Bioactive Microarrays Immobilized on Low-Fouling Surfaces to Study Specific Endothelial Cell Adhesion

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With the aim to study how to modulate the specific endothelial cell patterning and responses on biomaterials surfaces, bioactive microarrays were developed and validated for specific cell patterning. These microarrays were made of low-fouling surfaces, that prevent nonspecific cell adhesion, bearing bioactive molecules at given known locations by presenting specific ligands to cell receptors. Arrays of bioactive molecules (RGD, REDV, and SVVYGLR sequences and vascular endothelial growth factor (VEGF)) were immobilized on a carboxy-methyl-dextran low-fouling surface and were exposed to human endothelial cells and fibroblasts to screen for the effect of bioactive spot molecular composition on cell adhesion. Endothelial cells only were sensitive to RGD peptide co-immobilized with REDV or SVVYGLR sequences: they induced a reduction in cell spreading and a loss of actin stress fibers. RGD co-immobilized with VEGF also resulted in the reorganization of actin filaments and focal points in endothelial cells. Combination of RGD with these endothelial cell-selective biomolecules did not elicit a strong adhesion phenotype but rather one characteristic of migrating cells.

1. Introduction

Control over endothelial cell responses at the biomaterial interface is important in applications such as vascular prosthesis endothelialization and tissue engineering.[1] The formation of a continuous monolayer of endothelial cells on the luminal surface of vascular prosthesis helps to resolve the problem of graft thrombogenicity.[2] On the other hand, to construct or regenerate organs, invasion of a scaffold by endothelial cells and the subsequent formation of a capillary network is essential for tissue survival and growth.[3,4]

Endothelial cell adhesion to a biomaterial surface and subsequent cell survival, proliferation, or migration are mediated by cell−material interactions via cell adhesion receptors such as integrins.[5] Control of cell−material interactions, and thus cell behavior, may first be achieved by preventing nonspecific protein adsorption and cell adhesion and, second, by exposing specific cell ligands or bioactive molecules to optimize the adhesion, migration, and/or proliferation of desired cells.

Microarrays of extracellular matrix (ECM) proteins and growth factors have been used to screen molecules impact on cell adhesion, proliferation, and differentiation.[6−9] In the present study, arrays of bioactive molecules known to be specific for endothelial cells were fabricated to analyze their effect on endothelial cell adhesion. Bioactive molecules were immobilized on a low-fouling template layer to be able to directly observe only the effect of molecule−cell receptor binding on cell responses by efficiently eliminating nonspecific interactions. Layers made of carboxy-methyl-dextran (CMD), a dextran derivative, were used because they highly resist nonspecific protein adsorption and cell adhesion[10−14] and possess reactive groups convenient for the grafting of bioactive molecules such as peptides and proteins.[15]

RGD is the most effective and most often used peptide sequence to promote cell adhesion on synthetic surfaces.[16] The RGD sequence is present in many ECM proteins such as fibronectin, vitronectin, and collagen, and RGD is able to address more than one integrin receptor. The RGD peptide was immobilized on its own and in combination with other bioactive molecules specific for endothelial cells.

The REDV sequence, found within the alternatively spliced CS5 fibronectin domain, is specifically recognized by the integrin $\alpha 4\beta 1$.[17] REDV peptides grafted on synthetic surfaces were shown to induce selective adhesion of endothelial cells,[18,19] and the REDV sequence mediates endothelial cell migration on fibronectin via its $\alpha 4\beta 1$ receptor.[20] The SVVYGLR is a cryptic sequence exposed after osteopontin thrombin cleavage and is principally recognized by integrin $\alpha 9\beta 1$.[21] Grafted SVVYGLR induces endothelial cell adhesion, migration, and enhances angiogenesis in its soluble form.[22−24] REDV and SVVYGLR sequences are believed to be selective for endothelial cells as $\alpha 4\beta 1$ and $\alpha 9\beta 1$ expression is limited to a small number of cell types.[25,26]

Vascular endothelial growth factor (VEGF) is an endothelial-specific growth factor. VEGF-induced effects are principally mediated by interaction with its receptor VEGF-R2: it enhances endothelial cell proliferation, migration, and survival and is a potent angiogenic factor.[27,28] In vivo, endothelial cells respond to both soluble VEGF and immobilized VEGF, bound to the ECM. In vitro, immobilized VEGF induces endothelial cell migration and survival.[29,30]

In this study, microarrays made of bioactive molecules were exposed to human endothelial cells and human fibroblasts to investigate cell adhesion, spreading, cytoskeletal organization, and focal adhesion assembly with regard to bioactive spots molecular composition.

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2. Materials and Methods

2.1. Carboxy-methyl-dextran Layers. Detailed fabrication procedures of an optimized low-fouling CMD graft layer have been reported elsewhere.[12] CMD was prepared as follows. Five grams of 70 kDa dextran (Amersham Biosciences, Uppsala, Sweden, cat. no. 17-0280-01) were dissolved in 25 mL of a 1 M NaOH solution. Then, bromoacetic acid (Lancaster Synthesis Inc., Pelham, NH, cat. no. 3016) was added to a final concentration of 0.5 M. The solution was stirred overnight and dialyzed against Milli-Q water (Millipore Canada, Nepean, Canada), 0.1 M HCl, and finally Milli-Q water, for 24 h each. The solution was then lyophilized and stored at −20 °C. The carboxylation degree was determined by ^{1}H NMR to be 27.5%.

Borosilicate glass substrates (Assistent, Sondheim, Germany) were washed in Sparkleen solution (Fisher Scientific Canada, Ottawa, Canada), thoroughly rinsed with Milli-Q water, and dried with 0.2 μm filtered air. Clean substrates were surface-modified by plasma polymerization of *n*-heptylamine (99.5% purity, Sigma-Aldrich, Oakville, Canada, cat. no. 126802) in a custom-built plasma reactor.[31] Conditions of plasma polymerization were an initial monomer pressure of 0.040 torr, an ignition power of 80 W, an excitation frequency of 50 Hz, a deposition time of 45 s, and a distance between the electrodes of 10 cm. The resulting *n*-heptylamine plasma polymer (HApp) layers exposed functional amine groups that were used to graft CMD layers using carbodiimide chemistry.

A 2 mg/mL CMD solution was filtered with 0.45 μm pore size membrane to limit CMD aggregates and then activated with 17 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, Sigma-Aldrich, cat. no. E1769) and *N*-hydroxysuccinimide (NHS, Sigma-Aldrich, cat. no. H7377) for 10 min. Substrates freshly covered by HApp layers were rapidly immersed into the activated CMD solution, and reaction was allowed to proceed for 2 h under agitation. CMD surfaces were then rinsed with 1 M NaCl solution (2 × 12 h), with Milli-Q water (2 × 12 h), and autoclaved to remove any unbound molecules left. They were dried with 0.2 μm filtered air and stored in a desiccator until needed.

2.2. Fabrication of Arrays of Bioactive Molecules. Peptide sequence G−R−G−D−S and its inactive control G−R−G−E−S, named RGD and RGE (American Peptide Company, Sunnyvale, CA, cat. nos. 44-0-23 and 44-0-51, respectively), were dissolved in 150 mM PBS (pH 8.5) to a final concentration of 25 μg/mL (solutions R25 and RGE25). In some samples, G−R−E−D−V−D−Y or G−D−S−V−V−Y−G−L−R (named REDV and SVVYGLR, Celtek Bioscience, Nashville, TN) was added with a final concentration of 1 μg/mL to R25 solutions (solutions RE1 and SV1). Also, in other samples, recombinant human VEGF165 (Peprotech, Rocky Hill, NJ, cat. no. 100-20) was added to a final concentration of 2 μg/mL to R25, RE1, or SV1 solutions (solutions R25V, RE1V, and SV1V, respectively).

CMD surfaces were activated by a 200 mM EDC + NHS solution for 8 min, rapidly rinsed in Milli-Q water, and dried with 0.2 μm filtered air. Biomolecule solutions were placed in a 384-well plate and three individual spots of each biomolecule solution were deposited with a 1200 μm pitch on activated CMD surfaces using a noncontact dispensing robot (BioChip Arrayer, Perkin-Elmer Life and Analytical Sciences, Boston, MA) with arraying capabilities such as those used to manufacture DNA and protein chips. The piezo-dispensers were set to dispense 50 drops of solution per spot with 350 pL of sample per drop, resulting in ca. 700 μm diameter spots. The reaction was allowed to proceed overnight in a chamber filled with air saturated with humidity to maintain the substrates at dew point. Bioactive molecule arrays were rinsed in PBS (pH 8.5) coupling buffer (2 × 3 h) and then with PBS at pH 7.4. They were finally immersed overnight in sterile PBS containing antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin, Invitrogen, Burlington, Canada, cat. no. 15140) and stored at 4 °C until needed.

2.3. Testing Bioactive Arrays toward Cell Responses. Human umbilical vein endothelial cells (HUVECs) from Promocell (Heidelberg, Germany, cat. no. C-12200) were cultured in Medium 199 (M199, Sigma, cat. no. M5017) supplemented with 10% fetal bovine serum (FBS, Sigma, cat. no. F1051), 100 units/mL penicillin, and 100 μg/mL streptomycin, 90 μg/mL heparin (Sigma, cat. no. H1027), 2 mM L-glutamine (Invitrogen, cat. no. 25030), and 20 μg/mL endothelial cell growth supplement (ECGS, Sigma, cat. no. E2759). Human foreskin fibroblasts harvested from human biopsies with collagenase were maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, cat. no. 12100) with 10% FBS and antibiotics. Cells were incubated at 37 °C in a 5% CO_2 incubator.

2.4. Cell Adhesion Assay. Near confluence cells were harvested by a short trypsin−EDTA treatment (Invitrogen, cat. no. 25200), washed, and resuspended in serum-free culture media (M199 for HUVECs and DMEM for fibroblasts), incubated at 37 °C for 30 min, and seeded at 7500 cells/cm^2 on sterilized bioactive molecule arrays. Six hours after cell seeding, arrays were washed with PBS to remove nonadherent cells and were fixed with 3.7% formaldehyde during 15 min for further labeling. Images of each spot were taken using phase contrast microscopy (Nikon Eclipse TE2000-S) at 100× magnification. Cell number per spot was counted manually. The cell projected area was outlined manually using SigmaScan Pro software (SPSS Inc., Chicago, IL). Each biomolecule solution was deposited in triplicate on an array, and the assay was carried out in duplicate for each cell type.

2.5. Cell Labeling. Fixed cells were rinsed with PBS, permeabilized with 0.5% Triton X-100 for 5 min, and blocked with 2% bovine serum albumin (BSA, Sigma, cat. no. A7906). Samples were incubated for 1 h at room temperature with the primary mouse antivinculin antibody (1:25, Sigma, cat. no. V4505), rinsed with PBS, and incubated with Alexa Fluor 488 goat antimouse secondary antibody (1:1000, Invitrogen, cat. no. A11001) mixed with phalloidin−TRITC (1:300, Sigma, cat. no. P1951) for 1 h at room temperature. The samples were rinsed and mounted on glass slides and observed with an epifluorescence microscope (Nikon Eclipse) equipped with oil immersion objectives (60× and 100×).

2.6. Statistical Analysis. The adhesion results were presented as mean values ± standard deviations. Statistical analysis was performed using Student's *t* test for paired samples to compare bioactive spots to the RGD spots (R25). A *p*-value <0.05 was considered statistically significant.

3. Results

Surfaces bearing arrays of bioactive molecules were made by covalent immobilization of bioactive molecules on a low-fouling CMD layer (Figure 1). They were exposed to HUVECs and human fibroblasts to examine how grafted bioactive molecules affect cell adhesion, spreading, cytoskeletal organization, and focal adhesion assembly.

3.1. RGD Peptide Is Required to Initiate Cell Adhesion. Arrays were seeded with HUVECs or fibroblasts, and cell density was measured on each spot after 6 h. For both cell types, no cell adhesion was observed on the bare CMD layer or on RGE spots. Cells adhered and spread on spots bearing covalently immobilized RGD (Figure 2, parts A and B). However, no cell attached on spots where REDV, SVVYGLR (0.1−100 μg/mL), or VEGF (2−8 μg/mL) were grafted individually (data not shown). The co-immobilization of these molecules with RGD was necessary to induce HUVEC and fibroblast adhesion. Bioactive spots then supported cell adhesion and growth as illustrated in Figure 2C. Cells remained within the spot limits even after a few days in culture in the presence of serum.

3.2. REDV, SVVYGLR, and VEGF Specifically Affect Endothelial Cell Adhesion When Co-immobilized with RGD. Figure 3A reveals that co-immobilization of the RGD peptide with REDV (RE1) did not induce any effect on HUVEC attachment 6 h after seeding. However, the co-immobilization

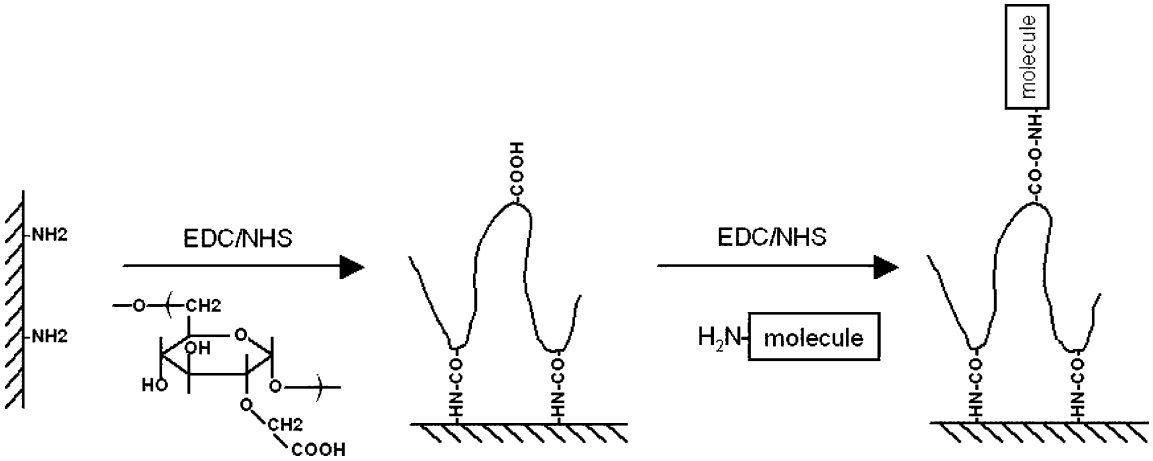

Figure 1. Reaction scheme for the grafting of carboxy-methyl-dextran (CMD) to the HApp-modified surface and subsequent bioactive molecule (peptide or growth factor) immobilization.

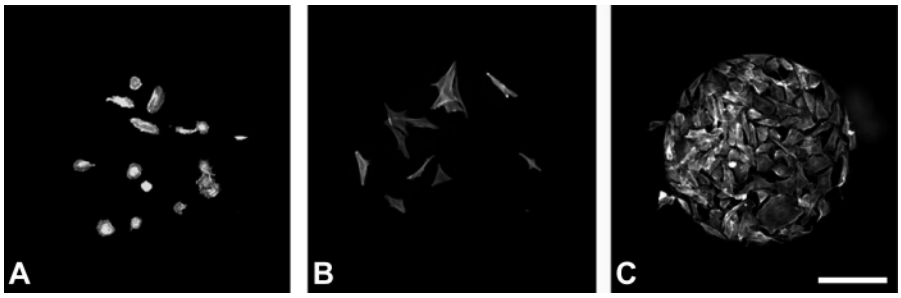

Figure 2. Microscopy images of phalloidin−actin labeling of (A) human umbilical vein endothelial cells (HUVECs) and (B) human foreskin fibroblasts adhering on RGD spots, 6 h after cell seeding, and of (C) confluent HUVECs cultured on a RGD spot for 5 days with 10% serum. Scale bar is 250 μm.

of RGD or RGD + REDV with VEGF (R25V and RE1V, respectively) leads to a significant increase in endothelial cell density. RGD co-immobilized with SVVYGLR (SV1) also significantly enhanced HUVEC adhesion. Addition of VEGF to this combination (SV1V) did not elicit an increase in endothelial cell attachment. On the other hand, the co-immobilization of RGD with either endothelial cell-selective peptides or VEGF induced no effect on fibroblast attachment (Figure 3B).

In vitro, initial cell attachment results in cell spreading. Projected cell area was measured for cells adhering on each bioactive spot, and results presented in Figure 4 suggest that HUVECs attached on spots exposing either REDV or SVVYGLR showed a reduction in spreading, while immobilized VEGF did not induce any effect 6 h after seeding. The molecular composition of bioactive spots did not affect fibroblasts spreading (data not shown).

3.3. REDV, SVVYGLR, and VEGF Affect Actin Filaments Organization and Focal Adhesion Assembly in Endothelial Cells. In vitro, cell adhesion is usually followed by formation of focal adhesions and reorganization of the actin cytoskeleton into stress fibers. Cells adhering to bioactive spots were fixed 6 h after cell seeding and labeled for their actin cytoskeleton and for vinculin, one of the major structural components of focal adhesions.[32] HUVECs adhering on spots made of RGD displayed a well-organized actin cytoskeleton with actin stress fibers associated with thick focal adhesions (Figure 5, parts E and F). However, cells on surfaces exposing REDV, SVVYGLR, or VEGF presented a different cytoskeletal organization. HUVECs on RGD + REDV (RE1) spots (Figure 5I) or RGD + SVVYGLR (SV1) spots (Figure 5M) displayed actin organized into thin filaments, but few actin stress fibers were observed; focal contacts were small (Figure 5, parts J and N). HUVECs adhering on spots bearing VEGF (R25V, RE1V, and SV1V) showed no stress fibers, and actin filaments were rather organized into cortical networks associated with membrane ruffling (Figure 5, parts G, K, and O). Focal contacts were small and mostly present at the cell edges (Figure 5, parts H, L, and P). In comparison, covalently immobilized VEGF did not seem to affect fibroblast actin cytoskeleton organization into stress fibers or focal adhesion assembly (Figure 5A−D).

4. Discussion

Arrays of RGD and bioactive molecules specific for endothelial cells were exposed to endothelial cells and fibroblasts to screen for their effect on cell adhesion, spreading, actin cytoskeleton organization, and focal adhesions assembly. Bioactive molecules were covalently immobilized on a low-fouling CMD layer, which prevents nonspecific protein adsorption and cell adhesion[12,14] and which possesses multiple functional carboxylic groups convenient for peptides and proteins grafting.[15]

HUVECs and human fibroblasts both adhered on bioactive spots bearing the RGD adhesion peptide, whereas no cell attached to the background CMD layer and to the RGE inactive peptide. This is in good agreement with a previous study in which RGE peptide did not support cell adhesion.[33] As expected, CMD layer properties prevented any unspecific cell adhesion. The introduction of a peptide containing charged groups may have altered surface properties, such as charge and hydrophilicity, and induced nonspecific cell adhesion. The absence of any cell adhesion on the inactive grafted RGE peptide proves that cell adhesion was directly mediated by specific interactions

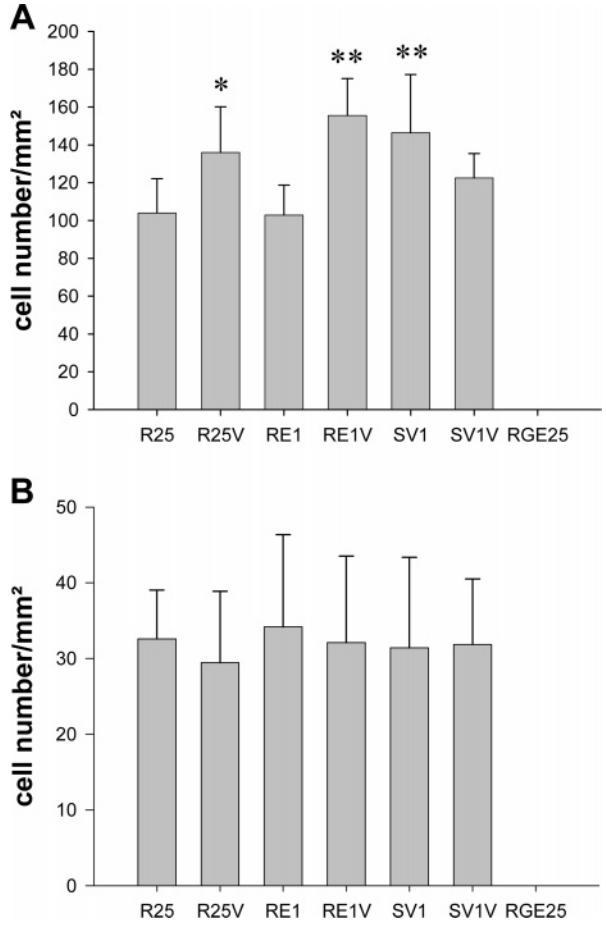

Figure 3. (A) Human umbilical vein endothelial cells (HUVECs) and (B) human foreskin fibroblasts adhesion on bioactive spots 6 h after cell seeding. Significant difference compared with RGD alone (R25) at $*p < 0.05$ or $**p < 0.01$.

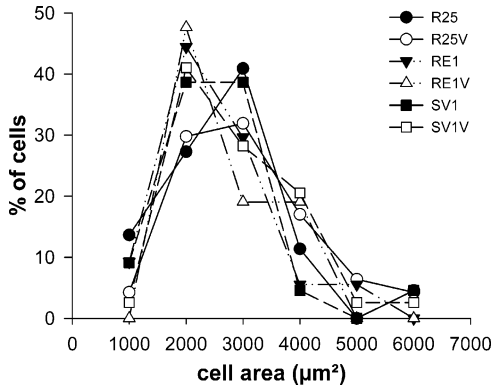

Figure 4. Spreading levels measured for human umbilical vein endothelial cells (HUVECs) on bioactive spots 6 h after cell seeding.

between the immobilized RGD sequence and its cell receptors. Moreover, the co-immobilization of RGD with either REDV, SVVYGLR, or VEGF molecules, believed to be rather specific for endothelial cells as their receptors are expressed by reduced number of cell types,[25,26] elicited changes in HUVEC adhesion, spreading, and cytoskeletal organization, whereas no change could be detected in fibroblasts behavior. It is then hypothesized that molecules immobilized on a low-fouling CMD layer are specifically recognized by their receptors and that the observed cell responses directly result from specific interactions between cell receptors and these immobilized bioactive molecules. Grafting molecules on a CMD layer may be a way to control elicited signals and to design bioactive surfaces that can specifically control cell responses and patterning in coculture systems, for instance.

When exposed to RGD, a peptide sequence recognized by integrins $\alpha v\beta 3$ and $\alpha 5\beta 1$ which are predominant in cell adhesion,[16,32] endothelial cells adhered, spread, formed focal adhesions and actin stress fibers indicative of a strong adhesion. However, REDV and SVVYGLR peptides were not able to promote HUVEC adhesion on their own (i.e., without the coimmobilization of RGD). The co-immobilization of RGD with either REDV or SVVYGLR induced a reduction in HUVEC spreading and affected actin filaments organization and focal adhesion assembly in adhering cells, with a reduced number of stress fibers and smaller focal adhesions than in cells adhering to RGD-modified spots. The known cell receptors for REDV and SVVYGLR are integrins $\alpha 4\beta 1$ and $\alpha 9\beta 1$, respectively.[17,21] Integrin $\alpha 4$ and $\alpha 9$ subunits are the sole members of a structural subfamily of integrin α subunits, and they share functional similarities as they both inhibit cell spreading and enhance cell migration.[34−36] $\alpha 4$ and $\alpha 9$ cytoplasmic domains directly bind to paxillin, a signaling molecule, inducing inhibition of cell spreading. Moreover, paxillin binding to the $\alpha 4$ cytoplasmic domain leads to focal adhesion disassembly and stress fibers disappearance.[34] As $\alpha 4$ and $\alpha 9$ share structural and functional similarities, ligand binding to $\alpha 9\beta 1$ integrin may also elicit focal adhesion disruption and stress fibers disappearance. Similarities between $\alpha 4$ and $\alpha 9$ functions and the observed morphology of cells adhering to spots bearing REDV and SVVYGLR (RE1 and SV1) suggest that there is a direct interaction between the immobilized peptide sequences and their receptors $\alpha 4\beta 1$ and $\alpha 9\beta 1$, leading to an intermediate state of adhesion.

Previous work on REDV and SVVYGLR sequences suggested that endothelial cells were able to attach on surfaces bearing these peptides.[18,19,22,23] The template layer used in this study is nonfouling, i.e., absolutely no cell adhered on the CMD surface. Previous studies on endothelial cell adhesion onto REDV- or SVVYGLR-modified surfaces usually revealed a minimal nonspecific cell adhesion on control surfaces without peptide.[18,22,23] This nonspecific adhesion may have been sufficient to initiate cell attachment on REDV- or SVVYGLR-modified surfaces. Furthermore, although cells adhering on RE1 and SV1 spots displayed similar changes in their spreading and cytoskeletal organization, only SVVYGLR co-immobilized with RGD could enhance HUVEC adhesion. If $\alpha 4$ and $\alpha 9$ seem to trigger similar signals involved in cell spreading and cytoskeletal organization, they may activate different signaling pathways related to cell adhesion. In addition, although some previous reports showed an enhanced endothelial cell adhesion on surfaces modified with the REDV sequence,[18,19] some were not able to observe any significant increase in endothelial cell adhesion.[37,38]

VEGF also enhanced cell adhesion when co-immobilized with RGD or RGD + REDV (RE1) but not with RGD + SVVYGLR (SV1). Surface-immobilized VEGF induces adhesion, migration, and survival of endothelial cells via integrins $\alpha v\beta 3$, $\alpha 3\beta 1$, $\alpha 9\beta 1$, and its receptor VEGF-R2.[29,30] When VEGF and SVVYGLR were co-immobilized, there might have been a competition between the two $\alpha 9\beta 1$ ligands, which may explain why combination of SV1 with VEGF did not enhance HUVEC adhesion. Nonetheless, as previously noticed,[29] endothelial cells adhering on spots where VEGF was exposed displayed few actin filaments arranged in a cortical network and peripheral focal

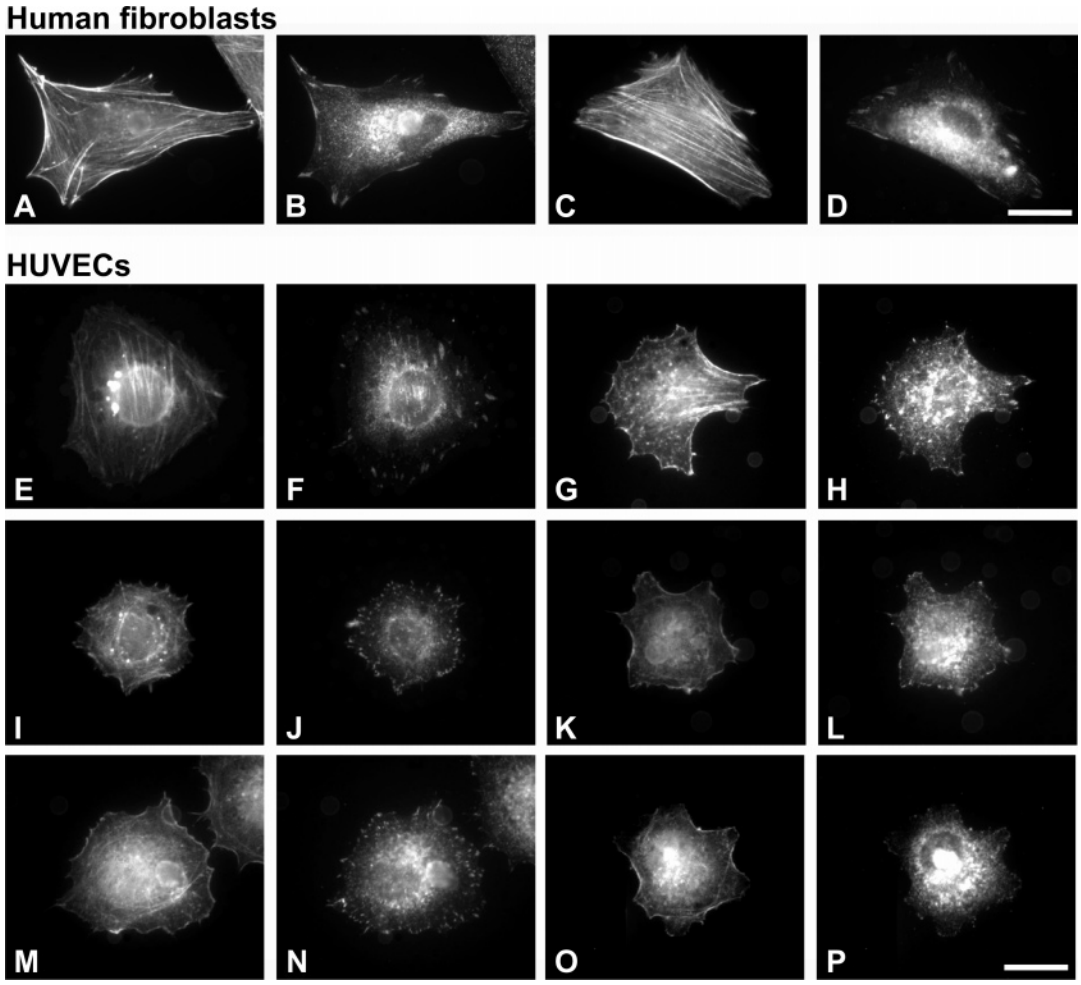

Figure 5. Actin filaments labeled with phalloidin (A, C, E, G, I, K, N, O) and focal adhesions labeled with antivinculin (B, D, F, H, J, L, N, P) in human foreskin fibroblasts and human umbilical vein endothelial cells (HUVECs) on R25 (A, B, E, F), R25V (C, D, G, H), RE1 (I and J), RE1V (K and L), SV1 (M and N), and SV1V (O and P) 6 h after cell seeding. Scale bars are 25 μm.

points. RGD co-immobilized either with VEGF, REDV, or SVVYGLR leads to a loss of stress fibers associated with membrane ruffling or lamellipodia extension and focal adhesion rearrangement into small and/or peripheral ones in adhering HUVECs. Immobilized VEGF has been shown to induce cell migration,[29,30] and integrins $\alpha 4\beta 1$ and $\alpha 9\beta 1$ also enhance endothelial cell motility.[20,34,36] Cell migration requires constant reorganization of the actin cytoskeleton and focal adhesions: extension of filopodia and lamellipodia is followed by attachment to the matrix via focal adhesions and formation of stress fibers to be able to contract the cell body to allow forward movement. Disruption of focal adhesions at the front edge is then necessary for the cell to migrate.[39] The observed morphology of HUVECs adhering on bioactive spots exposing REDV, SVVYGLR, and/or VEGF suggests that endothelial cell interaction with these bioactive molecules induces cell migration rather than a strong anchorage to the surface. Endothelial cell migration is required for in situ spontaneous endothelialization of vascular grafts or for construction or repair of a vascularized tissue, for instance.[2,3] Future work would then be to test the ability of these bioactive coatings to promote endothelial cells migration on a 2D-modified surface and to induce invasion of an engineered tissue substitute with formation of tubular structures. SVVYGLR and VEGF should be the chosen candidates as they both selectively enhance endothelial cell adhesion and their soluble forms have been shown to promote formation of capillary-like structures in vitro and blood vessels in vivo.[23,24,27]

5. Conclusions

This study reveals that low-fouling CMD layers constitute a good template to develop bioactive surfaces: their multivalence allows the immobilization of active biological ligands while their high resistance to unspecific adhesion allows and promotes specific cell receptor−ligand interactions.

Bioactive microarrays were made by covalent grafting of biologically active molecules specific for endothelial cells combined with RGD peptides. Arrays exposition to human endothelial cells and human fibroblasts revealed that fibroblasts were not affected by spot composition, whereas endothelial cells were.

While RGD initiated cell adhesion, the co-immobilization of RGD with the SVVYGLR sequence or with VEGF selectively enhanced endothelial cell adhesion, whereas the co-immobilization with either REDV or SVVYGLR induced a reduction in endothelial cell spreading. RGD combination with any of the bioactive molecules tested here resulted in a loss of stress fibers and rearrangement of focal adhesions, synonymous of a reduced strength of attachment, but probably associated with cell migration.

Further studies of bioactive coatings exposing SVVYGLR or VEGF will be done in regard to their ability to support invasion of a scaffold used for the (re)construction of a vascularized tissue. These bioactive microarrays are also an excellent tool to investigate cell patterning in coculture assays.

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References and Notes

(1) Ratner, B. D.; Hoffman, A. S.; Schoen, F. J.; Lemons, J. E. *Biomaterials Science, an Introduction to Materials in Medicine*, 2nd ed.; Elsevier: San Diego, California, 2004.
(2) Merzkirch, C.; Davies, N.; Zilla, P. *Anat. Rec.* **2001**, *263*, 379−387.
(3) Bouhadir, K. H.; Mooney, D. G. *J. Drug Targeting* **2001**, *9*, 397−406.
(4) Martin, Y.; Vermette, P. *Biomaterials* **2005**, *26*, 7481−7503.
(5) Rosso, F.; Giordano, A.; Barbarisi, M.; Barbarisi, A. *J. Cell. Physiol.* **2004**, *199*, 174−180.
(6) Flaim, C. J.; Chien, S.; Bhatia, S. N. *Nat. Methods* **2005**, *2*, 119−125.
(7) Watanabe, K.; Miyazaki, T.; Matsuda, R. *Zool. Sci.* **2003**, *20*, 429−434.
(8) Nakajima, M.; Ishimuro, T.; Kato, K.; Ko, I. K.; Hirata, I.; Arima, Y.; Iwata, H. *Biomaterials* **2007**, *28*, 1048−1060.
(9) Kato, K.; Sato, H.; Iwata, H. *Langmuir* **2005**, *21*, 7071−7075.
(10) McArthur, S. L.; McLean, K. M.; Kingshott, P.; St. John, H. A. W.; Chatelier, R. C.; Griesser, H. J. *Colloids Surf., B* **2000**, *17*, 37−48.
(11) Griesser, H. J.; Hartley, P. G.; McArthur, S. L.; McLean, K. M.; Meagher, L.; Thissen, H. *Smart Mater. Struct.* **2002**, *11*, 652−661.
(12) Monchaux, E.; Vermette, P. *Langmuir* **2007**, *23*, 3290−3297.
(13) McLean, K. M.; Johnson, G.; Chatelier, R. C.; Beumer, G. J.; Steele, J. G.; Griesser, H. J. *Colloids Surf., B* **2000**, *18*, 221−234.
(14) Monchaux, E.; Vermette, P. *J. Biomed. Mater. Res., Part A*, in press.
(15) Johnsson, B.; Lofas, S.; Lindquist, G. *Anal. Biochem.* **1991**, *198*, 268−277.
(16) Hersel, U.; Dahmen, C.; Kessler, H. *Biomaterials* **2003**, *24*, 4385−4415.
(17) Massia, S. P.; Hubbell, J. A. *J. Biol. Chem.* **1992**, *267*, 14019−14026.
(18) Hubbell, J. A.; Massia, S. P.; Desai, N. P.; Drumheller, P. D. *Biotechnology (N.Y.)* **1991**, *9*, 568−572.
(19) Plouffe, B. D.; Njoka, D. N.; Harris, J.; Liao, J.; Horick, N. K.; Radisic, M.; Murthy, S. K. *Langmuir* **2007**, *23*, 5050−5055.
(20) Garmy-Susini, B.; Jin, H.; Zhu, Y.; Sung, R. J.; Hwang, R.; Varner, J. *J. Clin. Invest.* **2005**, *115*, 1542−1551.
(21) Yokosaki, Y.; Matsuura, N.; Sasaki, T.; Murakami, I.; Schneider, H.; Higashiyama, S.; Saitoh, Y.; Yamakido, M.; Taooka, Y.; Sheppard, D. *J. Biol. Chem.* **1999**, *274*, 36328−36334.
(22) Nokihara, K.; Hamada, Y.; Takahashi, J.; Okazaki, M.; Matsuura, N. Development of Biomedical Materials Carrying Immobilized Functional Peptides and Discovery of an Angiogenic Peptide. In *Proceedings of the 38th Symposium on Japanese Peptide Symposium*; Nagasaki, Japan, October 3–5, 2001; Aoyagi, H., Ed.; Protein Research Foundation: Osaka, Japan, March 2002, pp 359–362.
(23) Hamada, Y.; Nokihara, K.; Okazaki, M.; Fujitani, W.; Matsumoto, T.; Matsuo, M.; Umakoshi, Y.; Takahashi, J.; Matsuura, N. *Biochem. Biophys. Res. Commun.* **2003**, *310*, 153−157.
(24) Hamada, Y.; Yuki, K.; Okazaki, M.; Fujitani, W.; Matsumoto, T.; Hashida, M. K.; Harutsugu, K.; Nokihara, K.; Daito, M.; Matsuura, N.; Takahashi, J. *Dent. Mater. J.* **2004**, *23*, 650−655.
(25) Liu, S.; Rose, D. M.; Han, J.; Ginsberg, M. H. *Trends Cardiovasc. Med.* **2000**, *10*, 253−257.
(26) Palmer, E. L.; Ruegg, C.; Ferrando, R.; Pytela, R.; Sheppard, D. *J. Cell Biol.* **1993**, *123*, 1289−1297.
(27) Yancopoulos, G. D.; Davis, S.; Gale, N. W.; Rudge, J. S.; Wiegand, S. J.; Holash, J. *Nature* **2000**, *407*, 242−248.
(28) Olsson, A. K.; Dimberg, A.; Kreuger, J.; Claesson-Welsh, L. *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 359−371.
(29) Hutchings, H.; Ortega, N.; Plouet, J. *FASEB J.* **2003**, *17*, 1520−1522.
(30) Vlahakis, N. E.; Young, B. A.; Atakilit, A.; Hawkridge, A. E.; Issaka, R. B.; Boudreau, N.; Sheppard, D. *J. Biol. Chem.* **2007**, *282*, 15187−15196.
(31) Martin, Y.; Boutin, D.; Vermette, P. *Thin Solid Films* **2007**, *515*, 6844−6852.
(32) Zamir, E.; Geiger, B. *J. Cell Sci.* **2001**, *114*, 3583−3590.
(33) Mann, B. K.; Tsai, A. T.; Scott-Burden, T.; West, J. L. *Biomaterials* **1999**, *20*, 2281−2286.
(34) Liu, S.; Thomas, S. M.; Woodside, D. G.; Rose, D. M.; Kiosses, W. B.; Pfaff, M.; Ginsberg, M. H. *Nature* **1999**, *402*, 676−681.
(35) Liu, S.; Slepak, M.; Ginsberg, M. H. *J. Biol. Chem.* **2001**, *276*, 37086−37092.
(36) Young, B. A.; Taooka, Y.; Liu, S.; Askins, K. J.; Yokosaki, Y.; Thomas, S. M.; Sheppard, D. *Mol. Biol. Cell* **2001**, *12*, 3214−3225.
(37) Holt, D. B.; Eberhart, R. C.; Prager, M. D. *ASAIO J.* **1994**, *40*, M858−M863.
(38) Nicol, A.; Gowda, D. C.; Parker, T. M.; Urry, D. W. Cell Adhesive Properties of Bioelastic Materials Containing Cell Attachment Sequences. In *Biotechnology and Bioactive Polymers*; Gebelein, C., Carraher, C., Eds.; Plenum Press: New York, 1994; pp 95−113.
(39) Lamalice, L.; Le, B. F.; Huot, J. *Circ. Res.* **2007**, *100*, 782−794.

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