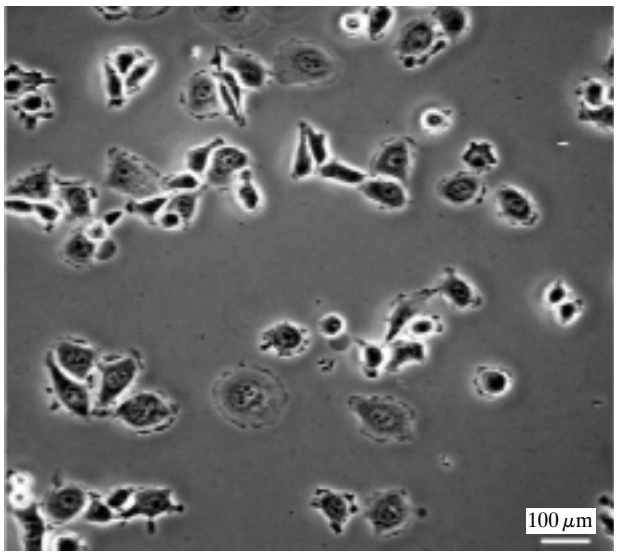

Figure 6. Cells interacting with FN adsorbed on sulfonated polystyrene surfaces. Image obtained using phase contrast microscopy. The cell-substratum interaction time t_{int} was equal to 4 h; FN surface density σ^{FN} = 500 ng/cm^2

culin staining; 1:64 dilution) or conjugated to FITC (FAK kinase staining; 1:40 dilution) for 1 h. The whole staining procedure was carried out at room temperature.

Image analysis of fluorescent stained cells. Cell images were obtained by an inverted Diaphot 300 microscope connected to a CCD camera (Bischke) and a frame-grabber (Matrox Meteor). Fluorescent stained cells were examined using an objective of × 100.

Cell image analysis was carried out using the *Bitanal* program prepared by Inkielman [42]. This program made it possible to acquire good-quality images of optical slices of the cell and to localize fluorescent structures in each of these slices. The distribution of cytoskeletal proteins and FAK kinase in the adhering cells was determined in sequences of fluorescent images of cell slices, obtained by focal plane shifts in 2.0 μm intervals. Special procedures were applied to enhance the image distinctness, such as the correction of the brightness intensity distribution and the contrast, the reduction of the background, and the deconvolution of images resulting in the elimination of out-of-focus information. Images of the optical slice at the cell-substratum interface are shown in the Results (Figures 8–14).

Statistical analysis. The values *a* occurring in this study are mean values of n measurements (n = 28) for each t_{int} obtained in at least four independent experiments. The dynamics of cell spreading was examined in three independent automatic sequential microscopic image registrations. The values *NS* obtained in selected time intervals t_{int} are mean values of *n* measurements (n = 20). For statistical evaluations, a Statistica 7.1 (Statsoft) program was applied. The statistical testing of experimental results distributions was verified by the Shapiro–Wilk test. Comparison of the mean values (within a variable) and the statistical significance of the differences between group means were performed using the Student's *t*-test. The data was reported as mean ± SD. A value of $p < 0.05$ was considered to be statistically significant.

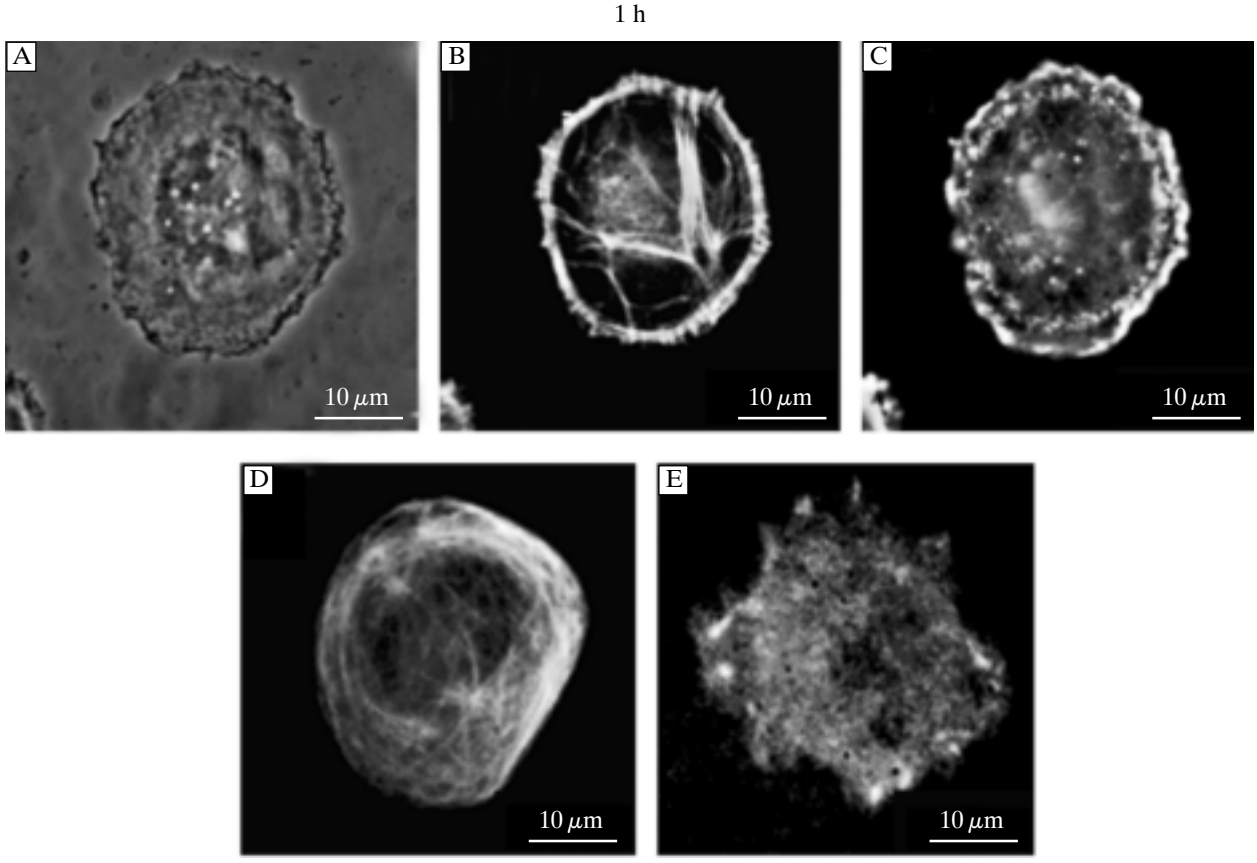

Figure 8. Pattern of localization of cytoskeletal proteins in discoidal cells adhering to FN adsorbed on sulfonated polystyrene surfaces within t_{int} = 1 h. FN surface density σ^{FN} was equal to 500 ng/cm^2. Cells examined by phase contrast (**A**). Localization of F-actin (**B**), α-actinin (**C**), tubulin (**D**) and vinculin (**E**). For F-actin visualization, fluorescence staining was carried out with FITC-Phalloidin (**B**). Immunofluorescence labeling for α-actinin was performed with monoclonal anti-α-actinin antibody (**C**); for tubulin with monoclonal anti-tubulin antibody (**D**); for vinculin with monoclonal anti-vinculin antibody (**E**), and subsequently with a TRITC-conjugated secondary antibody. Images of the optical slice at the cell-substratum interface. The number of individual cells analyzed was equal to 120

Results

Cell adhesion to fibronectin

In the initial phase, cell adhesion to fibronectin adsorbed on sulfonated polystyrene surfaces depends on the interaction time when $0 \leq t_{int} \leq 3$ min (Figure 3). When t_{int} reached 30 s, about one in three of the sedimented cells underwent adhesion, and when t_{int} was 3 min, approximately the entire population of cells was in adhesion. These results showed that under static conditions, the initial phase of cell adhesion to fibronectin is very dynamic.

No effect of fibronectin surface density on cell adhesion was found when σ^{FN} = 25, 75, 120 or 500 ng/cm^2.

The dynamics of cell spreading

In the present work, we examined not only cell adhesion but also the further stage of cell interaction with protein, i.e. cell spreading. The adhering cells were observed as projections on the substratum plane. Among these cells, we distinguished two qualitative classes with respect to the form of cell projections i.e. non-spread (and thus still quasi-spherical) cells and spread (and thus non-spherical) cells. Cells which belong to the non-spherical class were grouped into three subclasses: (1) discoidal; (2) quasi-triangle and quasi-rhomboidal; and (3) elongated (Figure 4).

We observed that in the early phase of cell spreading (within t_{int} = 30 min), the formation of thin and elongated fillopodii appeared. It may be that surface deformations and membrane undulations are displayed by these adhering cells in their response to fibronectin on the polystyrene surface [43]. The number *NS* of non-spherical cells increases in time and approaches the value of approximately 100% at t_{int} = 12 h (Figure 5).

The majority of cells appeared to be discoidal in shape with a characteristic ring of lamellar cytoplasm (Figure 6). A smaller part of the non-spherical cells

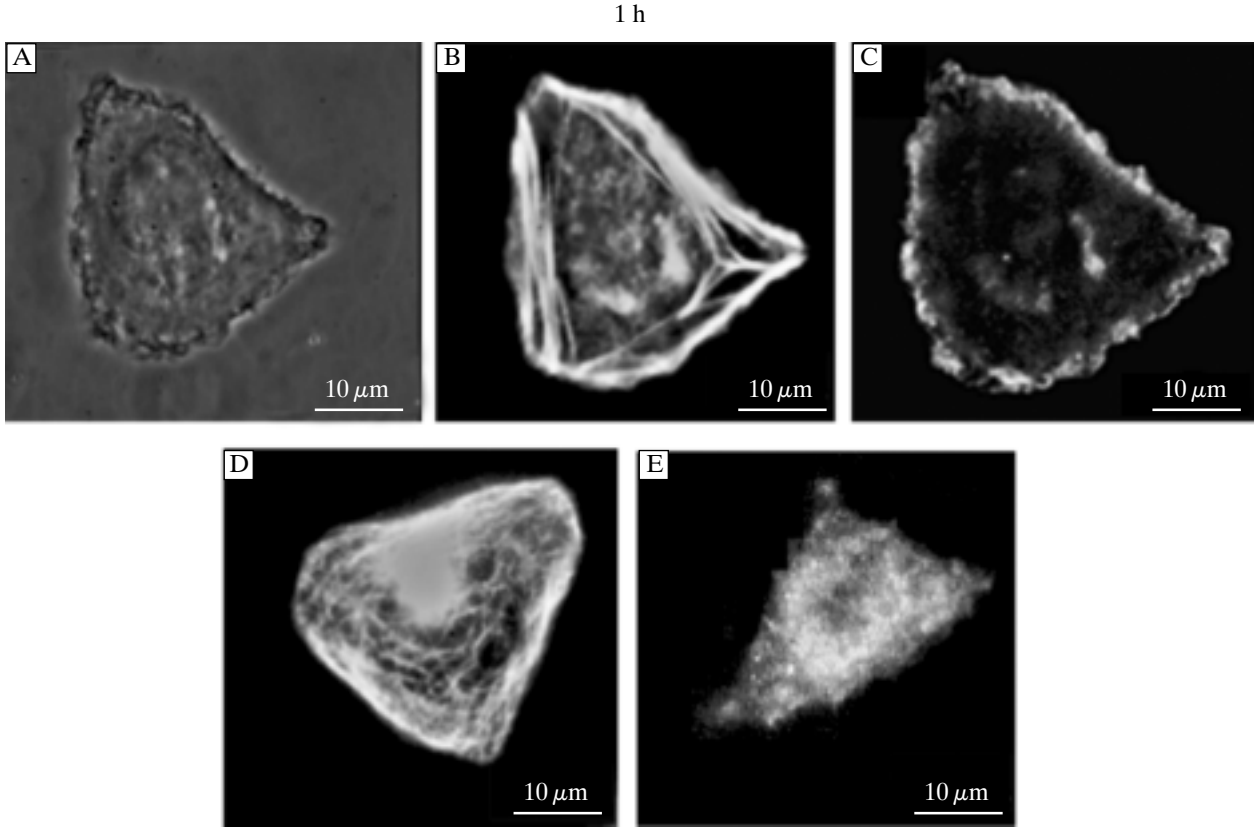

Figure 9. Pattern of localization of cytoskeletal proteins in quasi-triangle and quasi-rhomboidal cells adhering to FN adsorbed on sulfonated polystyrene surfaces within t_{int} = 1 h. FN surface density σ^{FN} was equal to 500 ng/cm^2. Cells examined by phase contrast (**A**). Localization of F-actin (**B**), α-actinin (**C**), tubulin (**D**) and vinculin (**E**). Staining of cells for visualization of individual proteins performed as described in the caption to Figure 7. Images of the optical slice at the cell-substratum interface. The number of individual cells analyzed was equal to 120

displayed another type of shape, e.g. quasi-triangle and quasi-rhomboidal, which may then change into an elongated shape.

At selected time intervals, it was observed that the number *NS* of non-spherical cells decreased, while that of quasi-spherical cells (*QS*) and the total number of cells increased (Figure 5). These results strongly suggest that cells exhibit an ability to proliferate.

Our results have shown that the dynamics of cell spreading depends on the fibronectin density σ^{FN} on polystyrene surfaces. Measurements were performed for σ^{FN} = 25, 75, 120, and 500 ng/cm^2 and t_{int} = 1, 4, 24 hrs (Figure 7).

Cytoskeleton organization in adhering cells

The analysis of fluorescent images of the adhered cells revealed that, as a result of cell interaction with fibronectin, induction of a signal cascade takes place, which leads both to the reorganization of cytoskeletal proteins (F-actin, α-actinin, vinculin and tubulin) and to the activation of FAK kinase (Figures 8–13). F-actin concentration at the sites of adhesion of the cells with fibronectin on the substratum surface was observed. In the case of quasi-spherical cells, F-actin was concentrated in the tips of microvilli at the cell-substratum interface and accumulated near the cell membrane region (Figure 14). In the case of non-spherical cells, F-actin was organized into a network with formed filament bundles accumulated near the cell membrane region and in the lamellipodium and filopodium edges. The formation of pronounced F-actin stress fibers suggests the existence of a strong interaction of cells with fibronectin adsorbed on polystyrene surfaces.

The α-actinin and vinculin were localized in the areas of high F-actin concentration at the sites of contact of cells with the substratum surface. However, pronounced vinculin reorganization was observed within t_{int} = 4 h.

Tubulin fibers were observed in the whole cell, but this protein was predominantly concentrated in the perinuclear area. Examples of cytoskeletal protein reorganization of the cells adhered to fibronectin are shown in Figures 8–13.

In the case of quasi-spherical cells, poorly-formed clusters of FAK protein were found in the whole cell. In contrast, for non-spherical cells, a change in ki-

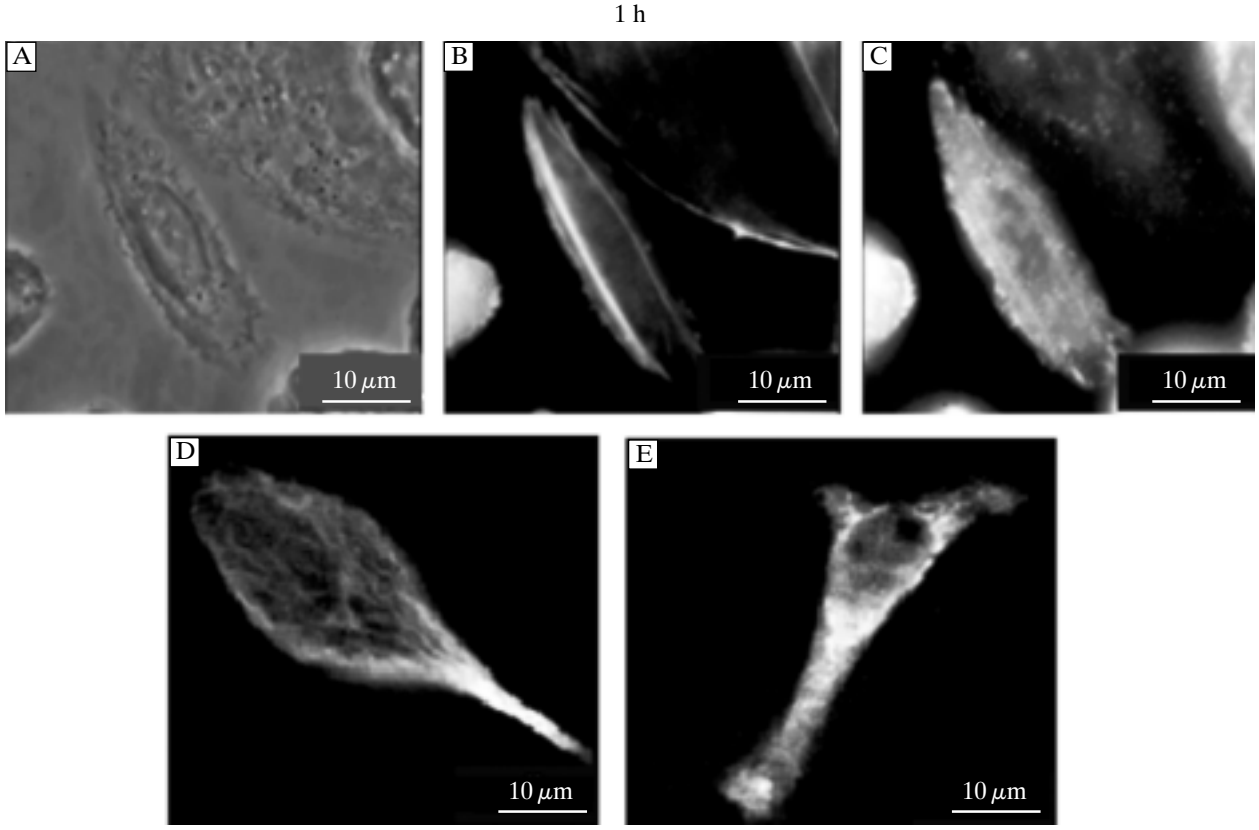

Figure 10. Pattern of localization of cytoskeletal proteins in elongated cells adhering to FN adsorbed on sulfonated polystyrene surfaces within t_{int} = 1 h. FN surface density σ^{FN} was equal to 500 ng/cm^2. Cells examined by phase contrast (**A**). Localization of F-actin (**B**), α-actinin (**C**), tubulin (**D**) and vinculin (**E**). Staining of cells for visualization of individual proteins performed as described in the caption to Figure 7. Images of the optical slice at the cell-substratum interface. The number of individual cells analyzed was equal to 120

nase distribution was observed. The FAK kinase was localized in areas of F-actin concentration, predominantly in focal adhesions, although their clusters were observed also in perinuclear areas in deeper cell slices. These results suggest that FAK kinase concentrations represent sites of cell-fibronectin interaction (Figures 11F and 12F).

Summing up, the pronounced F-actin, α-actinin, vinculin and tubulin reorganization at adhesion sites of cells with fibronectin, in the cell region near the cell--substratum interface, as well as the FAK-kinase activation, suggest that a strong interaction of cells with fibronectin exists. These results have shown, as expected, that cell response to the signal from fibronectin leads to the induction of the signaling cascade.

Discussion

One of the fundamental objectives of cell biology is to study the relationship between cell functions and the ability of cells to interact with the components of an extracellular matrix. Due to its ability to become bonded to more than 20 integrins on cell surfaces, fibronectin is known to play a very important role in normal and pathological processes, including those involving the formation of metastases [3, 6, 44].

The formation of metastases is a multi-stage process, in which adhesive properties and carcinoma cells' ability to actively migrate constitute important factors influencing cell invasiveness. Among the various cancer diseases, prostate cancer is one of the most mortal in men. Early detection makes it possible to treat prostate cancer effectively. However, in men with proved metastases, the chances of recovery are reduced. *In vitro* studies on cell lines have shed light on the pathogenesis of prostate cancer. One of the cell lines investigated is that of PC-3 epithelial cells originating from bone metastases, characterized by a high degree of invasiveness and resistance to hormonal therapy. The heterogeneity of PC-3 cells, in contrast to other prostate cancer lines [45], may lead to the formation of subpopulations with high plasticity and variability in response to factors blocking proliferation. This is why the strong invasive ability of the PC-3 cell line seems to provide a proper model for investigating cancer progression [45, 46].

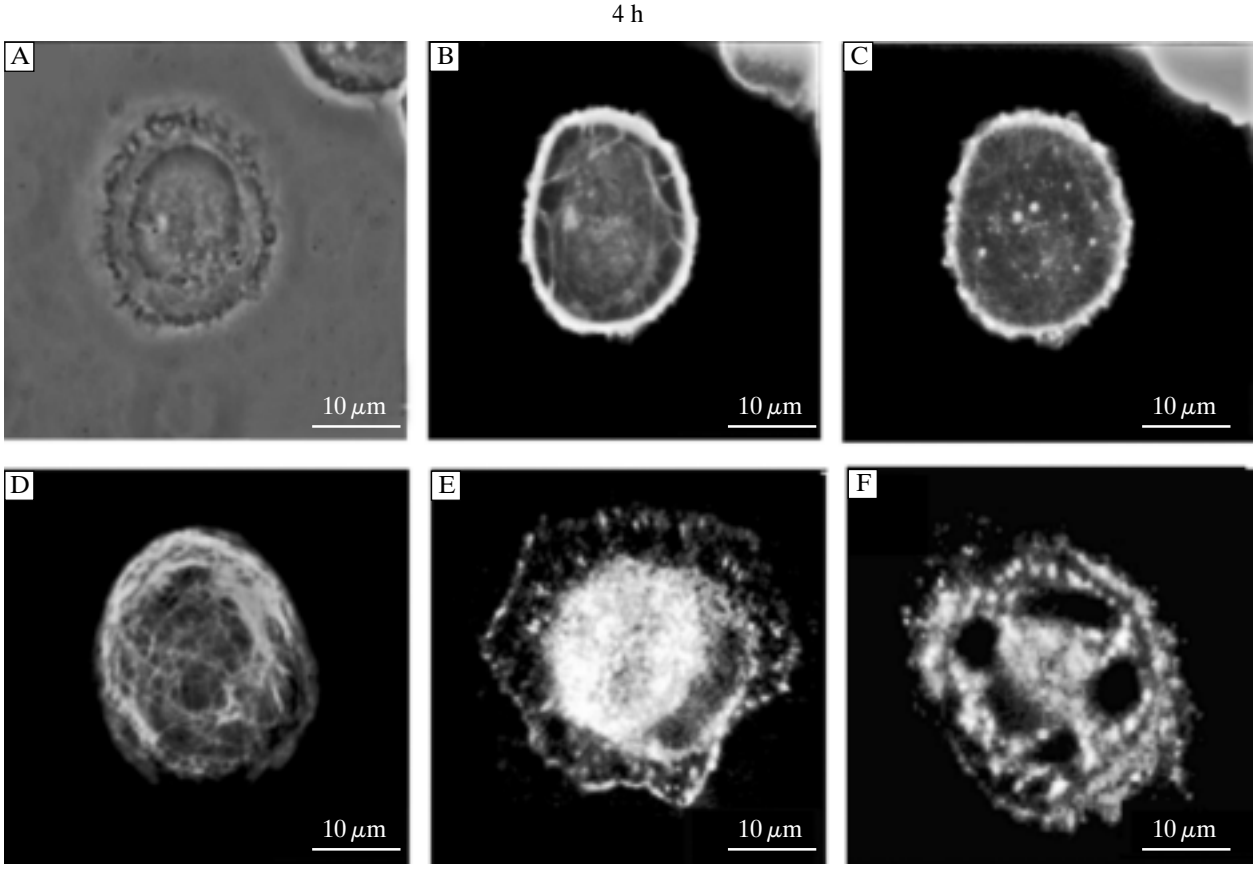

Figure 11. Pattern of localization of cytoskeletal proteins and FAK kinase in discoidal cells adhering to FN adsorbed on sulfonated polystyrene surfaces within t_{int} = 4 h. FN surface density σ^{FN} was equal to 500 ng/cm^2. Cells examined by phase contrast (**A**). Localization of F-actin (**B**), α-actinin (**C**), tubulin (**D**), vinculin (**E**), and FAK kinase (**F**). Staining of cells for visualization of individual cytoskeletal proteins performed as described in the caption to Figure 7. Immunofluorescence labeling was carried out with monoclonal anti-FAK kinase antibody and subsequently with FITC-conjugated secondary antibody, to visualize this protein (**F**). Images of the optical slice at the cell-substratum interface. The number of individual cells analyzed was equal to 120

A relatively small number of reports have been published on the interaction between PC-3 cells and single proteins adsorbed on planar substrata of well-characterized chemical composition and known surface topography. The studies carried out by Zheng et al. [30] and Cooper et al. [31] revealed that PC-3 cells interact with fibronectin and other proteins such as collagen or laminin. However, they did not report any data on the chemical composition of the substratum surface with which the cells interact, i.e. on the important parameter affecting protein conformation which has a role in the process of cell-protein interaction. The authors quoted above used the term 'adhesion' without distinguishing the early stage of protein interaction (in other words, *adhesion*), from the later stage of interaction, referred to as *spreading*.

Interesting results that have been reported in the literature on the interaction between PC-3 cells and proteins were carried out using matrigel membranes imitating ECM [32–35]. However, these results do not seem to provide a better understanding of the mechanism of interaction of cells with single proteins.

To the best of our knowledge, the present work is the first attempt to describe the interaction of PC-3 cells with biologically active human plasma fibronectin adsorbed on strongly polar polystyrene surfaces of a defined chemical composition and topography [14, 22].

In the earlier studies by Kowalczyńska et al. [12, 13], it was shown that sulfonic groups on styrene copolymer surfaces have a stimulatory effect on the adhesion of L1210 and 3T3 cells. In the case of 3T3 cells, the number of adhering cells correlated, while the number of non-spherical cells inversely correlated, with the surface density of sulfonic groups [17]. It was also found that the surface density of sulfonic groups has an effect on the reorganization of cytoskeleton proteins, both in the adhered normal and in cancer cells [13, 17, 21, 26].

In this work, we have investigated the early stage of cell adhesion to fibronectin, and, thanks to the use of automatic, sequential microscopic image registration, we are

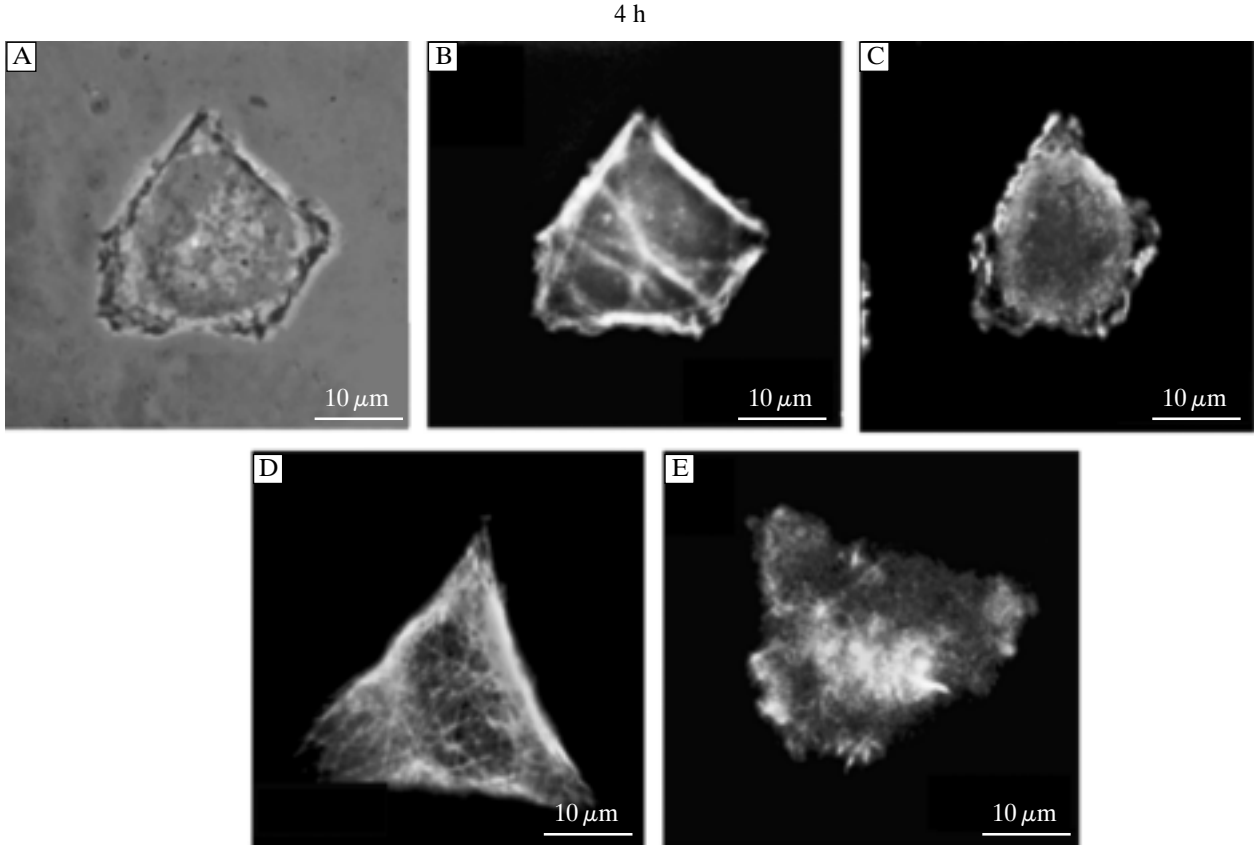

Figure 12. Pattern of localization of cytoskeletal proteins in quasi-triangle and quasi-rhomboidal cells adhering to FN adsorbed on sulfonated polystyrene surfaces within t_{int} = 4 h. FN surface density σ^{FN} was equal to 500 ng/cm^2. Cells examined by phase contrast (**A**). Localization of F-actin (**B**), α-actinin (**C**), tubulin (**D**) and vinculin (**E**). Staining of cells for visualization of individual proteins performed as described in the caption to Figure 7. Images of the optical slice at the cell-substratum interface. The number of individual cells analyzed was equal to 120

the first to present observations of the dynamics of PC-3 cell spreading and the cell shape during this process. It has been shown that the dynamics of cell spreading depends on the surface density of fibronectin. Moreover, the results of our studies on the interaction of PC-3 cells with fibronectin have confirmed earlier studies showing that fibronectin conformation adopted on sulfonated polystyrene surfaces stimulates cell adhesion.

The ability of cancer cells to interact with other cells and ECM proteins is an important feature of cancer progression, combined with changes in the cell shape resulting from the response of the cell to the signal from the substratum leading to the reorganization of cytoskeleton proteins, migration and proliferation. It can be assumed that a qualitative analysis of cytoskeletal protein organization in the cancer cells interacting with the ECM protein may reflect the degree of cancer cell heterogeneity, which in turn may contribute to a better knowledge of the mechanism of cell-ECM interaction [47–49]. Some authors have pointed out the effect of physicochemical properties of the substratum on the changes in cell shape and on the reorganization of cytoskeleton proteins in the adhering cells [50, 51].

Analysis of the cytoskeleton protein localization has revealed their reorganization in the PC-3 cells adhered to fibronectin adsorbed on the polystyrene surface. The results have shown that F-actin is organized in the form of stress fibers and is grouped at sites of cell-fibronectin adhesion. Moreover, α-actinin and vinculin, integrin-binding proteins with actin filaments, were found at sites of F-actin concentration. It has been reported that the expression of these proteins may affect the ability of cells to migrate [52–54].

In our study a large concentration of one of the key regulators of cell adhesion and migration, i.e. FAK kinase was found at sites where F-actin occurred in focal contacts, and in deeper layers in the perinuclear region. Enhanced expression and FAK protein activation was observed in cancer cells featuring a high degree of invasiveness [55, 56], and also in PC-3 cells as reported by Slack et al. [57], and Guan and Shalloway [58].

The large number of PC-3 cells adhering to fibronectin in a relatively short period of time, the high dynamics of the cell spreading and strong reorganization of cytoskeletal proteins in the adhering cells shown in this work, as well as the established ability of cells to

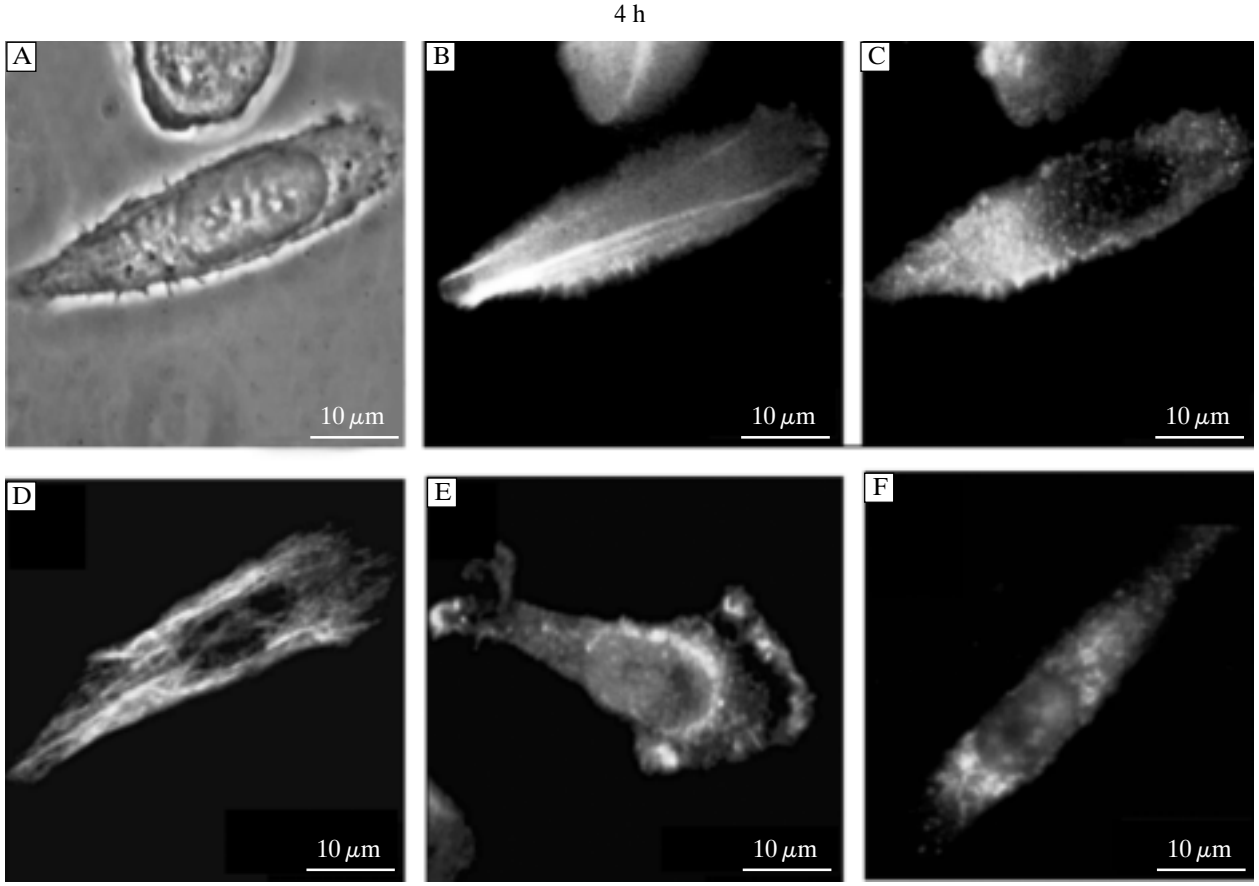

Figure 13. Pattern of localization of cytoskeletal proteins and FAK kinase in elongated cells adhering to FN adsorbed on sulfonated polystyrene surfaces within t_{int} = 4 h. FN surface density σ^{FN} was equal to 500 ng/cm^2. Cells examined by phase contrast (**A**). Localization of F-actin (**B**), α-actinin (**C**), tubulin (**D**), vinculin (**E**) and FAK kinase (**F**). Staining of cells for visualization of individual cytoskeletal proteins performed as described in the caption to Figure 7, and for FAK kinase visualization as described in the caption to Figure 10. Images of the optical slice at the cell-substratum interface. The number of individual cells analyzed was equal to 120

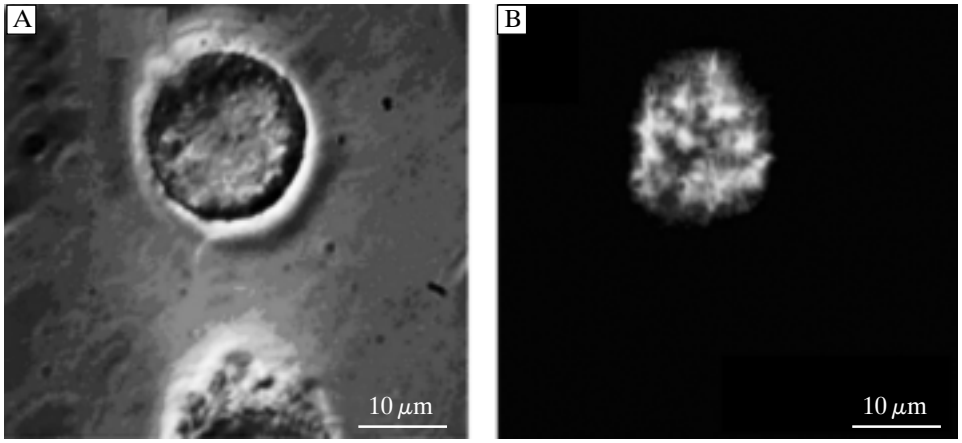

Figure 14. Pattern of F-actin localization in quasi-spherical cell adhering to FN adsorbed on sulfonated polystyrene surfaces. The cell-substratum interaction time t_{int} was equal to 15 min; FN surface density σ^{FN} was equal to 500 ng/cm^2; cell examined by phase contrast (**A**). For F-actin visualization, fluorescence staining was carried out with FITC-Phalloidin (**B**). Image of the optical slice at the cell-substratum interface. The number of individual cells analyzed was equal to 120

proliferate [see also 21], seem to indicate that the interaction process between PC-3 cells and fibronectin is both efficient and specific. These results add substantial weight to the claim that, due to the conformation of fibronectin molecules on the hydrophilic polystyrene surface, the cell-binding domain is exposed and well-recognized by fibronectin-specific integrins, which are found on the surface of PC-3 cells.

The PC-3 line is characterized by strong phenotype heterogeneity. Kaighn et al. [36] were the first to describe this cell heterogeneity of the PC-3 cell line, and the later study by Festuccia et al. [45] showed that it is the cell culture conditions and some experimental conditions such as the composition of matrigels that have a significant effect on cell heterogeneity. Line heterogeneity is reflected in the different degrees of adhesion ability and the different expression of specific receptors. The study by Tantivejkul et al. [59] confirmed that the morphological heterogeneity of PC-3 cells depends on the composition of the extracellular matrix. This is why the PC-3 line, due to its morphological heterogeneity and, consequently, functional differentiation, seems to be the best fit as a model for investigating prostate cancer progression.

Better understanding of the activation of the signal paths responsible for various PC-3 cell functions in the interaction with fibronectin, i.e. the ECM protein, may contribute to partial elucidation of the complex mechanism of prostate cancer cell functions. One might suppose that the investigations of the phenotype of PC-3 cells characterized by a high degree of variability will lead to a better knowledge of the function of these cells.

We have reason to hope that the adhesion and spreading processes analysis of the PC-3 cell line carried out in the present work has made it possible to better understand the biological nature of these cells. However, more research on cells' heterogeneity is needed.

Conclusions

The present work has focused on the following problem: the interaction (in a two-dimensional system) between the prostate cancer PC-3 cells and fibronectin adsorbed on sulfonated (i.e. hydrophilic) polystyrene surfaces of a defined chemical composition and topography.

We found that:

1. In the initial stage, cell adhesion is high and strongly dependent on the time of interaction with fibronectin.
2. The dynamics of cell spreading depends on the density of fibronectin adsorbed on polystyrene surface.
3. Reorganization of cytoskeleton proteins (F-actin, α-actinin, vinculin and tubulin) occurs at adhesion sites between the cell receptors and fibronectin, in the cell region near the cell-substratum interface, as well as the FAK kinase activation.

These results have shown, as expected, that cell response to the signal from fibronectin leads to the induction of the signaling cascade.

Acknowledgements

We are grateful to Jerzy Elbanowski M.Sc. for helpful discussions and for statistical computations. This work was supported by CMKP grant No 501-2-1-23--26/05 and partly by grant No 501-1-1-23-10/05.

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Submitted: 7 March, 2011
Accepted after reviews: 7 June, 2011