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ARTICLE

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Decorated surfaces by biofunctionalized gold beads: application to cell adhesion studies

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Abstract We describe a simple but versatile method to decorate solid surfaces randomly with colloidal gold particles to which ligands of cell receptors can be coupled to generate local attraction sites for the control of cell adhesion. A self-assembled monolayer of (3-mercaptopropyl)trimethoxysilane was deposited on glass slides. Gold beads were anchored to the functionalized surface through the sulfur group. We characterized the gold bead distribution on the functionalized surface with reflection interference contrast microscopy. The gold beads were functionalized with a disulfide-coupled cyclic pentapeptide containing an arginine-glycine-aspartic acid (RGD) tripeptide sequence which is selectively recognized by integrin receptors $\alpha_V\beta_3$ of endothelial cells. A blocking layer of bovine serum albumin was adsorbed onto the surface to prevent non-specific binding of the cells. We demonstrate that the RGD-functionalized colloidal gold beads act as local attraction centers, mediating rapid cell anchoring on a substrate impeding cell adhesion in the absence of attraction centers. Surprisingly, microinterferometry shows that after a time delay of about 1 h, the regions of the cell surface between the gold beads form close contacts with the substrate, which is attributed to strong van der Waals attraction after escape of repeller molecules from the contact surface.

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Introduction

Biopatterned surfaces have become of growing interest in biomaterial research and life sciences to generate biocompatible surfaces (e.g. for sensor applications) or mimetics of cell and tissue surfaces to study protein-protein recognition or cell adhesion processes (Dulcey et al. 1991; Dietrich et al. 1995; Chen et al. 1997; Sackmann and Tanaka 2000). Biotechnical applications require easily accessible, stable, and multifunctional biomaterials, which remain stable under physiological conditions (Turner et al. 1995; He 1998; Pum and Sleytr 1999). To achieve this goal, Goss et al. (1991) used monolayers of (3-mercaptopropyl)trimethoxysilane self-assembled on glass substrates to stabilize vapor-deposited gold on glass electrodes. Various attempts have been made to fabricate micro-patterned substrates. Colvin et al. (1992) described experiments for attaching semiconductor nanocrystals to metal surfaces with self-assembled organic monolayers as bridging compounds. Liu et al. (1998) developed a method to fabricate colloidal gold patterns on silicon surfaces using a photolithography technique. The size of the pattern was limited by the mask used for the photolithography technique. A very elegant technique to develop arrays of nano-gold on glass substrates was developed by Spatz et al. (1999). First, arrays of micelles of amphiphilic block co-polymers are deposited onto the substrate, which are then filled with gold. After photochemical deposition of the organic matrix, the nano-gold beads remain physisorbed on the substrate.

In the present work, we developed a simple method to form two-dimensional random patterns of gold beads on silanized glass surfaces. Glass substates are covered by monolayers of (3-mercaptopropyl)trimethoxysilane (MTS) which expose the SH end groups. Colloidal gold beads physisorb spontaneously on these monolayers, forming randomly distributed arrays, while the average

inter-bead distance can be adjusted through the density of the gold beads in the suspension. The problem of the randomness of the gold distribution can be overcome by the possibility to visualize the gold particles directly by dark field microscopy or microinterferometry. Since adsorbed particles expose their bare gold surfaces, they can be subsequently functionalized by biofunctional ligands through sulfur bonds. The simplicity and easy handling of the described technique facilitates its application in microdiagnostic devices (Schwarz et al. 1998).

In a first application, we attempted to apply the gold-patterned substrates to anchor endothelial cells locally by coupling a cyclic pentapeptide containing arginine-glycine-aspartate sequences (RGD ligands) to the gold beads through thiol-carrying anchors. These peptides are specifically recognized by endothelial integrin receptors $\alpha_V\beta_3$. The main aim was to mimic biological situations. It is well known that, under natural conditions, cells tend to adhere only locally to tissue surfaces by forming receptor clusters and by stabilizing the points of attachment though actin bundles coupled to the cytoplasmic domains of these integrin clusters through various coupling proteins (e.g. vinculin, talin). According to Chen et al. (1997), the growth and viability of endothelial cells requires that the cells can spread sufficiently on substrates, regardless of the type of extracellular matrix protein. If this is true, strong van der Waals attractions should be favorable for the well being of endothelial cells. On the other hand, endothelial cell layers on collagen I-coated solid substrates do not live more than 48 h. One possible reason for the finite lifetime is the strong van der Waals attraction between the cells and the solid substrates, which gives rise to strong membrane tensions. Moreover, van der Waals interactions do not play a role under most biological conditions, since cells are normally in contact with the soft tissue. They may therefore obscure many subtle aspects of cell adhesion. For this reason, studies of the effect of drugs or diseases on cell adhesion or cell locomotion performed under in vitro conditions are often questionable.

The main idea of the present work was therefore to minimize van der Waals attraction by separating the cells from the solid substrate by a few hundred nanometers by using the RGD-functionalized gold beads as spacers.

The adhesion of the cells on the transparent substrates was monitored by reflection interference contrast microscopy (RICM), which allows us to observe and analyze the contour of the cell surface facing the substrate with high optical resolution. We show that the gold beads covered on the top surface by RGD ligands act as attraction centers that form some type of anchor sites which attract and immobilize cells hovering over the surface. In the absence of such attraction sites, the cells do not adhere. However, we also demonstrated that the regions of the cell surface between the gold beads are eventually attracted by the solid substrate through van der Waals forces, resulting in strong cell-substrate contacts after about one hour.

Materials and methods

Silanization procedure

Glass cover slides (24 mm×24 mm, AL, Germany) were cleaned by successive ultrasonification, firstly twice in a 2 vol% aqueous solution of Hellmanex (Hellma, Mühlheim, Germany), and secondly twice in Millipore-filtered water (Millipore Milli-Q-System, Molsheim, France), for 30 min, respectively. Between each sonification step the glass slides were rinsed about 10 times with Millipore water. Then the samples were immersed in a 0.5 M KOH solution, kept at 40 °C for 30 min and rinsed again with Millipore water. Thereafter, the glass slides were dried for several hours at 75 °C. The clean and dry substrates were silanized with MTS purchased from Sigma (Germany). Self-assembled monolayers were generated by immersing the cleaned substrate for 12 h (and at room temperature) into a dehydrated toluene solution containing 10^{-3} M MTS (Liu et al. 1998). The MTS-coated substrates were then rinsed first with toluene and subsequently with ethanol (Fig. 1).

Ellipsometry

Optical ellipsometry was applied as a sensitive method to estimate the thickness of monomolecular films and to check the quality of the films formed on silicon wafers with a thermally grown oxide (~145 nm thick). Ellipsometry measurements were performed using a home-made ellipsometer with a 632.8 nm wavelength and with a fixed angle of incidence of 70° (Albersdörfer et al. 1998). Each sample was first evaluated after the washing procedure for reference and then after silanization.

Functionalization with colloidal gold

Suspensions of colloidal gold containing 250 nm diameter particles at a concentration of 3.6×10^8 particles/mL were purchased from British Biocell (Cardiff, UK). To vary the density of adsobed gold beads, we used different bead concentrations for the coupling procedure. For that purpose 10, 20, 30 and 40 μL of these gold suspensions were dispersed in 10 mL of ethanol. The MTS-coated glass substrates were immersed in this gold suspension at room temperature for at least 12 h, while shaking the glass slides on an incubator table. After this coupling procedure the samples were washed with ethanol and dried under vacuum (Fig. 1).

Synthesis of disulfide-coupled cyclic pentapeptide c(-RGDfK-)

Materials for synthesis

The disulfide derivative coupled to the cyclic RGD peptide [1,2-dithiolane-3-pentane-cyclo(-Arg-Gly-Asp-D-Phe-Lys-)] was prepared by coupling the DL-α-lipoic acid to the terminal amine of the protected peptide c[-R(Pbf)GD(OBu^t)fK-], which produced product **1**. Finally, all protecting groups were removed using an aqueous mixture of trifluoroacetic acid (TFA), yielding the molecule **2** (Fig. 2).

The DL-α-lipoic acid (1,2-dithiolane-3-pentanoic acid) derivative of the disulfide was purchased from Fluka; HATU [*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] was from PerSeptive Biosystems (Germany), and 2,4,6-collidine from Aldrich. All chemicals were used as purchased. The cyclic pentapeptide c[-R(Pbf)GD(OBu^t)fK-] was prepared by classical methods of peptide synthesis at the Chemistry Department of the Technical University of Munich (Geyer et al. 1994). All reactions were executed under argon. Reactions were monitored by thin-layer chromatography (TLC) using 0.25-mm silica gel plates (Merck, Darmstadt, Germany). Column chromatography was performed using silica gel-60 (particle size 0.040–0.063) (Merck).

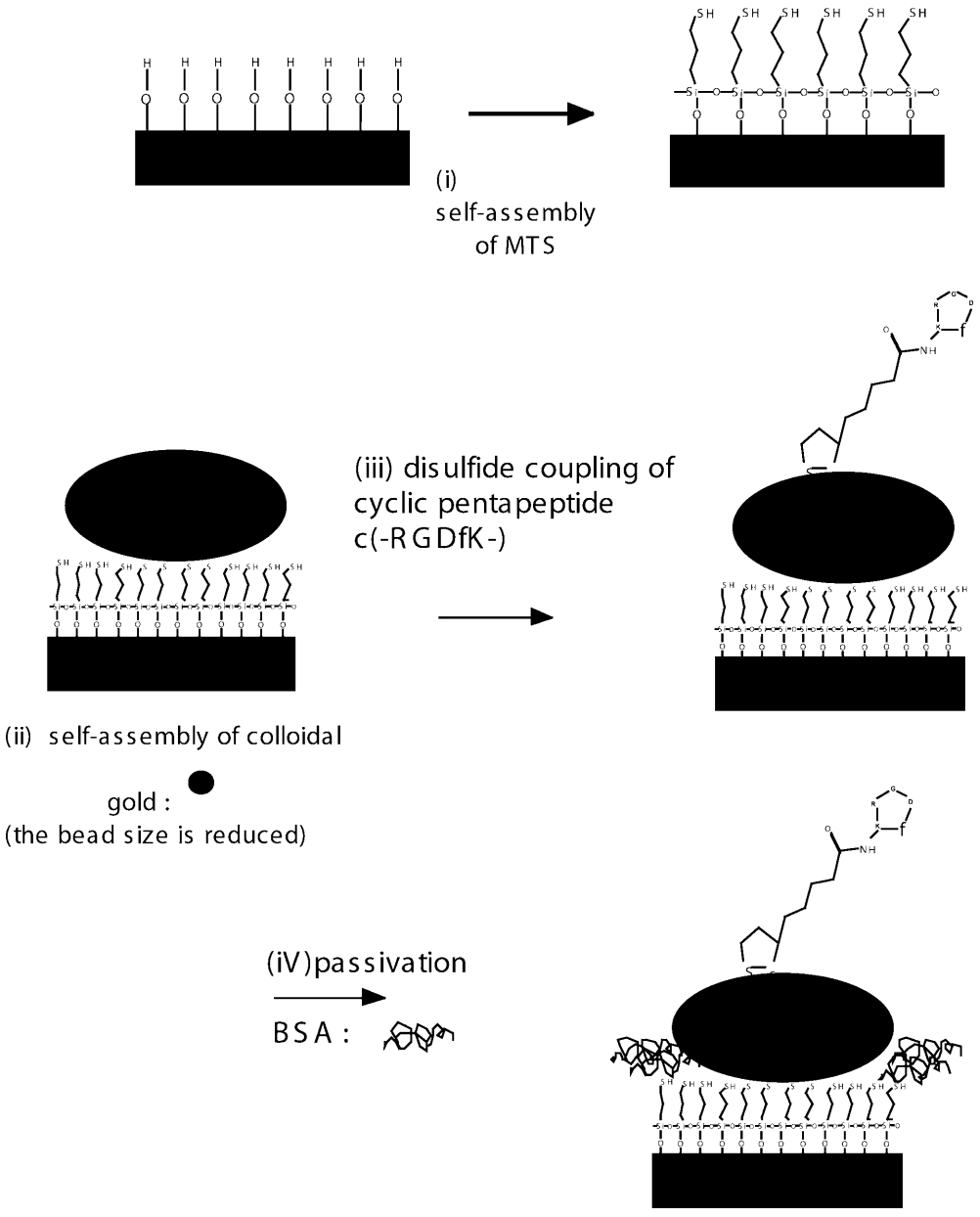

Fig. 1 Schematic representation of the steps followed for the preparation of the gold decorated surfaces: (*i*) silanization of the substrate, (*ii*) self-assembly of the colloidal gold beads, (*iii*) functionalization of the beads with the disulfide-coupled cyclic pentapeptide c(-RGDfK-), (*iv*) passivation with BSA

*Preparation of the disulfide-coupled cyclic pentapeptide c[-R(Pbf)GD(OBu^t)fK-] [1,2-dithiolane-3-pentane-cyclo [-Arg(Pbf)-Gly-Asp(OBu^t)-D-Phe-Lys-] (**1**)]*

A mixture of 1,2-dithiolane-3-pentanoic acid (33.9 mg, 0.164 mmol), c[-R(Pbf)GD(OBu^t)fK-] (150 mg, 0.164 mmol), HATU (62.3 mg, 0.164 mmol), and collidine (43 μL, 0.328 mmol) in anhydrous DMF (3 mL) was stirred for 10 h. After concentration in vacuum and column chromatography (10% MeOH in CH_2Cl_2), we obtained the product **1** shown in Fig. 2 (110 mg, 60.9%). The identity of the product was verified by mass spectrometry: the molecular weight calculated for $C_{52}H_{77}N_9O_{11}S_3$ was 1099.86; the measured *m*/*z* value was 1099.19.

*Preparation of the disulfide-coupled cyclic pentapeptide c(-RGDfK-) [1,2-dithiolane-3-pentane-cyclo (-Arg-Gly-Asp-D-Phe-Lys-) (**2**)*

A solution of TFA:H_2O (95:5, 500 μL) in anhydrous CH_2Cl_2 (2 mL) was used for cleavage of the protective group for the peptide product **1** (110 mg, 0.1 mmol). The mixture was stirred for 24 h. After evaporation of the solvent under reduced pressure, the crude product was crystallized at 0 °C in cold diethyl ether, yielding product **2** (54.4 mg, 68.7%). The identity of the product was verified by mass spectrometry: the molecular weight calculated for $C_{35}H_{53}N_9O_8S_2$ was 791.5, while the measured *m*/*z* value was 791.4.

Deposition of disulfide-coupled cyclic pentapeptide c(-RGDfK-) on gold beads

The MTS-covered substrates with adsorbed colloidal gold beads were immersed in a 1 mM solution of the disulfide-coupled cyclic pentapeptide c(-RGDfK-) (**2**) in ethanol for 12 h at room temperature. Then the substrates were rinsed twice with ethanol and dried under vacuum (Fig. 1).

Passivation of substrate by bovine serum albumin

The gold-free surface of the substrate covered with MTS was passivated by adsorption of bovine serum albumin (BSA), which is

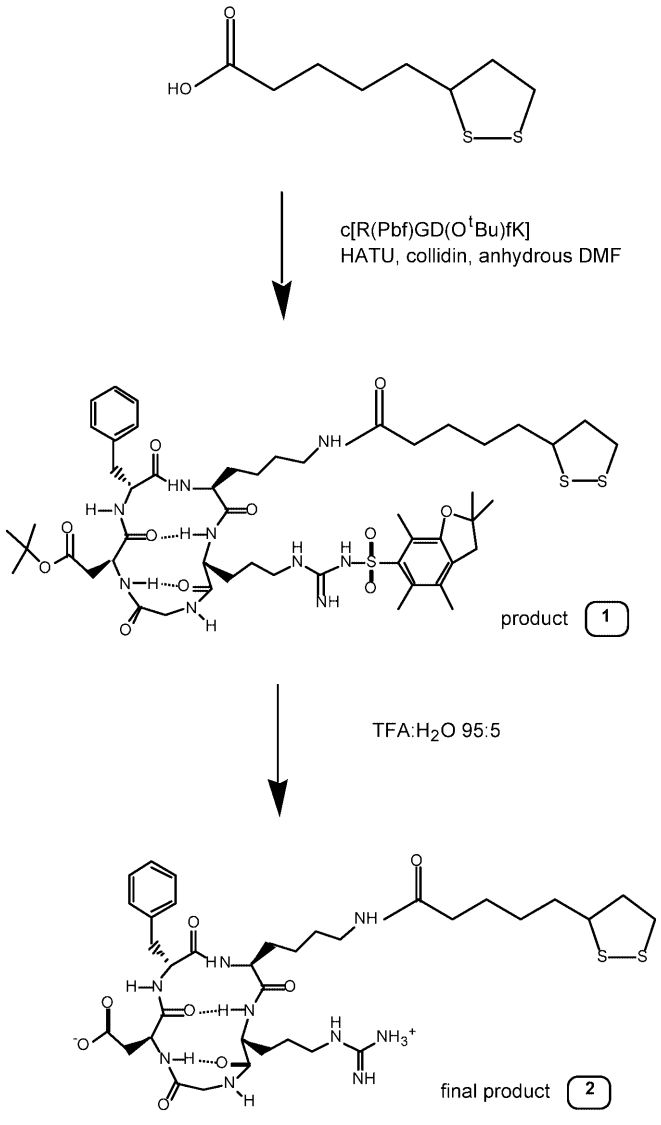

Fig. 2 Schematic view of steps taken during the synthesis of the disulfide-coupled cyclic pentapeptide c(-RGDfK-)

known to be well suited for the protection of glass surfaces against non-specific protein adsorption (Schwarz et al. 1998). For this procedure, substrates were immersed in a concentrated solution (0.2% w/v) of BSA (Albumin Bovine fraction V, Biomol Feinchemikalien, Hamburg). The sample was incubated for 1 h in this solution, then rinsed five times in Millipore water and immersed again for 1 h in Millipore water (Fig. 1). The surfaces are stable for at least one day.

Cell culture

Endothelial cells were obtained from human veins of the umbilical cord. The cells were cultivated in a humidified incubator at 37 °C and 5% CO_2 as described previously (Bausch et al. 2001). As culture medium we used endothelial cell growth medium (Promocell, Heidelberg, Germany), with 10% fetal bovine serum (Life Technologies, Eggenstein, Germany).

Every two days the medium of the tissue flasks was changed. Cells were removed from the tissue flask by washing twice with Ca^{2+}-free PBS (Life Technologies) and trypsin treatment (trypsin EDTA solution, Life Technologies). The suspended cells were diluted with culture medium to obtain a final concentration of about 100,000 cells/mL. In all our experiments we injected 200 μL of this cell preparation into our measuring chamber containing 1 mL of culture medium, which was kept at 37 °C to maintain the well being of the cells.

Dynamic reflection interference contrast microscopy

We analyzed the adhesion of the cells by reflection interference contrast microscopy (RICM). The image of the adhering cell surface is formed by the interference of light reflected from the glass surface (called the reference beam) and that reflected from the cell surface close to the substrate (called the object beam) (Zilker et al. 1992). The RICM micrographs were taken with a Zeiss Axiomat inverted microscope equipped with an antiflex oil immersion objective (Antiflex Neofluar 63/1.25). Illumination was performed with a high-pressure Hg lamp (Osram HBO, 100 W) and monochromatic light of $\lambda = 546$ nm was produced by a combination of interference and a cut-off filter (Schott A3-546 and OG 530) (Schindl et al. 1995). The use of an oil immersion objective was essential for the RIC microscopy in order to eliminate the strong reflection at the air-glass interface. All the measurements were carried out in cell growth medium at 37 °C to maintain the well being of the cells.

Results and discussion

Ellipsometric characterization of the silane monolayer

In order to determine the monolayer thickness, we assume that the refractive index of the silane (MTS) was considered to be constant and equal to $n \approx 1.5$ (Tillman et al. 1988). All reported values were averages of five measurements in different regions of the sample. In all measurements, values of the angles ψ and Δ were mapped, which are related to the ratio of the reflectivities of the light polarized perpendicular (r_s) and parallel to the plane of incidence (r_p) according to $r = r_p/r_s = \tan\psi \exp(i\Delta)$. The measured values of the angles ψ and Δ are given in Table 1. From these data the local thickness was determined by procedures described elsewhere (Albersdörfer et al. 1998). By averaging five measurements, the error of the thickness measurement is 0.1 nm. The thickness found of $d = 0.76 \pm 0.1$ nm corresponds well to the length of the stretched MTS molecule, which is 0.8 nm (Liu et al. 1998), suggesting that the self-assembled MTS forms a closed monolayer.

RICM study of endothelial cell adhesion

Visualization of gold particles

Typical RICM pictures of surfaces decorated with gold particles are shown in Fig. 3. It is seen that our technique leads to the formation of random distributions of fixed gold beads and that the surface density increases with increasing concentration of the colloidal gold particles in the suspension. The prepared surfaces are stable for more than one month. The RICM pictures

Table 1 Values of the ellipsometry angles ψ and Δ of the silicon wafer

(a) Values after the cleaning procedure (°)					
ψ	53.7636	53.7414	53.7608	53.8172	53.8385
Δ	82.1015	82.1579	82.1682	82.1732	82.0367
(b) Values after silanization with MTS (°)					
ψ	54.6781	54.6623	54.6730	54.7291	54.8390
Δ	82.4302	82.4360	82.4545	82.4702	82.3927

(Fig. 3a, b) show two different substrates prepared with 20 and 40 μL colloidal gold suspensions in 10 mL ethanol, respectively, observed after suspension in cell growth medium. As shown in Fig. 3c, the surface density (number of gold beads per μm^2) depends in a sigmoidal way on the concentration of the bulk suspension. We do not have an explanation for this deviation from Langmuir's adsorption law.

Cells on substrates with non-functionalized gold beads and passivated with BSA do not adhere

In a first control experiment, we demonstrated that the adsorbed protein (BSA) layer is effectively blocking the adsorption of cells from bulk solution. Figure 4a shows a typical result. Even after 55 min of incubation the observed endothelial cells do not bind to the surface, since no dark domains of adhesion are visible, only a shadow of the cells hovering over the surface. This is further confirmed by the observation that after 1 h 25 min of incubation time, the cells observed during this time start to escape again into the solution driven by Brownian motion (Fig. 4b). Detailed inspection of the whole substrate surface did not show any adhering cells. We can thus exclude the non-specific adsorption of cells on BSA-coated surfaces for the time over which our experiments were performed, although we cannot exclude adsorption of material from the serum.

Interaction of cells with substrate covered by RGD-functionalized gold beads

Figure 5 shows the behavior of cells after RGD functionalization of the gold beads for the case of a medium lateral density of beads (incubation with 20 μL of colloidal beads). Figure 5a shows that the cells start to adhere already after about 10 min. The cell hovering over the substrate forms filipodia-like protrusions which bind to the RGD-ligand-covered gold beads, thus forming local anchors. After this initial state the bulk of the cell surface adheres to the substrate. Figure 5b shows this situation (for a different cell) after 1 h. Obviously, the cells are completely spread and start to form pseudopods, which appear to end often at gold beads.

A closer inspection of Fig. 5b shows that the RICM image actually exhibits two sets of interference fringes

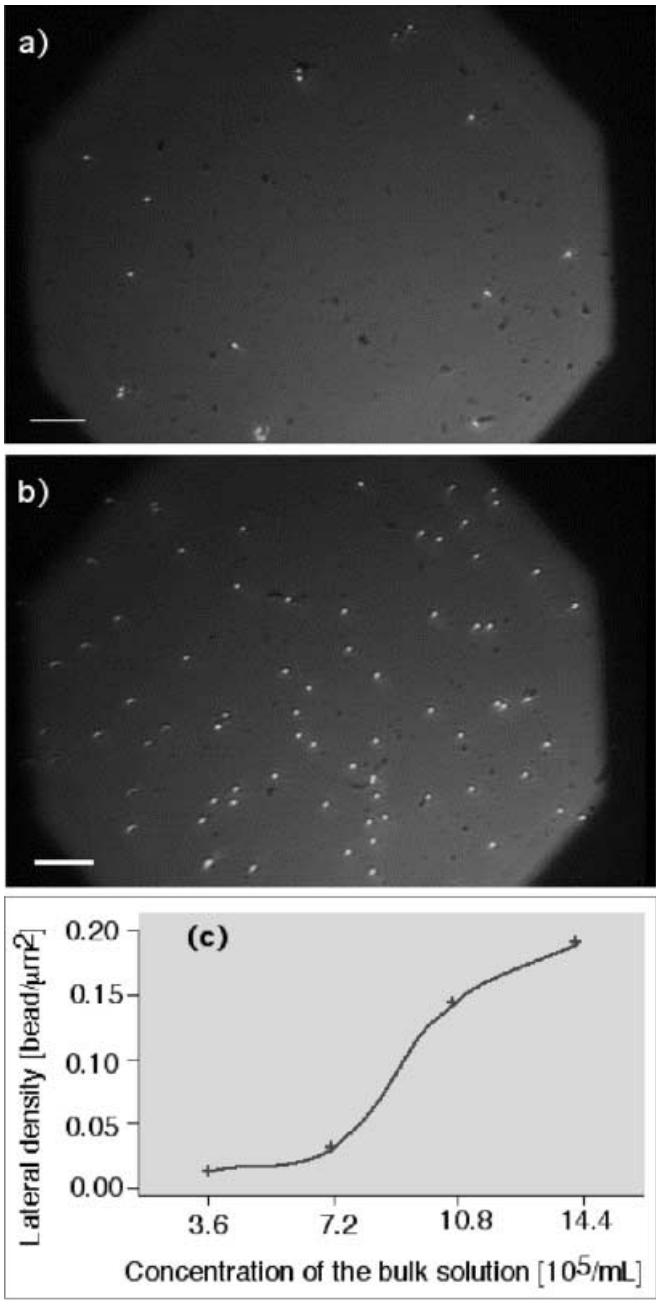

Fig. 3a–c RICM images of the glass surface decorated with gold beads by incubation of the substrates in solutions containing different concentrations of colloidal gold beads. Observation in cell growth medium. **a** Substrate immersed in a solution containing 7.2×10^5 gold particles per mL in ethanol. The *white bar* represents 5 μm. **b** Incubation with colloidal gold solution at 14.4×10^5 particles/mL ethanol. The *small dark spots* could be very small gold beads or aggregates from the cell growth medium. **c** Plot of the surface density of gold beads onto the surface as a function of the colloidal particle concentration in the bulk solution

(corresponding to lines of equal height). One set (indicated by a thick arrow and denoted as set I) is determined by the top contour of the cell as indicated in Figs. 6 and 7. Close to the center and the right side one sees a second series of concentric rings (denoted as set

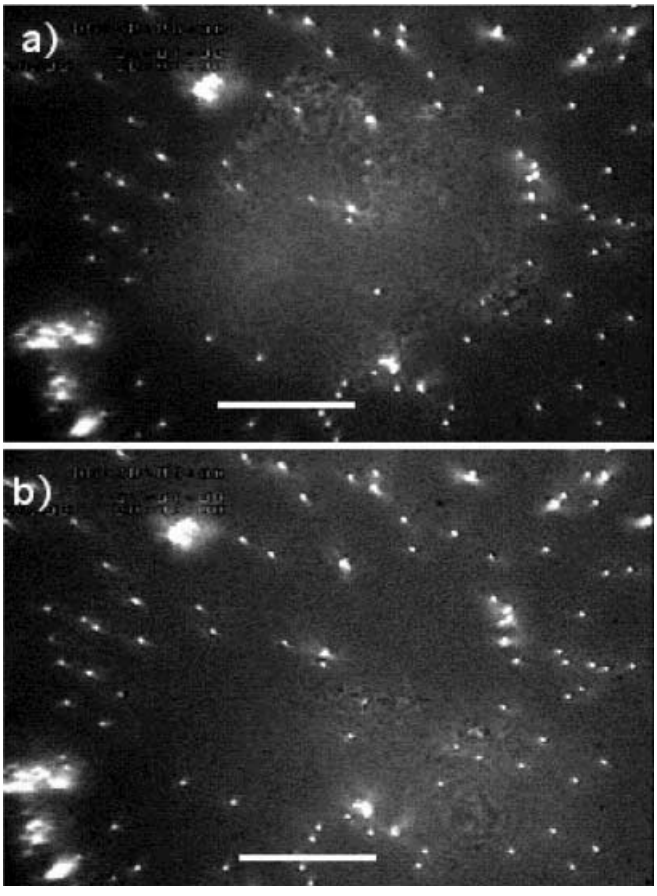

Fig. 4a, b Control experiment: observation of cell hovering over substrate decorated with gold beads by incubation in colloidal gold suspension of 14.4×10^5 particles/mL and passivated subsequently by incubation in BSA solution. **a** Situation after 55 min of incubation time. Note that endothelial cells are hovering over the surface but do not bind. *White bar* corresponds to 5 μm. **b** Situation of same site on substrate as in **a** after 1 h 25 min. Note that the cell starts to escape again due to Brownian motion after the image was taken

II). It is attributed to an indentation of the cell surface towards the cytoplasm, as indicated in Fig. 7. Detailed inspection of Fig. 5b shows that the gold beads are located beneath different fringes that are at sites of different height. This is a consequence of the polydispersity of the beads; the average bead size is 250 nm while the height difference corresponding to the distance of two dark lines is $\Delta h\approx250$ nm (Albersdörfer et al. 1997). Thus many of the beads are located close to the contour line corresponding to a height of 250 nm (second dark ring of set II). Some beads assemble at fringes corresponding to larger or smaller heights, which is attributed to the variation of the bead diameter or to the aggregation of two beads (cf. Fig. 6, bead 2 or Fig. 7).

Figure 6 shows the surface profile of the indentation of the cell along the sections AA′ (Fig. 6a) and BB′ (Fig. 6b) as obtained by analyzing the RICM interferogram of Fig. 5b following Albersdörfer et al. (1997). The beads of different size are located at sites where the cell-substrate distance agrees with the bead diameter. Note, for instance, that for bead B1 the lines of equal height are deflected laterally towards the center of the indentation, clearly indicating that the cell-substrate distance is adjusted to the bead size owing to the binding of the cell surface to the RGD ligands.

The formation of indentations of the cell surface towards the inside, at regions where gold beads are assembled, has been found many times. This leads to the conclusion that the cell membrane is locally closer to the glass surface at gold-free regions than at regions where several beads are assembled. Since these regions are passivated by BSA deposition, the cell has to be attracted by the glass surface through van der Waals attraction. By assuming that beads B1 and B4 have diameters of 250 nm, the distance between the glass and cell surface must be around 100 nm or smaller. Our experiments thus show that the RGD-functionalized gold beads are essential for the initiation of the cell adhesion process through the local anchoring of the cells, but that, at a later stage, van der Waals attraction by the whole substrate becomes important. This behavior can be explained in terms of the well-known fact that cell adhesion is a dynamic process (Springer 1994; Soler et al. 1998; Sperling et al. 1998). The glycocalix of endothelial cells contains a large number of transmembrane proteins with very large head groups extending up to 50 nm into the extracellular space. These include cell surface receptors with head groups composed of up to seven IgG domains [such as platelet endothelial cell adhesion molecules (PECAM) or glycoproteins with mucin head groups containing a high content of negatively charged sialic acid residues such as GLYCAMS (Springer 1994; Soler et al. 1998; Sperling et al. 1998)]. At such distances the van der Waals attraction between the membrane and the substrate surface is expected to be very small, since most of the mass of the glycocalix is composed of water. However, owing to this weak van der Waals attraction, the lipid/protein bilayer can be attracted gradually closer to the glass surface by expelling large repeller proteins from the cell-substrate contact area. Such dynamic adhesion has indeed been observed during interaction of white blood cells with endothelial cells (Soler et al. 1998). Further evidence for this interpretation has been provided by systematic studies of model systems (Bruinsma et al. 2000) demonstrating that adhesion is a first-order-like transition, which is initiated by a rapid nucleation process consisting in the formation of local centers of strong attraction between receptors and ligands, which is in general followed by the slow growth of these adhesion plaques until a large cell-substrate contact zone is formed. In these model systems the long-range repulsion forces generated by the cellular glycocalix were mimicked by embedding lipopolymers [lipids with large (polyethylene oxide) head groups] into the vesicle membrane and for undulation forces. Also, in this case, the formation of tight adhesion domains requires the expulsion of the repeller molecules from the contact

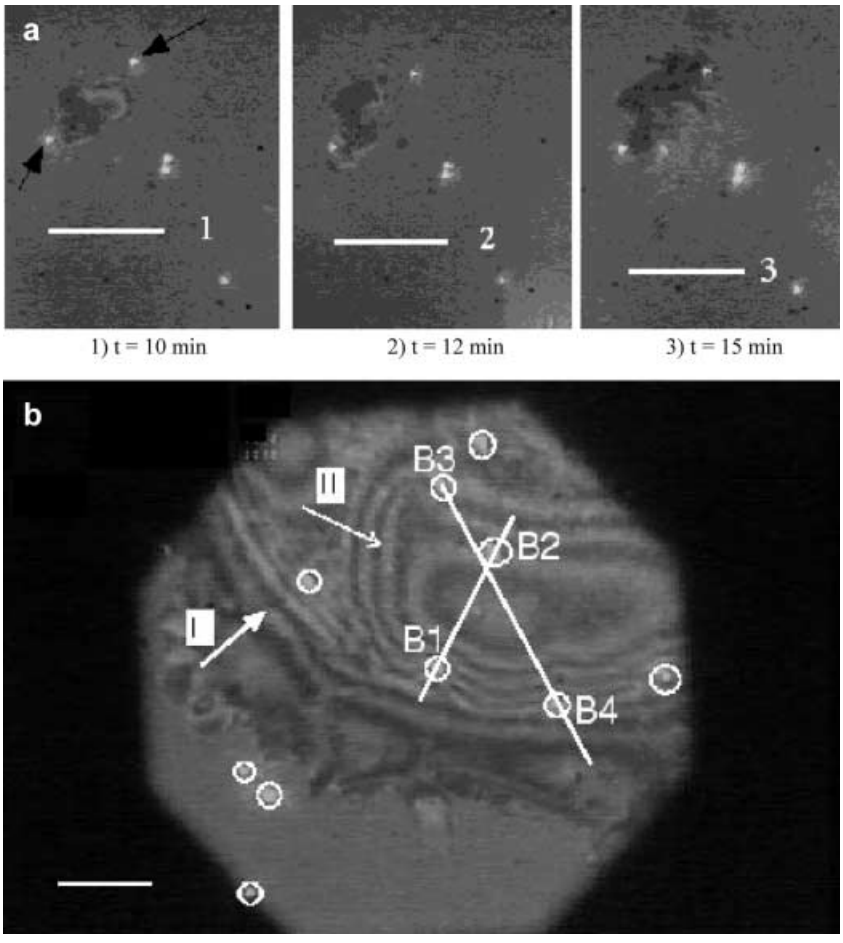

Fig. 5a, b Behavior of endothelial cell on surface decorated with gold beads biofunctionalized with RGD ligands. The gold beads were deposited by incubation in a suspension containing 7×10^5 particles/mL. The gold was coupled to the disulfide-coupled cyclic pentapeptide c(-RGDfK-) and the remaining surface was passivated by covering with BSA. At time $t=0$, the solution containing the endothelial cells was injected into the measuring chamber. **a** Initial step of the adhesion process observed between 10 and 15 min of incubation time. The *white bar* corresponds to 5 μm. **b** Endothelial cell on the surface after 1 h 5 min of incubation time

zone (Z. Guttenberg, unpublished work from Doctoral Thesis, Technical University of Munich, 2001).

Conclusion

A simple and versatile method for generation of a biofunctional pattern on solids suited for cell adhesion studies is presented. It does not require sophisticated nano-structuring techniques such as the beautiful method of gold pattern formation developed by Spatz's group (Spatz et al. 1999). The method to couple the gold beads to surface-grafted monolayers, exposing thiol groups, is universal and leaves the exposed side of the beads free to anchor functional groups onto the top surface again through thiol chemistry. The technique enables the use of a large variety of bead sizes. Since the beads can be easily visualized by microscopic techniques, the disadvantage of the randomness of the bead distribution can be compensated. However, regular arrays gold beads could also be prepared by photolithographic formation of a regular thiol pattern.

One major motivation for the present work was to generate a method for the local attachment of cells through adhesion domains in order to mimic a typical situation of cell adhesion: the formation of adhering domains due to clustering of receptors (e.g. integrins). Judged from numerous optical studies, focal adhesion sites exhibit diameters of the order of a few hundred nanometers (Smilenov et al. 1999), similar to the size of the gold beads. These local pinning centers are stabilized through actin stress fibers which are attached to the intracellular domains of the receptors through focal complexes (Smilenov et al. 1999). On the other hand, the lifetime of umbilical endothelial cells on functionalized solids is much shorter than under physiological conditions.

Experiments in the authors' laboratory showed that umbilical endothelium adhering on glass substrates which was functionalized by self-assembled monolayers of silanes carrying RGD head groups start to show pronounced membrane flickering, similar to erythrocyte membrane flickering (Zilker et al. 1992), after a few hours, which is a consequence of the decoupling of the lipid/protein bilayer from the actin cortex. One possible reason for this effect could be the strong van der Waals attraction between the membranes and solid substrates (Bruinsma et al. 2000). We hoped that separating the cells from the solid surface by a few hundred nanometers would impede the tight binding due to the van der Waals attraction and thus avoid some disadvantages of the microprinting technique. However, our present experi-

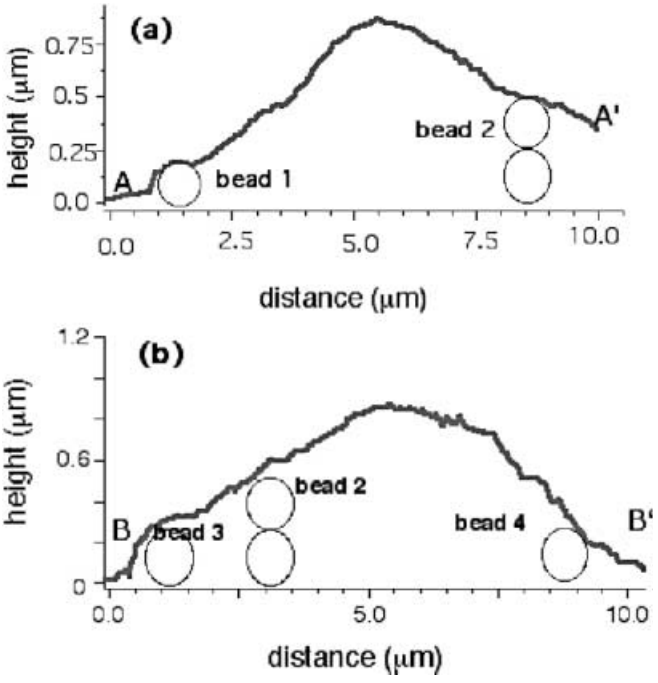

Fig. 6a, b Reconstruction of cell surface profiles for indentations of a cell over clusters of gold beads. **a** Reconstruction of the cell surface profile along section AA′. **b** Reconstruction of cell surface profile along section BB′

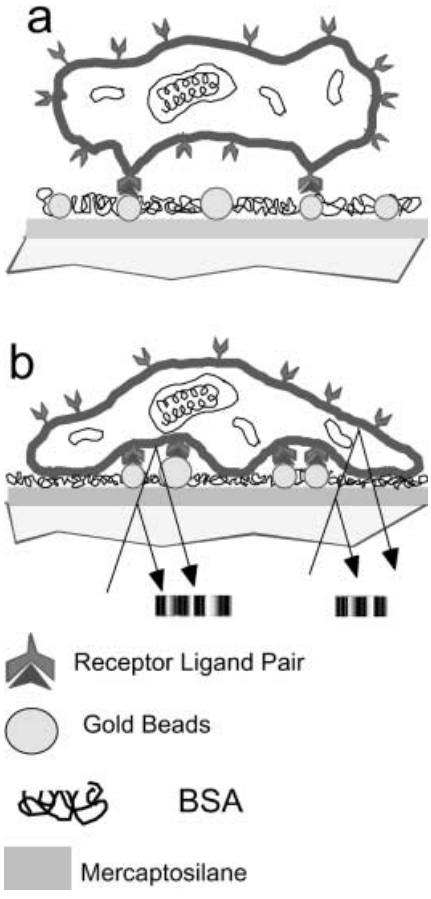

Fig. 7a, b Schematic view of an endothelial cell adhering to the functionalized surface. **a** Initial state of adhesion. **b** Later state of adhesion associated with the formation of a tight membrane-substrate contact generated by strong van der Waals attraction of the cell by the solid substrate

ments show that the van der Waals forces are strong enough to induce the formation of tight contacts of the cell membrane with the solid surface by gradual expulsion of repeller proteins of the cell glycocalix from the contact zone. Judged from the reconstruction of the cell surface profile (see Fig. 6c, d), the distance of the cell surface from the substrate is $d \leq 50$ nm. This estimate is consistent with the size of head groups of receptors such as integrins (exhibiting a maximum height of the head group of 15 nm).

The main purpose of the present work was to demonstrate the formation of random biofunctional patterns by self-assembly of gold beads through thiol chemistry. The method is highly versatile since both the size of the gold particles and their average separation distance may be adjusted, while the disadvantage of the random arrangement of the biofunctional centers may be overcome by optical observation of their functionality. An advantage of the present decoration technique compared to the contact printing technique (cf. Chen et al. 1997) is that the bulk of the cell surface can be separated from the solid support by a few hundred nanometers in order to avoid van der Waals attraction. This non-specific interaction may be very strong (Bruinsma et al. 2000) and it corresponds to a non-biological situation. Since the tissue is a soft material, the van der Waals attraction is negligible. On the other hand, owing to such non-specific interatomic mechanisms, studies of cell adhesion or cell locomotion under in vitro conditions are often questionable. It is hoped that the present technique can be applied to a more stress-free immobilization of cells by covering the space between the gold beads with thin biocompatible polymer films of hyaluronic acid, following Albersdörfer and Sackmann (1999). Stress-free immobilization is essential for potential applications of cells as monitors for the detection of toxins or other cell damaging effects (Hillebrandt et al. 2001) or to stimulate tissue growth on surfaces.

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