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Remodeling of fibrinogen by endothelial cells in dependence on fibronectin matrix assembly. Effect of substratum wettability

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The endothelization of cardiovascular implants is desirable to improve their blood compatibility. The capacity of the endothelial cells to attach, migrate, proliferate and function on the implant surface depends on the presence of matrix proteins such as fibronectin (FN) and fibrinogen (FNG). In this study, we show that the deposition of fibrinogen into extracellular matrix-like structures by human umbilical vein endothelial cells (HUVEC) is dependent on FN matrix formation. We found further that the process of organization of both adsorbed and soluble FN and FNG is dependent on the wettability of materials since it was observed only on a hydrophilic and not on a hydrophobic model surface. β_3 integrin was involved in the process of cell attachment to adsorbed FNG, while the mechanism of FNG fibrillogenesis required the activity of the β_1 integrin. Studies of EC morphology showed the predominant peripheral organization of actin filaments and the formation of distinct leading and trailing cell edges suggesting a motile phenotype of cells when they are seeded on FNG. In summary, we concluded that adsorbed fibrinogen may enhance the motility of HUVEC and that soluble FNG requires FN matrix assembly to be organized in fibrilar structures.
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Introduction

The endothelization of cardiovascular implants is an important task for biomedical engineering as it could overcome many clinical problems connected with the surface-induced thrombosis [1]. Particularly, seeding of blood-contacting implants with endothelial cells (EC) might result in the development of a confluent cell monolayer to mimic the function of the normal vascular endothelium. The benefits of this approach however, are not realized yet, as most of the materials used for cardiovascular prosthesis are not designed to promote cellular interaction [2]. Most of them are hydrophobic in order to lower the undesired adhesion of platelets and inflammatory cells [3]. There is abundant evidence that hydrophilic, or wettable materials may facilitate cell adhesion in contrast to hydrophobic surfaces. Since fibronectin (FN) and vitronectin, and more recently fibrinogen (FNG), were found to be the main soluble matrix proteins in blood that cells can recognize, one possible explanation for this different biological reactivity might be the distinct adsorption and conformation of these proteins on biomaterial surfaces [4]. Until now, however, this hypothesis was clearly proven only for FN employing fibroblasts as cellular model [5], while little data for EC have been provided.

Fibronectin is the major soluble extracellular matrix (ECM) component, which readily induces cell adhesion when adsorbed on material surfaces [6]. The main FN receptor is the $\alpha_5\beta_1$ integrin, which belongs to the integrin family of cell adhesion molecules [7]. Usually the cells not only adhere to FN, but also secrete and organize this protein in the extracellular matrix (ECM) [8]. Thus, fibroblasts may organize substratum bound, or secreted FN *in vitro* into a specific fibrilar pattern [9–11], presumably as an attempt to form a matrix on the material surface [12]. We have recently shown that the ability of fibroblasts to organize such FN fibrils is dependent on the surface wettability, and hence it might be a characteristic for the biocompatibility of materials [5, 12]. EC can also organize FN into fibrils [11], but no data about the role of material surface wettability have been provided till now.

Further, we have shown that antibodies that bind β_1 integrin may induce a specific linear rearrangement of

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this cell receptor on the dorsal cell surface of living fibroblasts, presumably also reflecting the process related to the initial FN fibrillogenesis [13]. As, this activity was altered on hydrophobic substrata [13], we hypothesized that in order to be biocompatible, materials need to adsorb FN loosely so that cells can easily remove it from the substratum and organize into matrix-like structures [12] via the coordinated function of integrins [13]. In contrast, hydrophobic materials possess lower tissue compatibility, as they adsorb FN much stronger and change its biological conformation [14]. As a result, fibroblasts cannot organize a FN matrix, because of the local arrest of integrin function on such substrata [13].

On the other hand, fibrinogen (FNG), which is abundantly available in blood, was shown to strongly influence EC adhesion, motility and growth during events associated with blood coagulation and wound repair [15, 16]. FNG and its derivative fibrin, play an important role for the maintenance of the balance between prothrombotic and fibrinolytic properties of endothelium during events associated with blood coagulation and wound repair [17, 18]. Many studies have shown that the adhesion of endothelial cells to FNG is also mediated by integrins, particularly by the $\alpha_v\beta_3$ integrin [19, 20], which recognizes a single RGD-containing sequence near the C-terminus of the α-chain of the FNG molecule [19]. In addition, FNG can follow FN fibrillogenesis, as it was shown for epithelial cells [21], and may form a provisional matrix with FN during wound healing [22, 23]. Based on this, we hypothesized that EC may also use a FNG matrix for their proper interaction and growth on foreign substrata, especially during the initial steps of colonization of implants. However, since the role of EC for FN matrix organization is rather well studied [11, 14, 24] there are no data whether these cells organize FNG into matrix-like structure.

For that purpose, we initiated more detailed studies on the matrix remodeling activities of EC using human umbilical vein endothelial cells (HUVEC) as a model system. Assuming that the different surface wettability might cause a distinct conformational/orientation state of adsorbed FNG [4] which in turn affects cell adhesion [25] we used as a model hydrophilic (glass) and hydrophobic (ODS-coated) substrata to prove this possibility. Indeed, we found that EC were able to organize adsorbed FNG in a specific fibrilar pattern depending on the wettability of the substrata. Fibrillogenesis of soluble FNG on the dorsal cell surface was mediated by β_1 and not by β_3 integrins, and co-localized with FN fibrils. These findings allow us to conclude that the leading and/or initiating factor for FNG fibrillogenesis is the FN remodeling into matrix-like structures by EC. Details of this study are given herein.

Materials and methods

Hydrophilic and hydrophobic surfaces

To obtain hydrophilic surfaces glass slides (Superior-Marienfeld, Germany) were cleaned in 80% ethanol for 15 min. After extensive washing with distilled water, the glass slides were dried at 120 °C for 120 min and kept in dry places until use. To obtain hydrophobic surfaces, glass slides were treated first with solution of conc. H_2SO_4 and H_2O_2 in proportion of 3 : 1 for 15 min. After extensive washing, the slides were dried at the same conditions as above. Then the slides were treated with dimethyloctadecylchlorosilane (ODS) as described before [13]. Briefly, the slides were incubated in 2% (v/v) of ODS (purchased from Fluka, Neu-Ulm, Germany) in n-hexane (Merck, Darmstadt, Germany) for 1 h, then rinsed with hexane and ethanol (until the slides became transparent), washed with distilled water, and air-dried. The wettability of the materials was assessed by the sessile drop method measuring the static water contact angle on three different slides for each material. The water contact angle (CA) for hydrophilic glass was found to be $24 \pm 2.04°$, while the CA for ODS glass was $86 \pm 3.88°$.

Antibodies and proteins

Monoclonal antibodies against the human β_1 integrin (clone K20), β_3 integrin (clone SZ21) and fibronectin (clone 120-5) were obtained from Immunotech (Marseille, France). Secondary goat anti-mouse IgG antibody-conjugated with Cy2 was purchased from Jackson Immuno Research Laboratories (West Grove, PA, USA). Human plasma FN (Roche Diagnostics GmbH, Mannheim, Germany) and FNG purified also from human plasma, fraction I, type III (Sigma, Deisenhofen, Germany) were used in this study. When indicated, the proteins were labeled with fluorescein (FITC, Sigma) or Rhodamine Red (Molecular Probes, Leiden, The Netherlands) according to the manufacturer's protocol.

Cell culture and substrate coating

Human umbilical vein endothelial cells (HUVEC) were used between passages 2 and 8 to avoid senescence of cells. They were cultured in endothelial cell growth medium (Cell Lining, GmbH, Berlin, Germany) supplemented with 2% fetal calf serum (FCS), basic fibroblast growth factor (bFGF, 1 ng/ml), endothelial cell growth supplement/heparin (ECGS, 0.4%), Amphotericin/Gentamicin (50 ng/50 μg) at 37 °C and 5% CO_2. Cells from about confluent cultures were harvested with 0.05% trypsin/0.6 mM EDTA (Sigma). Trypsin was neutralized with FCS. When indicated, the slides were precoated for 30 min at room temperature (RT°) with FITC–FNG or FITC–FN (40 μg/ml), or intact FNG or FN (20 μg/ml), respectively. For some experiments Rhodamine-conjugated FNG was used to coat the slides at 40 μg/ml as described above.

Cell morphology

For studying the overall cell morphology, approximately 3×10^4 cells were incubated in endothelial cell growth medium for 2 h in six-well tissue culture plates (Falcon, Becton Dickinson, USA) containing the slides. Actin staining using BODIPY 558/568-conjugated phalloidin (Molecular Probes) was applied to visualize the overall cell morphology and the organization of actin cytoskeleton as previously described [5]. Briefly, cells were fixed

in 3% paraformaldehyde (PFA) for 15 min, permeabilized with 0.5% triton X-100 (5 min), saturated with 1% BSA in PBS (30 min), pH 7.4 and incubated for 30 min at RT° with 4 $U ml^{-1}$ BODIPY-conjugated phalloidin. Then samples were washed and mounted in Mowiol [26], and studied with confocal laser scanning microscopy (CLSM).

Remodeling of substratum-bound or soluble fibronectin and fibrinogen

Reorganization of substratum-bound FN and FNG was observed by incubation of HUVEC on hydrophilic or hydrophobic slides precoated with FITC–FN or Rhodamine–FNG. After 4 h of incubation in 10% serum-containing medium, cells were fixed with 3% PFA, washed and mounted in Mowiol.

For evaluating of the organization of soluble FN and FNG cells were incubated for 1 h on FN coated substrata and then FITC–FN or FITC–FNG (100 μg/ml) was added for an additional 2 h of incubation. Subsequently the samples were fixed, mounted and viewed with CLSM.

Distribution of integrin receptors on the ventral and dorsal cell surface

To detect integrin clustering, cells were incubated for 1 h in serum-free medium (basal endothelial cell growth medium, Cell Lining) on glass and ODS slides. The slides were coated with FN (20 μg/ml) for visualization of the β_1 integrin, and with FNG (20 μg/ml) for β_3 integrin, respectively. Cells were then fixed, permeabilized, saturated with 1% BSA as described above, and incubated for 30 min with monoclonal anti-β_1 or monoclonal anti-β_3 antibodies, respectively, and visualized with goat anti-mouse IgG–Cy2-conjugated as a secondary antibody. For detection of integrins on the dorsal cell surface only, the cells were processed as described but without the permeabilization step. The samples were studied with CLSM.

Co-localization experiments

To detect co-localization between FNG and FN or integrins, HUVEC were incubated on FN (20 μg/ml) coated slides for 1 h. Soluble FNG-Rhodamine (100 μg/ml) in the presence of 10% FCS was added and the cells were further incubated for 2 h. After the washing procedure the cells were fixed and saturated with 1% BSA to suppress non-specific antibody binding.Then cells were incubated with primary monoclonal anti-FN and anti β_1, or β_3 antibodies, as specified above for 30 min, then washed. The distribution of labeled proteins was visualized with goat anti-mouse IgG–Cy2 conjugated secondary antibody using CLSM.

Results

Overall cell morphology

Fig. 1 shows the morphology of HUVEC adhering on FN and FNG coated hydrophilic and hydrophobic substrata, after 2 h of incubation. Significant differences in the cell shape were found depending on the wettability of substrata and the type of coating. The cells attached to the FN coated hydrophilic glass were well spread (Fig. 1A) containing prominent linear arrays of actin bundles. On hydrophobic ODS surface (Fig. 1B) the cells were less spread and exhibited predominantly circumferential organized actin filaments. On FNG substrata cells exhibited a quite different morphology (Fig. 1C and D). The number of adherent cells was visibly higher on glass (Fig. 1C) than on ODS (Fig. 1D), and the cells also spread better on glass (Fig. 1C), although many of them possessed an irregular shape indicating an enhanced motility on this substrate. The peripheral organization of actin filaments and the formation of distinct leading and trailing cell edges were another sign for a higher motility of cells, interestingly, better pronounced on the hydrophobic ODS substrata (Fig. 1D).

Reorganization of substratum – bound FN and FNG

FITC-conjugated FN (FFN) and FNG (FFNG) were adsorbed on glass and ODS, and the substrata were incubated with HUVEC in EC medium containing 10% FCS, for 4 h. We used this technique of direct fluorescent labeling of protein, instead of antibody tagging techniques, considering the limiting antibody accessibility beneath the cells [9, 10]. As was shown in Fig. 2A, a significant amount of adsorbed FFN was readily removed by the cells from the hydrophilic glass and accumulated in fibrilar structures (arrows in Fig. 2A) along the cell margins or beneath the cells. In marked contrast, no removal and no reorganization of FFN by HUVEC was found, when it was adsorbed to ODS. On FFNG coated glass the removal was less pronounced in comparison to FFN, but still well visible dark patches and streaks on the bright fluorescent background of adsorbed FFNG were observed (Fig. 2C). Again, no removal of FFNG on ODS was detected (Fig. 2D), although some accumulation of fluorescent FNG was observed around the cell nucleus, which can be interpreted as non-specific staining. Some internalization of the fluorescent protein however, cannot be excluded. The uniform and slightly visible dark zones around some of the cells revealed the possibility of an increased proteolytic cellular activity on ODS surfaces.

To check whether labeled protein affect cell adhesion and overall morphology to a certain extent, the separate experiments with non-labeled FN and FNG were performed and no difference was observed (data not shown).

Remodeling of soluble FN and FNG

To determine whether exogenous (soluble) FN or FNG can be organized by HUVEC, the cells were plated onto FN coated glass and ODS and allowed to spread for 1 h. FFN or FFNG conjugates were added for additional 2 h. Fig. 3A shows that FN was readily organized in fibril-like structures on glass as it was expected and shown previously [5]. Confocal images showed that FN fibrils span several cells forming a complex FN matrix. On ODS substrate the FN fibrillogenesis was considerably

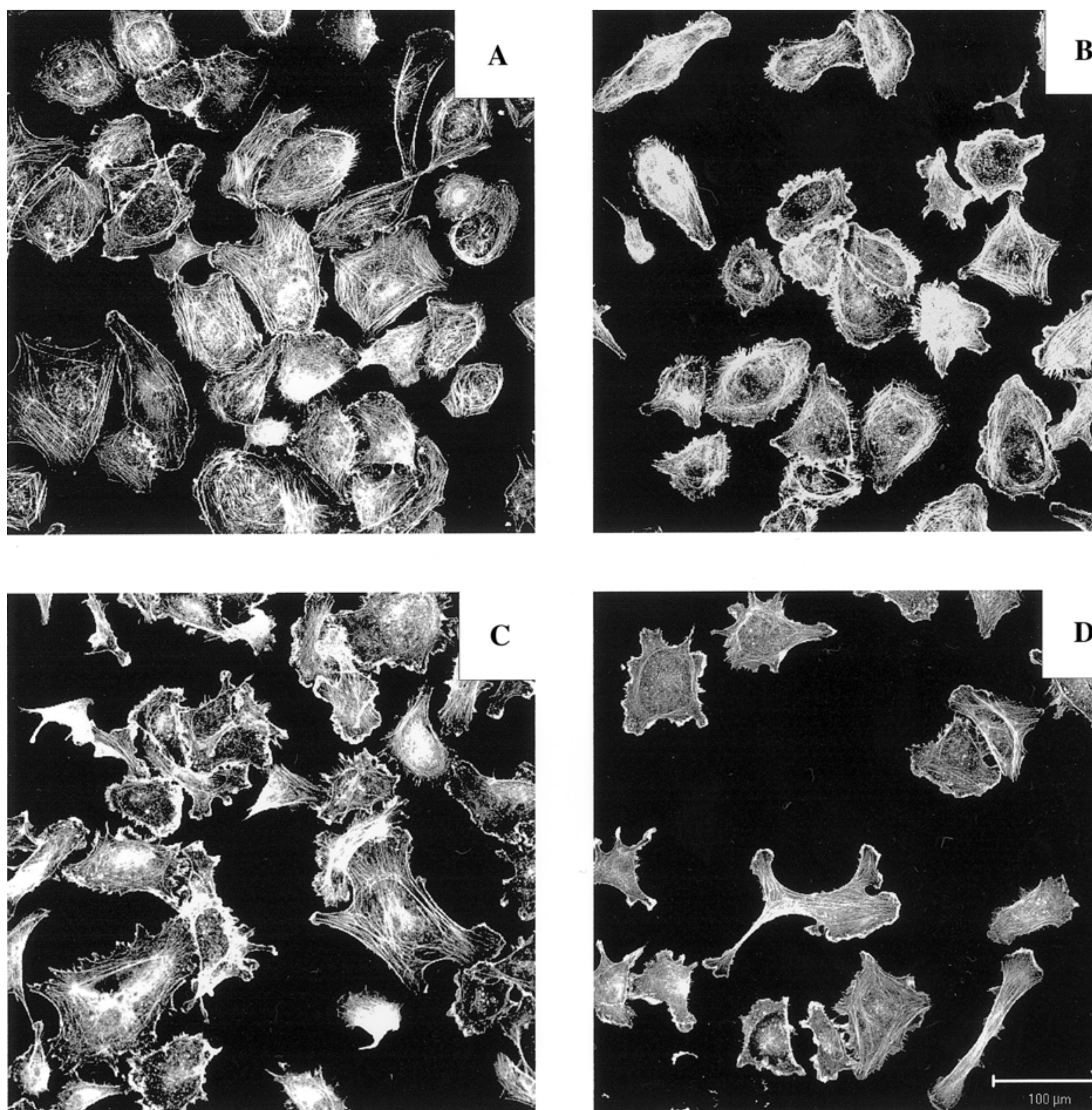

Figure 1 Overall cell morphology of HUVEC. Glass (A and C) and ODS (B and D) slides were coated with 20 µg/ml FN (A and B) or with 20 µg/ml FNG (C and D). The cells were allowed to spread on the coated slides in EC growth medium for 2 h, then fixed, permeabilized, saturated and stained for actin using BODIPY-phalloidin. Bar is 100 µm.

reduced (Fig. 3B) and only short streaks were observed around the cell margins. In contrast to FN, soluble FNG had a tendency to become less organized. It was only deposited in linear structures mostly at the cell periphery as it is shown in Fig. 3C (arrows in C) for hydrophilic glass. Many aggregates of FFNG were also detected in these preparations. Some internalization of the aggregates because of the obvious perinuclear (Goldgi) accumulation could not be excluded (Fig. 3C, bold arrows). On ODS only some perinuclear accumulation of FFNG was found, again suggesting some internalization activity, but not any fibrilar organization was visible (Fig. 3D). Control experiments were performed in which FNG was used as substratum for cell attachment, to study the possible influence of the surface coating on the organization of both, FN and FNG. The obtained results showed that the type of coating does not play a significant role in the reorganization of soluble FN and FNG (data not shown).

FN and FNG receptor clustering in HUVEC

To better characterize the EC interactions with the above substrata, we studied the organization of β_1 integrin, as constituent of the FN receptor ($\alpha_5\beta_1$), and of β_3 integrin, as constituent of the FNG receptor ($\alpha_v\beta_3$), on permeabilized cells. HUVEC were allowed to attach for 1 h on the respective ligands (FN or FNG), coated on hydrophilic or hydrophobic substrata, than the cells were fixed, permeabilized and stained to visualize integrins. Fig. 4 (upper panel) represents a typical view of EC on FN coated glass, where numerous β_1-rich streaks of focal adhesions were found (presumably on the ventral cell surface) when HUVEC were spread on hydrophilic glass (Fig. 4A). On FN-coated ODS (Fig. 4B) however, β_1 integrins were more dot-like distributed and only single streaks of focal adhesions were observed.

As is shown on the lower panel of Fig. 4, on FNG-coated substrata EC represented much less organized β_3 integrins, mostly as dot-like structures, but on the

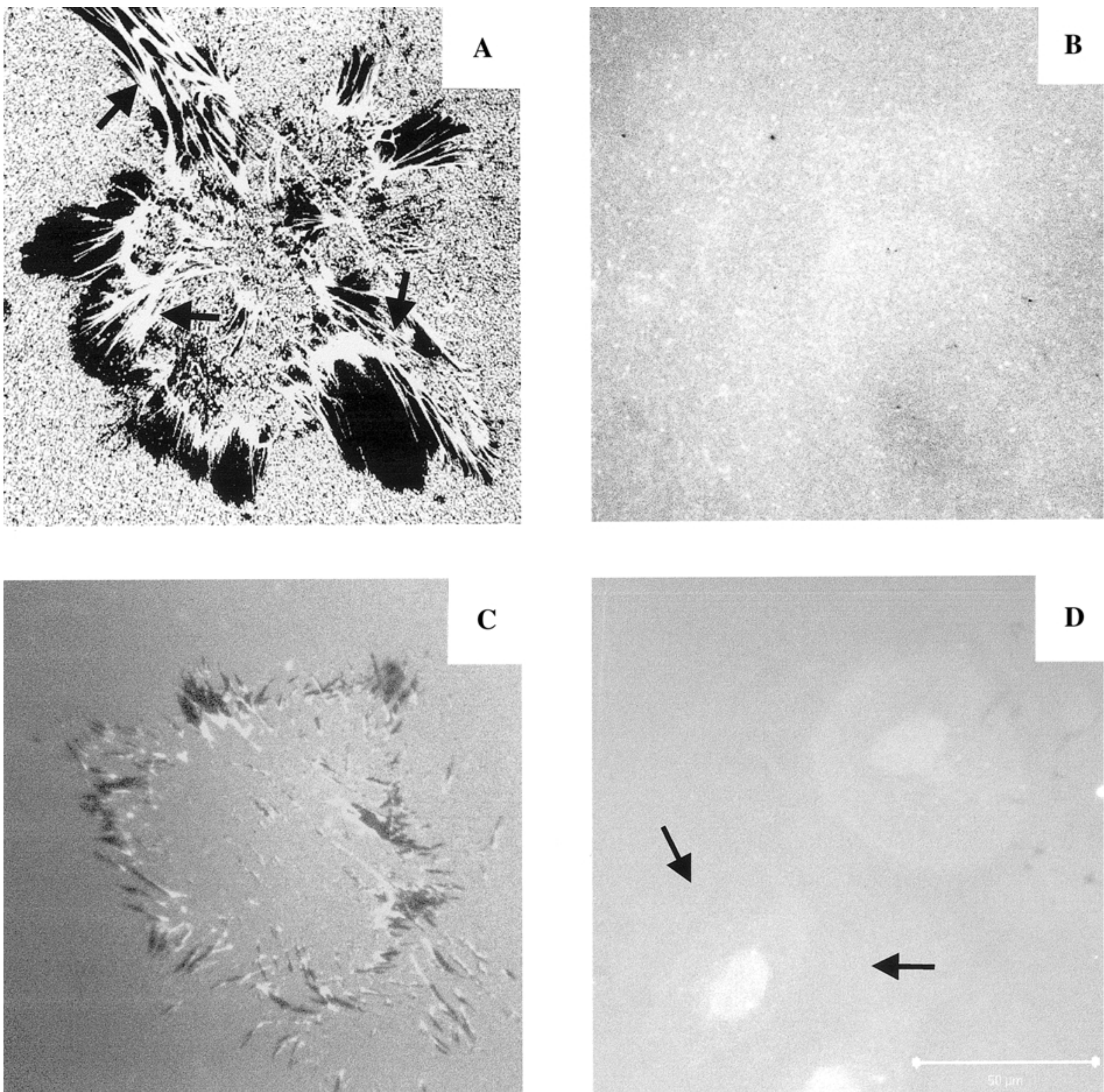

Figure 2 Reorganization of substratum-bound FN and FNG. Glass (A and C) and ODS (B and D) were coated with 40 µg/ml FITC–FN (FFN, A and B) or with 40 µg/ml Rhodamine Red–FNG (FFNG, C and D). Adsorbed FFN on glass (A) was organized in fibrilar structures at the cell periphery (arrows in A). No removal of adsorbed FFN was detected on ODS (B). FFNG was well organized on glass (C), while on ODS (D) only an accumulation of adsorbed FFNG beneath the cell center was observed. Uniform dark zones (arrows in D) were slightly visible. Bar is 50 µm.

hydrophilic glass some of them also formed elongated streaks (Fig. 4C) located at the cell edges. Clustering of β_3 was not observed on FNG-coated ODS (Fig. 4D).

Organization of β_1 and β_3 integrins on the dorsal cell surface

To study the possible role of β_1 and β_3 integrins for FN and FNG remodeling on the dorsal cell surface, we cultured the HUVEC on the respective protein coated glass or ODS substrata. The cells were incubated in serum-free medium, to avoid the effect of other proteins, for 1 h and further fixed and stained (without permeabilization) for the above integrins. Fig. 5 shows the typical linear pattern of the dorsal organization of β_1 integrin on FN coated glass (Fig. 5A). The strong fluorescent signal in the middle of the cell is presumably an artifact from aggregates of anti β_1 integrin antibodies. On ODS the β_1 integrin was much less organized (Fig. 5B). In contrast on FNG coated substrata, the β_3 integrin exhibited only a punctual staining for both hydrophilic (Fig. 5C) and hydrophobic ODS substrata (Fig. 5D).

Co-localization of β_1 or β_3 integrins with FNG fibrils

To address the question which integrins were involved in the fibrilar organization of FNG on the dorsal cell surface on hydrophilic substrata, double staining experiments were conducted. HUVEC were incubated on FN coated substrata for 1 h and Rhodamin-conjugated FNG was added for the next 2 h of incubation. The cells were fixed and stained for β_1 or β_3, integrins, respectively using Cy2-conjugated secondary antibody. Since we did not find any fibrilar organization of FNG on ODS surfaces these experiments were performed with glass surfaces

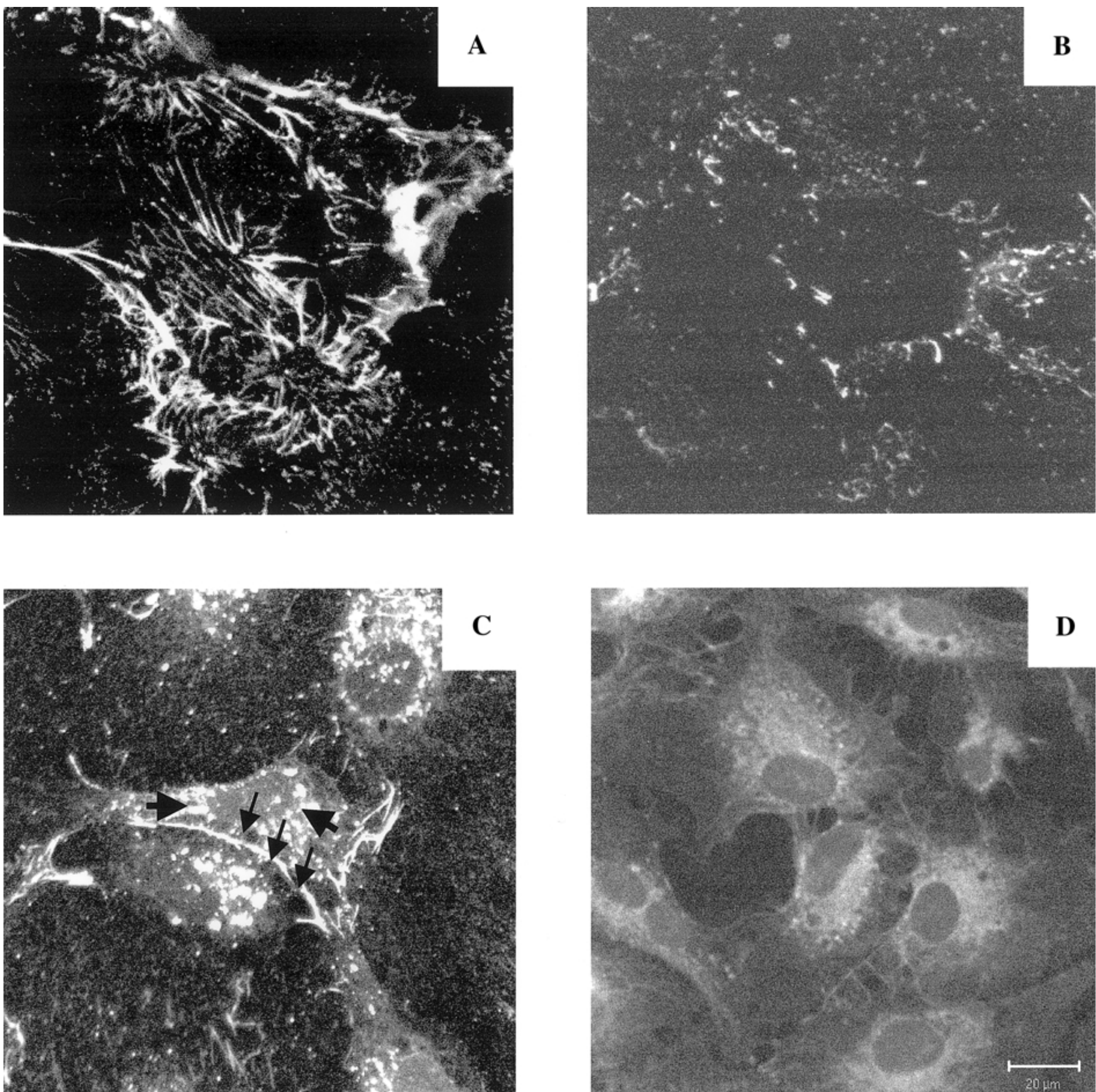

Figure 3 Reorganization of soluble FN and FNG. HUVEC were incubated on FN (20 µg/ml) coated glass (A and C) and ODS (B and D). 100 µg/ml FITC–FN (A and B) or FITC–FNG (C and D) were added for further 2 h of incubation. FN fibrils on glass (A) spanned several cells organizing a FN matrix. On ODS (B) only short FN streaks mostly at the cell margins were observed. FNG on glass (C) showed strong linear structures along the cell body (arrows in C), and aggregates in cell cytoplasm (bold arrows in C). On ODS (D) only an amorphous distribution of fluorescent FNG was visible on the cell body. Bar is 20 µm.

only. It was found that FNG fibrils co-localized with β_1 integrin and not with β_3 intergin. As can be seen in Fig. 6 (left panel, C) the FNG fibrils co-localized with the elongated β_1-rich streaks at the cell periphery (inset in Fig. 6, left panel, C), while for β_3 integrin such co-localization was absent (Fig. 6, middle panel; C).

Co-localization of FNG and FN fibrils on the dorsal cell surface

Because FNG fibrils were found to co-localize with the β_1 subunit of the main FN receptor on EC [27], it was interesting to test whether there was some relation between FN and FNG fibrillogenesis. For that purpose HUVEC were seeded on FN coated glass and incubated for 1 h. Then Rhodamine-labeled FNG was added for subsequent 2 h. Cells were fixed and stained for extracellular FN using a monoclonal anti-FN antibody visualized by secondary Cy2-conjugated antibody. After the incubation FNG fibrils were already deposited and assembled in patches (Fig. 7A). It was observed that FN matrix fibrils (Fig. 7B) co-localized with FNG fibrils, but only at the cell periphery (Fig. 7C) suggesting their transient association.

Discussion

Seeding of cardiovascular implants with endothelial cells is a desirable effect in order to improve the blood compatibility of these devices. Since FNG and FN are the natural constituents of the wound bed at the sites of vascular injury and new vessel formation [28], the interaction with these proteins when adsorbed to material surfaces could affect EC seeding, especially on poor

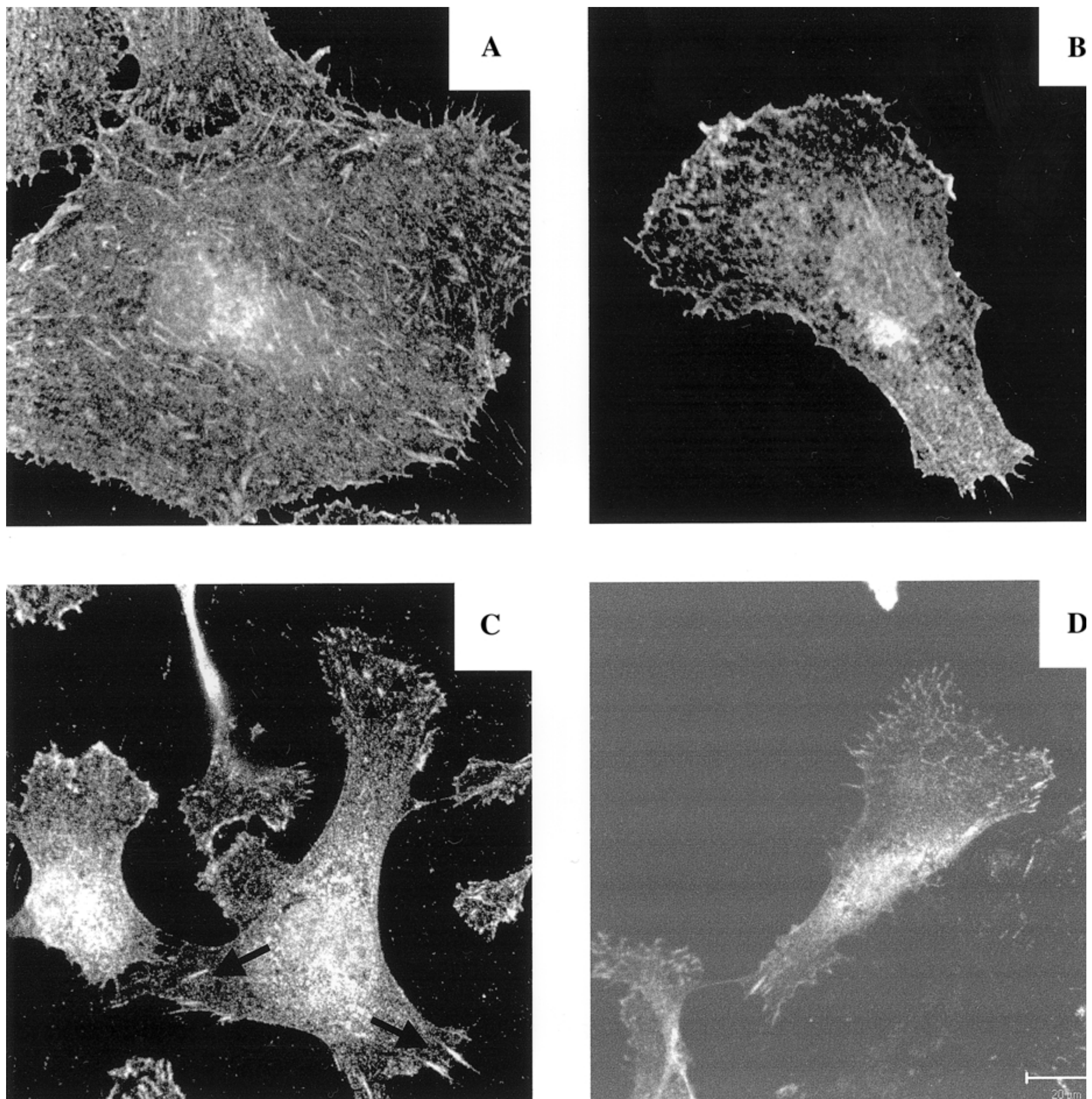

Figure 4 Detection of integrin clustering. Glass (A and C) and ODS (B and D) slides were coated with 20 μg/ml FN (A and B) or with 20 μg/ml FNG (C and D). The cells were stained for β_1 (A and B) or for β_3 (C and D). β_1 on glass (A) was localized in numerous linear streaks representing focal adhesions, while on ODS (B) only a few adhesion plaques were visible. On glass (C) β_3 was localized in the form of streaks and spots (arrows in C) at the cell edges or in rather elongated streaks (bold arrows in C). The clustering of β_3 integrin on ODS (D) was diminished in single short streaks at the cell endings. Bar is 20 μm.

wettable substrata. However, cells not only adhere to matrix proteins, but also organize them into matrix-like structures [8, 12]. It is well known that the adsorption of matrix proteins is affected by the substratum wettability [3], but how this reflects the ability of cells to organize their own matrix, particularly FN, was one of the main topics of our research during the last decade [5, 12, 13]. Recently, we postulated that in order to be biocompatible, materials need to adsorb FN loosely, so that it can be easily reorganized by cells into matrix-like fibrils [5]. Here, we extended our previous studies on fibroblasts to the ability of endothelial cells to reorganize matrix components on foreign substrata.

The main observation of this study was that EC were able to organize both adsorbed and soluble FNG in specific fibrilar structures, similar to the FN matrix. Recently, Pereira *et al.* [29] have demonstrated that human fibroblasts can organize FNG, and so do alveolar epithelial cells [21]. However until now there are no data available for EC. Although the ability of EC to organize a FN matrix was studied previously [11, 14, 24], here we provide evidence that this is inhibited on hydrophobic substrata. Thus, fibrillogenesis is altered on hydrophobic substrata, which in general confirmed our previous observations with human fibroblasts [5]. However, the important finding here was that the wettability of substrata affects the FNG remodeling activity of EC.

The incorporation of FNG into the extracellular matrix of epithelial cells was shown to be dependent on the active assembly of a FN matrix [23]. Dejana *et al.* [28] reported that endothelial cell spreading on FNG was affected by cellular fibronectin synthesized by themselves. This suggests a possible joint mechanism for interaction. Our data support this finding with the observation of a different pattern of integrin organization during the interaction with substratum-bound and

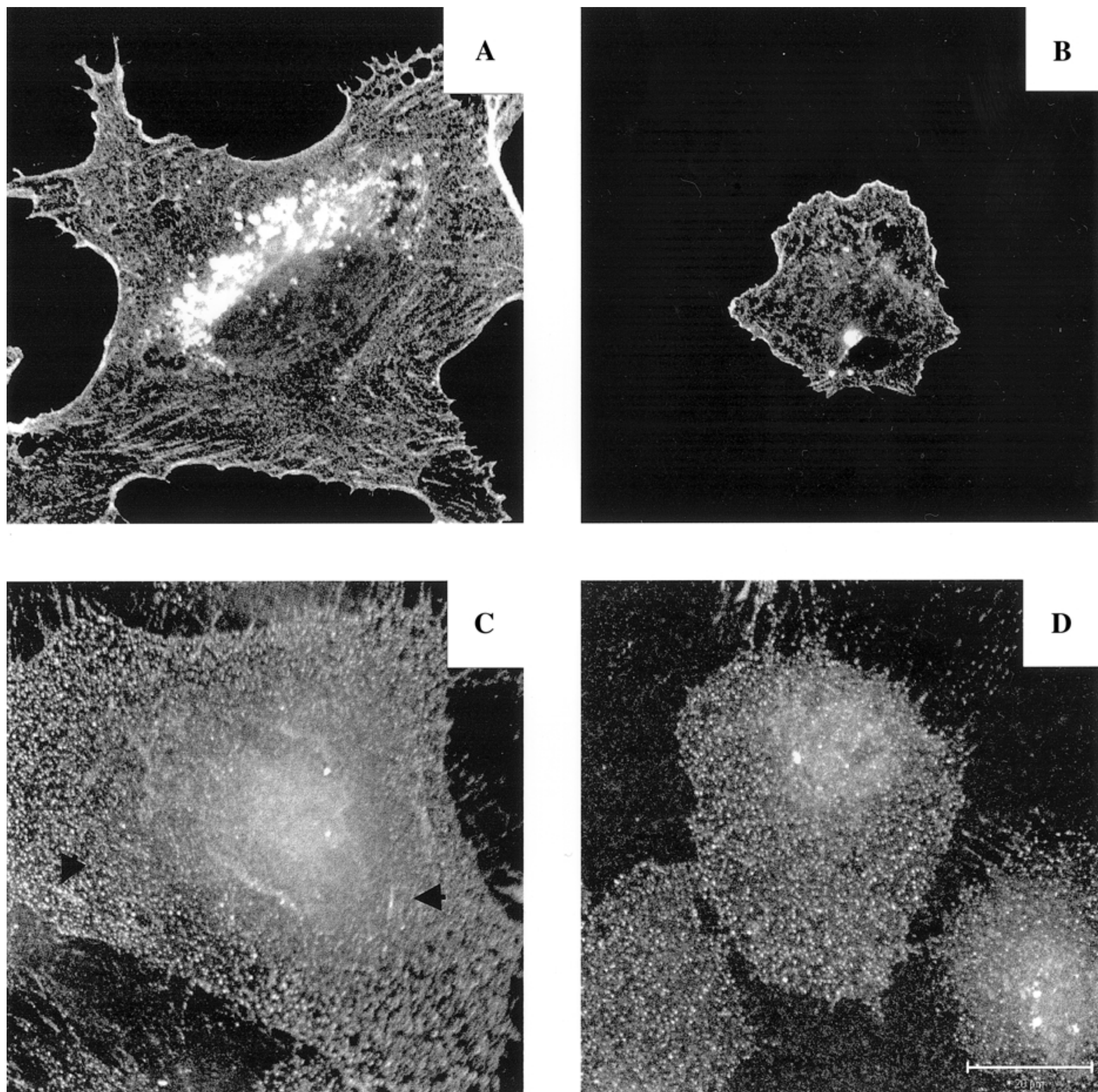

Figure 5 Distribution of β_1 and β_3 on the dorsal cell surface. HUVEC were incubated on FN (20 μg/ml) coated glass (A and C) and ODS (B and D). The cells were stained for β_1 (A and B) or β_3 (C and D). Defined linear pattern of organization of β_1 on glass (A), was greatly reduced on ODS (B). β_3 showed a punctuate staining on glass (C) with few linear streaks (arrows in C). Diffusive β_3 distribution was found on ODS (D). Bar is 20 μm.

soluble FNG. It is known the adhesion of HUVEC to adsorbed FNG is mediated by $\alpha_v\beta_3$ integrin [20]. Indeed, our data also showed that β_3 integrin clusterized in structures resembling focal adhesion contacts when EC adhere to FNG substrata. Conversely, on the dorsal cell surface FNG fibrils were not co-localized with β_3 integrin, representing a punctuate distribution, in contrast to β_1 integrin, which showed a well pronounced linear pattern of organization. The absence of β_1 integrin from the focal adhesion plaques reported previously by Dejana *et al.* [28], is an indication that the FN receptor does not participate in EC adhesion to FNG. Integrin β_1 however, has clearly shown to be involved in FN fibril formation [28–30]. Co-assembly of FNG and FN was also shown for both epithelial cells [21] and fibroblasts [29]. Thus, the existence of such joint fibrillogenesis was very probable for EC as well. Indeed, we found a clear morphological evidence for the co-localization of FN and FNG fibrils on the dorsal cell surface of HUVEC. It should be noted also, that the incorporation of FN into matrix fibrils starts from the distinct place at the cell periphery, near to the focal adhesions [29]. All these facts suggest the leading role of FN in this process.

The influence of substratum wettability was clearly outlined since on the poor wettable ODS the remodeling of adsorbed FNG was significantly abolished, correlating with less β_3 integrin clustering. We hypothesize that it is due to the higher affinity of the substrata for FNG [4] preventing this protein to be removed and reorganized by EC, as we have shown previously for FN [5]. The lack of linear β_3 integrin reorganisation on the dorsal surface of cells plated on hydrophobic ODS could be also explained by the strongly bound ligand. For example, FN as well as FNG adsorbed strongly on hydrophobic surfaces, as previously suggested, may immobilize the corresponding integrins on the ventral cell surface thus preventing their translocation to the dorsal cell surface [13, 32].

On the other hand, it was shown previously [15, 19], that adsorbed FNG may induce motility and growth of endothelial cells. Moreover, other matrix proteins such as

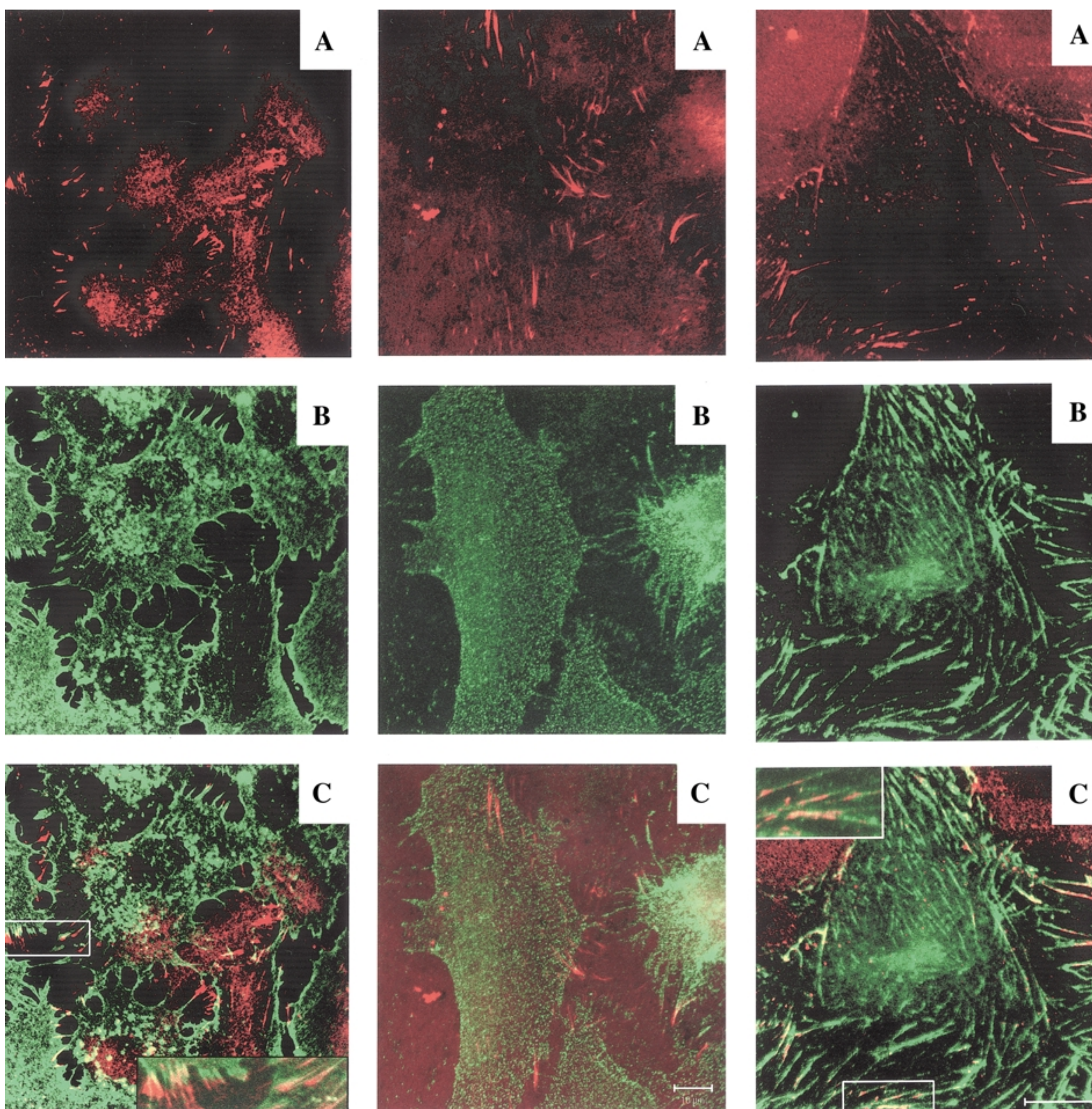

Figure 6 Co-localization of β_1 or β_3 with FNG fibrils. Glass slides were coated with FN (20 μg/ml). Soluble FNG-Rhodamine Red (100 μg/ml) was added to the living cells for further 2 h. The fixed cells were stained for β_1-middle panel, or for β_3-middle panel. Left panel (C-superimposed image) shows the co-localization (inset in C) between FNG fibrils (red channel) and β_1 linear streaks (green channel) mostly at the cell periphery. In the right panel is visualized the fibrilar organization of FNG (A), dot-like organization of β_3 (B) and lack of co-localization between FNG and β_3 (C-superimposed image). Bar is 10 μm.

Figure 7 Co-localization between FNG and FN fibrils (right panel). HUVEC were incubated on FN (20 μg/ml) coated glass slides. Soluble FNG–Rhodamine Red (100 μg/ml) was added to the living cells for further 2 h. The cells were stained for FN using anti-FN monoclonal antibody. FNG fibrils are spanned around the cell periphery (A-red channel), while FN formed linear net of fibrils over the entire cell surface (B-green channel). The FNG and FN fibrils co-localized mostly at the cell periphery (C-superimposed image). Bar is 50 μm.

FN and vitronectin [28] may enhance this motility. Thus, both substratum adsorbed and already fibrilized FNG and FN may affect the colonization of implants. Our data also suggest that HUVEC acquired a motile morphology on FNG substrata. However, on hydrophobic ODS, although more FNG may be adsorbed [4], integrins clusterize less efficiently than on glass, which might be due to a defect signal transfer to the cell interior via integrins [33]. All this, taken together with the observed less FNG/FN matrix formation, may explain why hydrophobic cardiovascular implant materials are difficult to become colonized by EC.

In conclusion, our results provide new insight into the ability of EC to interact and remodel FN and FNG in a spatially organized and coordinated manner in contact to biomaterial surfaces. These cellular events seem to be extremely important for the biocompatibility of cardiovascular implants, as they were dramatically altered on hydrophobic substrata, which resemble most of the current cardiovascular implant materials.

References

1. A. TASSIOPOULOS and H. GREISLER, *J. Biomater. Sci. Polymer Edn.* **11** (2000) 1275.

2. A. SCHNEIDER, R. MELMED, H. SCHWALB, M. KARCK and G. URETZKI, *J. Vasc. Surg.* **15** (1992) 649.
3. J. BRASH, *J. Biomater. Sci. Polymer Edn.* **11** (2000) 1135.
4. R. TZONEVA, M. HEUCHEL, T. GROTH, G. ALTANKOV, W. ALBRECHT and D. PAUL *ibid.* (in press).
5. G. ALTANKOV and T. GROTH, *J. Mater. Sci. – Mater. M.* 5 (1994) 732.
6. R. HYNES, in ''Fibronectins'' (Springer-Verlag, New York, 1990).
7. R. HYNES, *Cell* 48 (1987) 549.
8. E. HAY, in ''Cell Biology of Extracellular Matrix, 2nd edn.'', edited by E. Hay, (Plenum Press, New York, 1991) p. 468.
9. F. GRINNELL, *J. Cell Biol.*, **103** (1986) 2697.
10. Z. AVNUR and B. GEIGER, *Cell* **25** (1981) 121.
11. R. CHRISTOPHER, A. KOWALCHYK and P. MCKEWON-LONGO, *J. Cell Sci.* **110** (1997) 569.
12. G. ALTANKOV, F. GRINNELL and T. GROTH, *J. Biomed. Mater. Res.* 30 (1996) 385.
13. G. ALTANKOV, T. GROTH, N. KRASTEVA, W. ALBRECHT and D. PAUL, *J. Biomater. Sci. Polymer Edn.* **8** (1997) 721.
14. D. IULIANO, S. SAAVEDRA and G. TRUSKEY, *J. Biomed. Mater. Res.* **27** (1993) 1103.
15. E. DEJANA, L. LANGUINO, N. POLENTARUTTI, G. BALCONI, J. RYCKEWAERT, M. LARRIEU, M. DONATI, A. MANTOVANI and G. MARGUERIE, *J. Clin. Invest.* **75** (1985) 11.
16. M. CHANG, B. WANG and T. HUANG, *Thromb. Haemost.* **74** (1995) 764.
17. M. GE, G. TANG, T. RYAN and A. MALIK, *J. Clin. Invest.* **90** (1992) 2508.
18. M. WISSINK, R. BEERNINK, A. POOT, G. ENGBERS, T. BEUGELING, W. VAN AKEN and J. FEIJEN, *Biomaterials* **22** (2001) 2283.
19. D. CHERESH, *Proc. Natl. Acad. Sci.* **84** (1987) 6471.
20. D. CHERESH, S. BERLINER, V. VICENTE and Z. RUGGERY, *Cell* **58** (1989) 945.
21. G. GUADIZ, L. SPORN and P. SIMPSON-HAIDARIS, *Blood* **90** (1997) 2644.
22. R. CLARK, J. LANIGAN, P. DELLAPELLE, E. MANSEAU, H. DVORAK and R. COLVIN, *J. Invest. Dermatol.* **79** (1982) 264.
23. D. DONALDSON, J. MAHAN, D. AMRANI and J. HAWIGER, *J. Cell Sci.* **94** (1989) 101.
24. D. MOSHER, J. SOTTILE, C. WU and J. MCDONALD, *Curr. Opin. Cell Biol.* **4** (1992) 810.
25. N. HALLAB, K. BUNDY, K. O'CONNOR, R. CLARK and R. MOSES, *J. Long Term Eff. Med. Implants* **5** (1995) 209.
26. E. HARLOW and D. LANE, in ''Antibodies'' (Cold Spring Harbor, NY, 1988) p. 726
27. E. DEJANA, in ''Vascular Endothelium: Interactions with Circulating Cells'', edited by J. L. Gordon (Elsevier Science Publishers BV, 1991) p. 31.
28. E. DEJANA, M. LAMPUGNANI, M. GIORGI, M. GABOLI and P. MARCHISIO, *Blood* **75** (1990) 1509.
29. M. PEREIRA, B. RYBARCZYK, T. ODRLJIN, D. HOCKING, J. SOTTILE and P. SIMPSON-HAIDARIS, *J. Cell Sci.* **115** (2002) 609.
30. Z. ZHANG, A. MORLA, J. VOURI, J. BAUER, R. JULIANO and E. RUOSLAHTI, *J. Cell Biol.* **12** (1993) 235.
31. R. PANKOV, E. CUKIERMAN, B. KATZ, K. MATSUMOTO, D. LIN, S. LIN, C. HAHN and K. YAMADA, *ibid.* **148** (2000) 1075.
32. T. GROTH, G. ALTANKOV, A. KOSTADINOVA, N. KRASTEVA, W. ALBRECHT and D. PAUL, *J. Biomed. Mater. Res.* **44** (1999) 341.
33. A. GARCIA and D. BOETTIGER, *Biomaterials* **20** (1999) 2427.

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