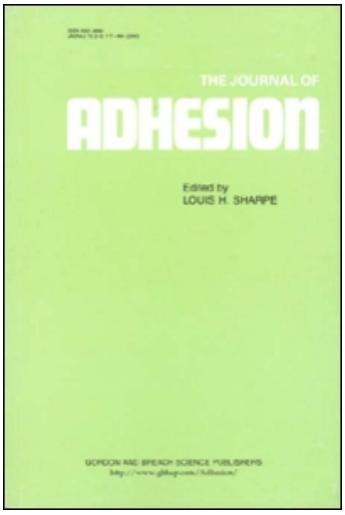

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CELL ADHESION TO POLYMER SUBSTRATES CHARACTERIZED BY THE MICROPIPETTE ASPIRATION TECHNIQUE

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A micropipette suction method was adapted for the characterization of cell adhesion to polymer surfaces. The instrument was applied in experiments probing the adhesion of human erythrocytes to polymer films which had been precoated with monoclonal antibodies against two different transmembrane proteins of the cells. Cells were impinged on the polymer substrates and subsequently removed by stepwise micropipette aspiration. Variations of shape and contact area of the cells during micropipette aspiration-driven detachment were evaluated to determine the separation energy. A strong increase of the separation energy with decreased contact area was observed and explained by the smoothing of the cell membrane at elevated membrane tensions. The results indicate that the overall strength of attachment was determined by the amount and availability of the adsorbed antibodies, while the separation of the cells from the polymer substrates occurred, in general, due to dislocation of the transmembrane proteins from the membrane.

Keywords: Cell adhesion; Micropipette; Glycophorin; Antibodies; Polymer

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One of a collection of papers honoring A. W. Neumann, the recipient in February 2004 of *The Adhesion Society Award for Excellence in Adhesion Science, Sponsored by 3M*.

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INTRODUCTION

Cell adhesion to artificial surfaces plays a key role in a wide variety of demanding products and technologies such as medical implants or bioreactor systems [1–3]. Accordingly, several experimental and theoretical approaches have been developed to unravel the mechanisms of cell-materials interactions and to identify the relevance of the surface characteristics of materials with that concern [4–6]. The latter is expected to enable the rational design of materials capable of inducing desired features of adherent cellular systems [7–11].

More and more knowledge was recently gained on the identification and functional characterization of the relevant cell adhesion molecules occurring associated with the cell membrane on the cellular side or as the extracellular matrix [12–17]. Beyond that, an increasing number of studies could convincingly demonstrate that mechanical forces arising due to the cell-matrix adhesion are most important, not only with respect to the cell anchorage but also for the activation of distinct cellular functions and even cellular fate decisions [18–22]. In that context, the experimental quantification of cell adhesion deserves paramount attention. Different concepts have been developed to probe the binding strength of cells to artificial surfaces, including a variety of shear stress experiments [23–28], centrifugation assays [29] and the application of optical tweezers [30].

Encouraged by experiments performed in the laboratories of A. W. Neumann [31, 32], we recently adapted a micropipette aspiration technique for the characterization of the detachment of elastically deformable cells from biomedical polymer substrates. The approach was pioneered earlier in several elegant biophysical studies by the group of E. Evans to unravel the mechanical properties of cell membranes and vesicle capsules as well as for the analysis of cell–cell or cell–vesicle interactions [33]. The method was, furthermore, recently extended to permit a novel type of experiment designated as dynamic force spectroscopy, which provides most valuable insights on the load-dependence of the strength of individual bonds between cellular receptors and their ligands [34].

A dedicated setup was designed, built, and tested to permit the suction-pressure–controlled separation of individual cells from polymer substrates. The instrument was utilized in a series of experiments probing the separation energy of erythrocytes from three different polymer thin films—cellulose, polystyrene, and a plasma-deposited fluoropolymer—where they had been attached *via* preadsorbed monoclonal antibodies against the membrane proteins Glycophorin A and Glycophorin C, respectively. By comparison of the separation energy

data with the surface concentrations of the adsorbed monoclonal antibodies, we were able to draw conclusions on the anchorage effect arising from the individual antigen–antibody bonds.

MATERIALS AND METHODS

Polymer Substrates

Thin films of cellulose, polystyrene, and a plasma-deposited fluoropolymer were prepared on top of glass beads (radius 20–50 μm) molten out at the tips of glass microcapillaries and on planar reference carriers (Si wafer). Cellulose (MW 200.000 $gmol^{-1}$, dissolved in dimethylacetamide/lithium chloride, kindly donated by AKZO Research Laboratories, Obernburg, Germany) was solution casted and subsequently rinsed with deionized water to remove the salt. Polystyrene (MW 55.000 $gmol^{-1}$, Polyscience, Warrington, PA, USA) was dissolved (2 wt% in tetrahydrofuran, Fluka, Deisenhofen, Germany) and solution casted, and the plasma-deposited fluoropolymer was prepared in a dedicated argon-glow process or from tetrafluoroethylene vapour at the Institute of Energy Problems of Chemical Physics (Chernogolovka, Russia) as described elsewhere [35]. The polymer films on the reference carriers were thoroughly characterized by means of ellipsometry (M45, Woolam, Lincoln, NE, USA), Fourier transform infrared spectroscopy in attenuated total reflections (ATR-FTIR) (FS66, Bruker, Karlsruhe, Germany), X-ray photoelectron spectroscopy (XPS) (Escalab 2, Kratos, Durham, UK), atomic force microscopy (AFM) (Nanoscope II, Digital Instruments, Santa Barbara, CA, USA), contact angle measurements (axisymmetric drop shape analysis, ADSA, in-house design according to Rotenburg *et al.* [36]) and electrokinetic measurements (EKA, A. Paar GmbH, Graz, Austria). Selected results of the film characterization reflecting important properties of the films with respect to protein adsorption are summarized in Table 1.

Antibodies

Monoclonal antibodies (IgG1) with specificity for Gylcophorin A (BRIC256) and Glycophorin C (BRIC4) were purchased from the International Blood Group Reference Laboratory (Bristol, UK) and diluted in phosphate-buffered saline (Sigma-Aldrich GmbH, Steinheim, Germany) for the adsorption experiments. The adsorbed amounts of BRIC256 and BRIC4 obtained by ellipsometry according to an evaluation procedure recently described in Werner *et al.* [37] for antibody

TABLE 1 Surface Characteristics of the Polymer Substrates and Adsorbed Amounts of Antibodies Used in the Cell Adhesion Experiments

	Cellulose (CE)	Polystyrene (PS)	Plasma-deposited fluoropolymer (PDFP)
Layer thickness/ refractive index	127.5 ± 0.3 nm 1.547 ± 0.02	126.15 ± 0.77 nm 1.532 ± 0.01	110.2 ± 0.42 nm 1.368 ± 0.02
R_a roughness	7.5 nm	2.1 nm	3.7 nm
Water contact angle	(captive bubble)	(sessile drop)	(sessile drop)
(advancing)	$56.7^\circ \pm 1.7^\circ$	$91.0^\circ \pm 0.3^\circ$	$118.9^\circ \pm 1.2^\circ$
(receding)	$23.6^\circ \pm 0.8^\circ$	$78.7^\circ \pm 0.8^\circ$	$100.0 \pm 0.9^\circ$
Isoelectric point (0.003 m KCl)	4.05 ± 0.05	4.05 ± 0.05	4.05 ± 0.05
Adsorbed amount of BRIC256/ 25 μg cm^{-3}	0.07 ± 0.015 μg cm^{-2}	0.11 ± 0.015 μg cm^{-2}	0.16 ± 0.015 μg cm^{-2}
Adsorbed amount of BRIC256/ 250 μg cm^{-3}	0.12 ± 0.015 μg cm^{-2}	0.28 ± 0.015 μg cm^{-2}	0.32 ± 0.01 μg cm^{-2}
Adsorbed amount of BRIC4/ 25 μg cm^{-3}	0.06 ± 0.015 μg cm^{-2}	0.12 ± 0.015 μg cm^{-2}	0.15 ± 0.015 μg cm^{-2}
Adsorbed amount of BRIC4/ 250 μg cm^{-3}	0.12 ± 0.02 μg cm^{-2}	0.27 ± 0.01 μg cm^{-2}	0.3 ± 0.01 μg cm^{-2}

solution concentrations of 25 and 250 μg cm^{-3} after 14 h at room temperature are given in Table 1.

Cells

Human erythrocytes were collected by dilution of 50 μl freshly drawn human whole blood obtained by finger-prick with phosphate-buffered saline (PBS) containing 0.5 wt% bovine serum albumin (Sigma, Deishofen, Germany) to prevent osmotic stress.

Cell Detachment Experiments

Referring to the experimental design applied in the earlier and extensive work of Evans [38], an inverted light microscope was modified and assembled with peripherals required to perform micropipette suction experiments [39]. The setup is schematically shown in Figure 1 and is designated as *Membrane Force Transducer Apparatus*, referring to Evans [38]. Micropipettes with tapered tips and

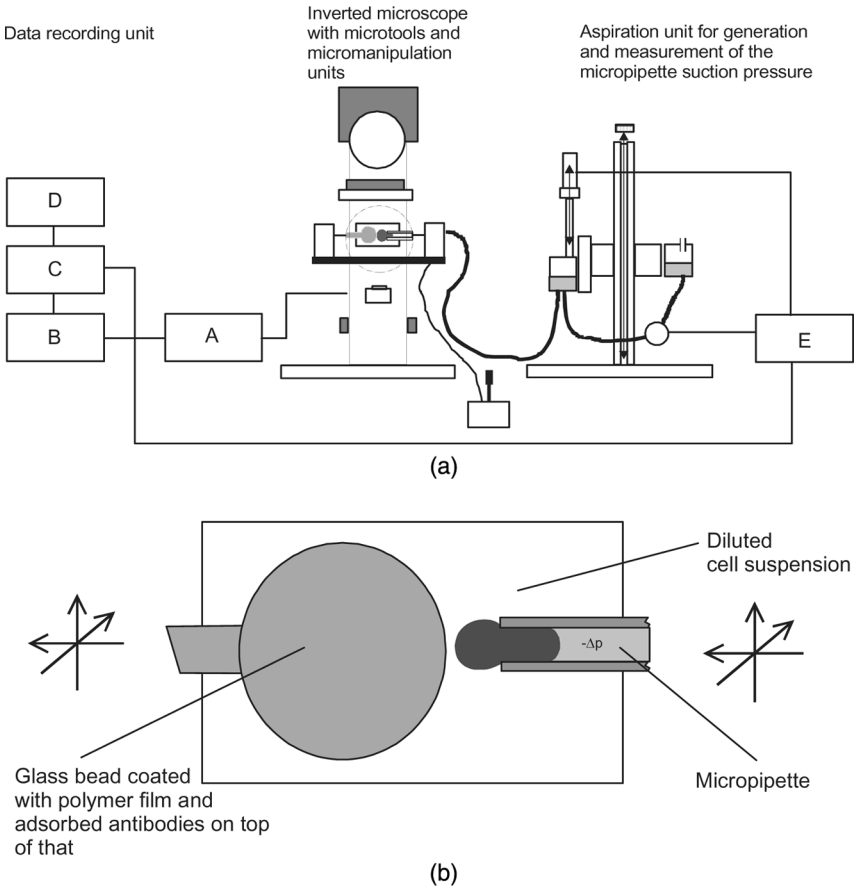


FIGURE 1 (a) Membrane force transducer apparatus, schematic representation of the components. A, CCD camera; B, video recorder; C, data transfer unit; D, PC with frame grabber; E, PC for measurement and recording of the suction pressure. (b) Scheme of the assembly of the microtools in the microscopy chamber of the membrane force transducer apparatus.

aperture diameters of 3.5–4.5 μm (determined by a Zeiss Gemini scanning electron microscope (Zeiss, Oberkochen, Germany) with a resolution of 0.05 ± 0.01 μm) were prepared from glass capillaries (borosilicate mxn00034, Narishige, Tokyo, Japan) using a Flaming-Brown-Puller (P87, Sutter Instruments, Novato, CA, USA). The tip orifice was fire-polished with a microforge (MF14, Narishige), and the pipettes were prefilled with aqueous solution by condensation of water vapour. Suction pressure was applied *via* a tubing system

connected to the micropipette and generated by the motor-micrometer-screw-driven vertical displacement of a liquid container filled with aqueous solution. The pressure was quantified with two transducers (DP15, DP105, Validyne, Los Angeles, CA, USA), determining the pressure difference against a liquid reservoir fixed at the level of the microscope stage (0.25% accuracy, according to manufacturer). The experiments were performed in a droplet of the cell suspension deposited in the microscopy chamber formed between two cover slips. The chamber designed allowed the introduction of the glass capillary carrying the glass bead with the sample surface (*i.e.*, polymer film coated with adsorbed antibody layers prepared as described above) and the micropipette on the same axis from two opposite sides. The microscope (Wilovert-Hund, modified by Thalheim Spezialoptik, Pulsnitz, Germany) was equipped with a $40\times$ objective (Nikon, Tokyo, Japan). Microscopic images (resolution about 0.2 μm) of the detachment steps of the cells were gained with a charge–coupled device (CCD) camera (CCD446, Narishige, Tokyo, Japan) and recorded simultaneously with the suction pressure on videotape (2000XC, Sony, Tokyo, Japan).

Cells were captured and maneuvered by the suction micropipette, gently impinged on the polymer substrates, and subsequently removed by stepwise increase of the aspiration pressure. Experiments comprised the variation of the polymer substrate, type and surface concentration of the adsorbed Glycophorin-antibody, and duration and force of impingement, as well as the dynamics of the suction-driven separation (see Table 2). The evaluation of the detachment experiments

TABLE 2 Summary of the Experimental Settings Applied in the Micropipette Cell Separation Experiments

Polymer substrate	PDFP, PS, CE
Preadsorbed antibody	anti-Glycophorin A (BRIC 256), anti-Glycophorin C (BRIC 4) adsorbed during 14 h from solutions of 25 or 250 $\mu g\,cm^{-3}$ antibody in PBS at room temperature, subsequently rinsing with PBS
Contact phase	contact force: 0.15 nN or 3 nN contact time: 10 s or 300 s initial contact diameter: 4.0 μm ± 0.5 μm or 2.0 μm ± 0.5 μm (for systems with adsorption from solutions of 250 $\mu g\,cm^{-3}$ antibody, contact force 0.15 nN, contact time 300 s)
Separation	separation dynamics 0.2 nN min^{-1}or 2.0 nN min^{-1} (for systems with adsorption from solutions of 250 $\mu g\,cm^{-3}$ antibody, contact force 0.15 nN, contact time 300 s)

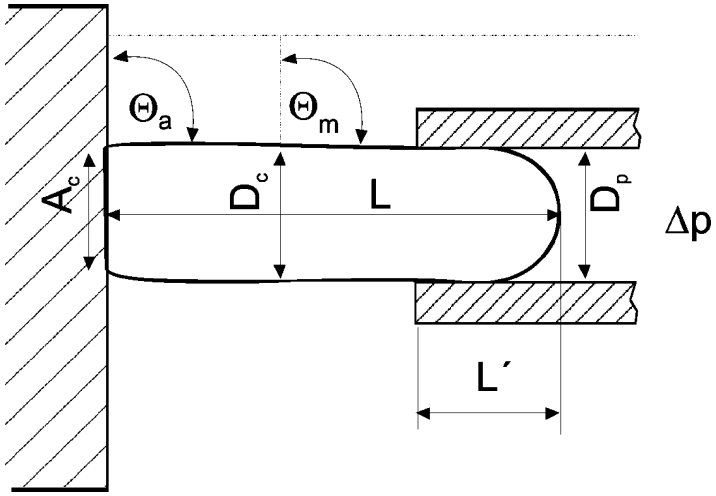


FIGURE 2 Survey on data determined by the evaluation of each step (*i.e.*, suction pressure) of the cell–substrate separation experiment: A_C, cell–substrate contact diameter; θ_a, cell–substrate contact angle; θ_m, meridian angle of the axisymmetric cell contour; D_C, meridian diameter of the axisymmetric cell contour; L, total cell length; L′, aspiration length of the cell; D_p, pipette diameter; Δp, suction pressure.

was performed with the Optimas5.2. Image Analysis Software (Optimas, San Diego, CA, USA) based on the analysis of selected image sequences using digitized images. The measured features of the detached cells are summarized in Figure 2.

Referring to the analysis developed by Berk and Evans [40] and by Tözeren *et al.* [41] for the separation of large cell–cell contact areas established by a multitude of isolated molecular attachment points, the data obtained were further utilized to determine the separation energy, ω_f, according to

$$\omega_f = \tau_m(1 - \cos\theta_a), \tag{1}$$

where τ_m is the membrane tension at the cell-substrate contact perimeter and θ_a is the contact angle of the cell membrane at the substrate. The membrane tension, τ_m, can be obtained as

$$\tau_m = \frac{\Delta p r_P^2 + p_C r_A^2}{2 r_A \sin\theta_m}, \tag{2}$$

with Δp aspiration pressure at the tip of the pipette, r_P radius of the pipette tip, p_C pressure of the cytoplasma, r_A radius of the

cell-substrate contact area, and θ_m angle between the nonaspirated cell contour and the pipette axis.

p_C cannot easily be determined experimentally, but, it can be reasonably approximated for osmotically swollen erythrocytes according to [42]

$$p_C \cong \frac{\Delta p r_P}{r_A}\left[\frac{(r_P - r_A \sin\theta_m)}{(r_P \sin\theta_m - r_A)}\right]. \quad (3)$$

RESULTS AND DISCUSSION

Table 2 summarises the experiments performed in this study. The choices, of the polymer substrates, the type and surface concentration of the preadsorbed antibodies, and the conditions of cellsubstrate contact and separation, were taken in view of the fact that cells adhering to artificial surfaces *via* specific bonds to preadsorbed ligand proteins are most important in the application of medical devices and biomedical products. As in many practically relevant examples, such as the adhesion of cells *via* integrin ligands binding to extracellular matrix proteins like fibronectin, the binding strength of the molecular attachment points, *i.e.*, the connection of the antibodies BRIC256 (anti-Glycophorin A) and BRIC4 (anti-Glycophorin C) to their cell membrane associated antigens, can be considered to be similarly irreversible at the experimental conditions applied in this study, while the density and anchorage of the physisorbed antibodies on the polymer films and the Glycophorins in the cell membrane were graduated within the set of samples investigated.

No adhesion of erythrocytes was observed on any of the polymer substrates in albumin-containing PBS without precoating of the surfaces with the anti-Glycophorin antibodies. This lack of nonspecific adhesion can be expected since red blood cells are circulating in high quantities in blood and attachment to interfaces would impair their functions. Even at polymer substrates with preadsorbed layers of the antibodies BRIC256 or BRIC4, which specifically bind to the erythrocyte transmembrane proteins Glycophorin A and Glycophorin C, no spontaneous spreading of the cells on the surfaces was observed, and extended attachment areas of the cells were established only upon impingement of the cells. This hysteresis effect of the adhesion can be attributed to distances of the molecular attachment points on the cell membrane exceeding the range of the attractive interactions [38, 43] (Figure 3).

Accordingly, cell–substrate adhesions were induced by moving partially aspirated cells in close proximity to the substrate surface and

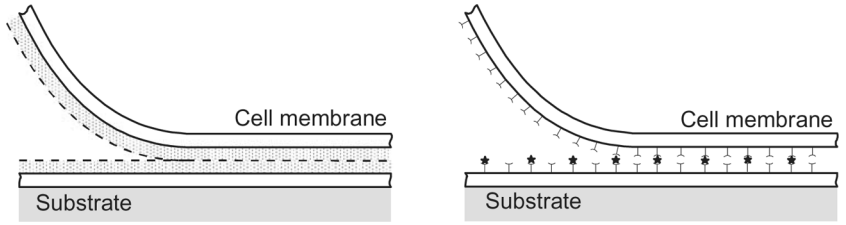
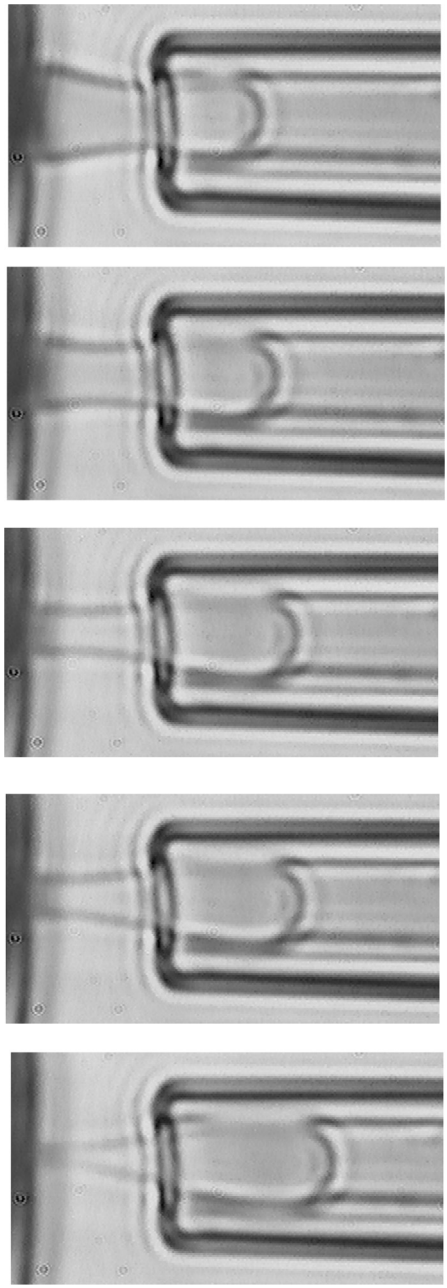


FIGURE 3 Schematic drawing of continuous (left) and discontinuous (right) distribution of interaction centers inducing cell–substrate adhesion (note that the distinction of "continuous" and "discontinuous" is based on the range of attractive interaction forces).

subsequent application of a positive pressure, forcing a large area of the cell membrane to contact the polymer surface. Different contact times between the cell membrane and the solid surface were realized prior to detachment of the cells by stepwise increase of the aspiration pressure applied through the micropipette. The conditions of the contact formation have been varied, as is summarized in Table 2. An exemplary series of images recording the suction pressure–driven cell–substrate separation is given in Figure 4. The results of the experiments were evaluated to give the energy of separation as a function of the contact area as shown for two selected examples in Figure 5.

The separation energy per unit contact area was found to increase strongly with the reduction of the contact area in all performed experiments. This effect occurred for both anti-Glycophorin A–and anti-Glycophorin C–attached cells without any influence of the separation dynamics. Referring to earlier studies on experiments where Glycophorin antibodies have been applied to agglutinate erythrocytes, the apparent strengthening of the adhesion during separation can be attributed to the smoothening of the cell membrane with increased membrane tension [42]. This can be expected to cause an increase of the frequency of molecular attachment points up to a boundary value at which all of the available molecules at the contact periphery are connected (see Figure 3). At this stage, a spontaneous separation of the cell from the substrate should occur without further increase of the aspiration pressure. Several of our experiments confirmed that phenomenon.

Table 3 summarizes the maximum separation energy data per contact area ($\omega_{\mathrm{f\ max}}$) obtained for the analysed systems. Comparing the different substrates, $\omega_{\mathrm{f\ max}}$ was, for a given solution concentration of the antibodies during adsorption, the highest for cells adhering to polystyrene surfaces and the lowest for cells on cellulose films. As



the substrates do not exhibit any significant morphological structures on the micrometer scale and do not contain any dissociating functionalities, this can be related to the graduation of the hydrophobicity of the polymer films; the adsorbed amount of the antibodies increased with increasing substrate hydrophobicity [44]. However, the maximum separation energy per amount of preadsorbed antibody was found to decrease in the order cellulose > polystyrene > plasma-deposited fluoropolymer, pointing at a better availability of the antibodies at the more hydrophilic substrates. Since we concluded that the separation of the cells did not occur by detachment of the antibodies from the substrate (see below), this trend might be attributed to conformational changes and unfavourable orientations of the antibodies at the more hydrophobic surfaces (see Table 3 and the subsequent discussion).

In general, higher values of $\omega_{f\,max}$ were obtained in the case of any given substrate for binding *via* Glycophorin C/BRIC4 as compared with Glycophorin A/BRIC256 (see Table 3). Furthermore, in line with the increasing surface concentration of the antibodies, $\omega_{f\,max}$ increased for any substrate and antibody for the higher solution concentration of the antibody during the adsorption (note that only solutions containing 25 and 250 $\mu g\,cm^{-3}$ have been compared in this study). For the higher surface concentrations of both antibodies, $\omega_{f\,max}$ of the cells follows in the trend cellulose > plasma-deposited fluoropolymer > polystyrene.

The detachment of the cell can occur due to the dislocation of the physisorbed antibody from the substrate, the break of the antibody-antigen bond, or through separation of the Glycophorin molecules from the cell membrane (Figure 6). While the antigen–antibody bond can be considered to be tight and irreversible at the applied experimental conditions [45], the detachment of the adsorbed antibody from the substrate or the dislocation of the Glycophorins from the membrane both could give rise to the separation of the cell from the carrier. Since Glycophorin C—which is present on the cell membrane at levels of only about 10% of the Glycophorin A concentration but, unlike Glycophorin A, is linked to the cytoskeleton [46, 47]—gives rise to significantly higher separation energies, we can relate the separation to the

FIGURE 4 Example of a series of images collected during stepwise separation of an erythrocyte from a polymer substrate upon increase of the suction pressure (polystyrene, preadsorbed anti-Glycophorin C from 250 $\mu g\,cm^{-3}$ solution, initial contact diameter 4.2 μm, separation dynamics 2 nN/min, separation energies increasing from upper to lower image: 33, 162, 283, 360, $455 \times 10^{-6}\,Nm^{-1}$).

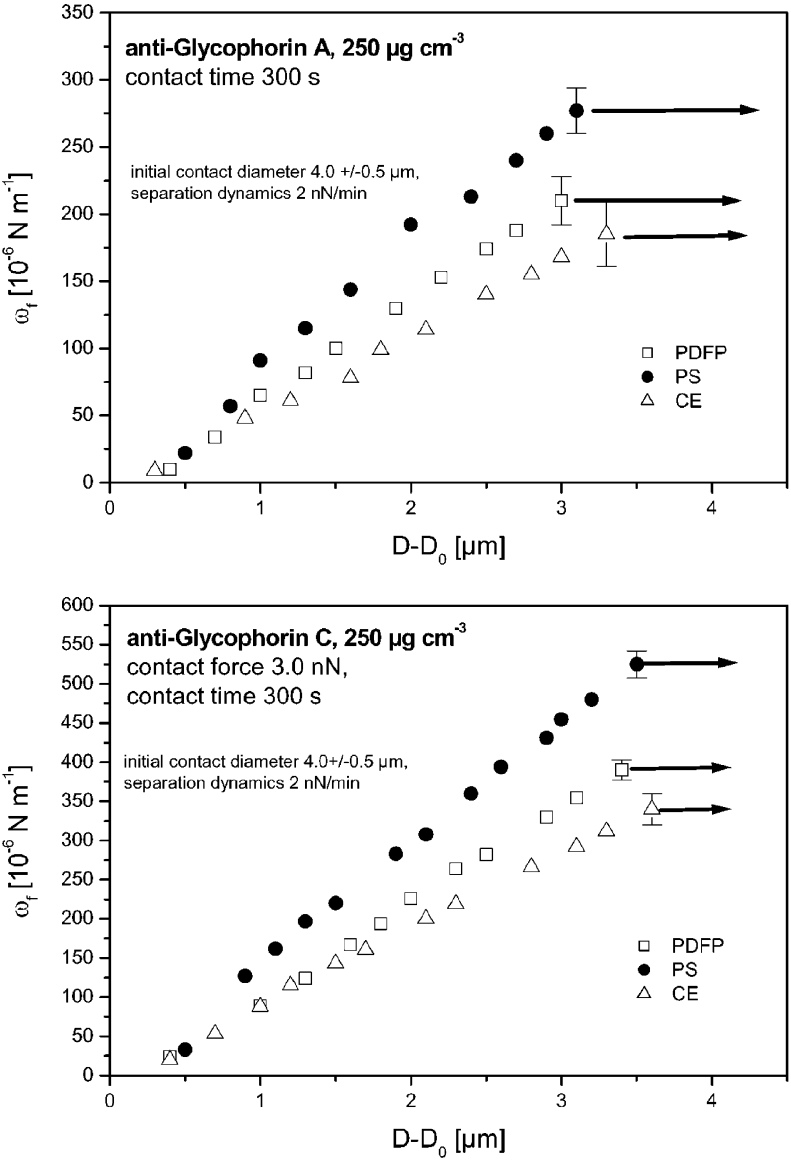


FIGURE 5 Examples of separation energy data versus reduction of the cell–substrate contact diameter. Above: Cells attached *via* anti-Glycophorin A (BRIC256). Below: Cells attached *via* anti-Glycophorin C (BRIC4). Arrows indicate spontaneous separation of the remaining contact area without further increase of the suction pressure. For explanation of the evaluation see the Materials and Methods section of the text.

TABLE 3 Results of the Evaluation of the Maximum Separation Energy of Erythrocytes Attached to Different Substrates via Antibodies to Glycophorin A and Glycophorin C

c_B ($\mu g\,cm^{-3}$)	Γ_{OF} AB ($\mu g\,cm^{-2}$)	N_{OF} AB (μm^{-2})	N_{OF} AG (μm^{-2})	$\omega_{f\,max}$ ($\times 10^{-20} J \mu m^{-2}$)	$\omega_{f\,max}$/AB ($\times 10^{-20} J$)	$\omega_{f\,max}$/AG ($\times 10^{-20} J$)
PDFP						
A 25	0.16	6350	3700	135	2.1	3.6
A 250	0.32	12700	3700	210	1.6	5.7
C 25	0.15	5953	370	307	5.1	82.9
C 250	0.30	11906	370	390	3.3	105.4
PS						
A 25	0.11	4365	3700	140	3.2	3.8
A 250	0.28	11112	3700	277	2.5	7.5
C 25	0.12	4762	370	420	8.8	113.5
C 250	0.27	10715	370	525	4.9	141.9
CE						
A 25	0.07	2778	3700	122	4.4	3.3
A 250	0.12	4762	3700	185	3.9	5.0
C 25	0.06	2380	370	280	11.7	75.7
C 250	0.12	4762	370	340	7.1	91.9

C_B, type and solution concentration of the antibodies during adsorption (A-BRIC256, C-BRIC4); Γ_{OF} AB, surface concentration of the antibodies; N_{OF} AB, number of adsorbed antibodies per area; $\omega_{f\,max}$, maximum separation energy per contact area; $\omega_{f\,max}$/AB, maximum separation energy per antibody; $\omega_{f\,max}$/AG, maximum separation energy per Glycophorin molecule.

anchorage of the Glycophorin in the cell membrane for Glycophorin A. Furthermore, for the separation of erythrocytes agglutinated by similar anti-Glycophorin antibodies, peak forces of 20–30 pN for the anti-Glycophorin A linkages and 80–90 pN for the anti-Glycophorin C bonds were recently reported. Since the ratio of the separation energy levels in our experiments was higher then the ratio of these peak forces, we may conclude that also for the Glycophorin C–attached systems the dislocation of the transmembrane protein is the most probable location of the bond failure.

The maximum separation energy per area, $\omega_{f\,max}$, can be further recalculated to estimate the separation energy for the individual attachment points (Table 3) assuming either the surface concentration of the Glycophorin or the antibody surface concentration to be the limiting quantity. Published data for the expression of Glycophorin A ($5–9 \times 10^5$ per cell) and Glycophorin C ($5–10 \times 10^4$ per cell) [46, 47] (to give N_{OF} AG, see Table 3 for definition) and the results of the antibody surface concentrations determined by ellipsometry

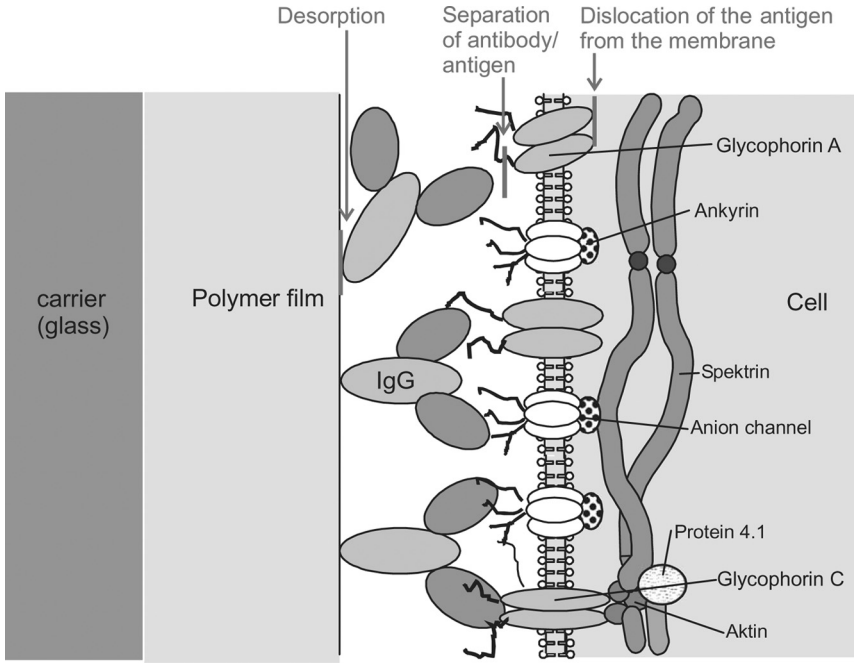


FIGURE 6 Schematic view of the cell–substrate adhesion zone, indicating possible locations of the bond failure upon separation of the cell from the substrate. For explanation see the Results and Discussion section of the text.

(the adsorbed amount per area was recalculated using the known molecular weight of the antibodies to give N_{OF} AB, see Table 3 for definition) were utilized. Only experiments where $\omega_{f\,max}$ data correspond to spontaneous separation (*i.e.*, full bond saturation) were evaluated. The obtained representation of the data demonstrates that $\omega_{f\,max}$ per antibody ($\omega_{f\,max}$/AB) is the higher the more hydrophilic the substrate is, which reflects a larger fraction of binding antibodies. The latter is in line with a recent study on the immobilization of antibodies at substrates with different hydrophilicity [48]. Since the surface concentrations of the antibodies can be considered to exceed the Glycophorin density in our experiments, the $\omega_{f\,max}$/AB data will overestimate the separation energies. However, as the increased surface concentration of the antibodies (achieved with increased solution concentrations in the adsorption) is accompanied by an increase of $\omega_{f\,max}$, the number of molecular attachment points can be concluded to be limited by the density of available antibodies on the polymer films (for the systems with antibody layers adsorbed from solutions of

$25\,\mu g\,cm^{-3}$antibody). This observation demonstrates that the available fraction of the antibodies is increased with their surface concentrations on the polymer films. The fact that even at antibody layers formed in solutions of $250\,\mu g\,cm^{-3}$ the $\omega_{f\ max}$ per Glycophorin ($\omega_{f\ max}$/AG) differs for the compared samples shows that the available antibody concentration is still limiting the anchorage of the cells in these cases.

CONCLUSION

Micropipette aspiration was demonstrated to provide detailed insights into energetic aspects of cell adhesion to artificial surfaces. For an exemplary study utilising this approach we selected adhesion of human erythrocytes on different polymer films precoated with antibodies to two of their transmembrane proteins (Glycophorin A and Glycophorin C). Adhesion between erythrocytes and the analysed polymer substrates (plasma-deposited fluoropolymer, polystyrene, and cellulose) required preceding adsorption of the antibodies and external force to establish extended contact areas. In all cases ω_f was found to increase during separation of the cells from the substrate. This effect was attributed to the submicroscopic smoothing of the cell membrane upon increase of the membrane tension and the resulting increase of the frequency of molecular attachment points up to a value related to the full binding of all available molecules. $\omega_{f\,max}$/AB was found to increase with the hydrophilicity of the polymer substrates, pointing to a better availability of the antibodies at the more hydrophilic surfaces. For all of the analysed cases we conclude that separation of the cells from the substrate occurs due to the dislocation of the Glycophorins from the cell membrane.

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