Published in final edited form as:

Biomaterials. 2009 January ; 30(3): . doi:10.1016/j.biomaterials.2008.08.042.

The development of peptide-based interfacial biomaterials for generating biological functionality on the surface of bioinert materials

Steven R. Meyers^a, Xiaojuan Khoo^a, Xin Huang^b, Elisabeth B. Walsh^c, Mark W. Grinstaff^{a,*}, and Daniel J. Kenan^{d,*}

^aDepartments of Biomedical Engineering and Chemistry, Boston University, Boston, MA 02215, USA.

^bDepartment of Biomedical Engineering, Duke University, Durham, NC 27708, USA.

^cDepartment of Chemistry, Duke University, Durham, NC 27708, USA.

^dDepartment of Pathology, Duke University Medical Center, Durham, NC 27710, USA.

Abstract

Biomaterials used in implants have traditionally been selected based on their mechanical properties, chemical stability, and biocompatibility. However, the durability and clinical efficacy of implantable biomedical devices remains limited in part due to the absence of appropriate biological interactions at the implant interface and the lack of integration into adjacent tissues. Herein, we describe a robust peptide-based coating technology capable of modifying the surface of existing biomaterials and medical devices through the non-covalent binding of modular biofunctional peptides. These peptides contain at least one material binding sequence and at least one biologically active sequence and thus are termed, "Interfacial Biomaterials" (IFBMs). IFBMs can simultaneously bind the biomaterial surface while endowing it with desired biological functionalities at the interface between the material and biological realms. We demonstrate the capabilities of model IFBMs to convert native polystyrene, a bioinert surface, into a bioactive surface that can support a range of cell activities. We further distinguish between simple cell attachment with insufficient integrin interactions, which in some cases can adversely impact downstream biology, versus biologically appropriate adhesion, cell spreading, and cell survival mediated by IFBMs. Moreover, we show that we can use the coating technology to create spatially resolved patterns of fluorophores and cells on substrates and that these patterns retain their borders in culture.

Keywords

biomaterial; engineered peptides; peptide coating; bioactive surface; interfacial biology; surface modification; RGD; apoptosis; patterning

© 2008 Elsevier Ltd. All rights reserved.

*To whom correspondence should be addressed: Prof. Daniel Kenan, Department of Pathology, Duke University Medical Center, Box 3020, Durham, NC 27710-3020. dan.kenan@duke.edu Fax: 919-684-9825 and Prof. Mark Grinstaff, Departments of Biomedical Engineering and Chemistry, Boston University, 590 Commonwealth Ave., Boston, MA 02215. mgrin@bu.edu Fax: 617-358-3186. .

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Implanted medical devices are increasingly used to treat a wide range of medical conditions [1]. Historically, the choice of materials used in medical devices has been based primarily on the passive mechanical and chemical requirements of each component with less emphasis placed on promoting integration with the surrounding tissue.[2] For example, orthopaedic devices must be capable of withstanding intermittent high mechanical loads over a period of decades while at the same time resisting corrosion and preventing the leaching of toxic chemicals into the human body [3, 4]. The metals classically selected for this application certainly fulfill these requirements, but they possess no mechanism to promote interaction with native tissue, instead relying on their inertness. However, poor integration and a lack of proper interaction often lead to sub-optimal implant performance, resulting in device failure and adverse outcomes to the patient. Examples of unwanted biological responses include bacterial colonization, [5-9] the foreign body response and fibrous capsule formation on implants, [10-14] biofouling of sensors, membranes, and drains by protein adsorption, [15-18] and thrombosis and restenosis on intravascular devices [19-21]. Future biomaterials will solve these problems in part by incorporating surface modifications that promote active interaction with the surrounding biology, and in some cases supporting remodeling into normal tissues.

We have created multi-domain peptides for use as coatings to endow material surfaces with desired biological activities. We term such peptide-based functional coatings “Interfacial Biomaterials” or IFBMs. Specifically, an IFBM is a modular peptide containing at least one material binding domain and at least one bioactive functionality (Figure 1). Unique peptide sequences that bind specifically and with high affinity to various materials or biological targets were identified using a combinatorial phage display screening process [22-28]. These affinity peptides were subsequently conjugated to a functional moiety with distinct biological activity, affording a biomimetic bridging macromolecule capable of promoting specific interactions with cells and biomolecules at the surface of a material. We have previously reported the use of IFBMs as cytophobic, non-fouling coatings for plastics [29, 30] and as RGD fusion peptides to promote adhesion of endothelial cells to titanium [31] and poly(glycolic acid) substrates [32]. Another example of a bioactive peptide identified through phage display was reported by Sanghvi et al. where a polypyrrole-binding peptide was conjugated to the integrin-binding sequence GRGDS to create a modular peptide capable of mediating adhesion of neuroblastoma cells to conducting polypyrrole [33]. Despite the demonstrated efficacy of such chimeric peptides in mediating cell adhesion, their application to directing more complex biological processes has not yet been reported. Here, we extend the concept of an IFBM beyond simple cell adhesion to encompass a spectrum of surface-directed biology including adhesion, cell spreading, and cell signaling and survival. We also demonstrate additional applications of IFBMs including biomolecule and cell patterning.

2. Materials and Methods

2.1. Peptide Synthesis

Peptides were commercially synthesized by solid-phase peptide synthesis techniques. The resultant peptides were purified to at least 95% purity and included high performance liquid chromatography (HPLC) and mass spectrometry analysis. The biotinylated peptide for affinity constant calculation was synthesized using a C-terminal biotin attached through the epsilon amide of the C-terminal lysine residue.

2.2. Quartz Crystal Microbalance (QCM)

5 MHz, AT-cut QCM-D quartz crystals coated on one side with 100 nm polystyrene were purchased from Q-sense (Q-Sense AB; Gothenburg, Sweden). All measurements were performed in a temperature-stabilized chamber (Q-Sense E4 system) at a working temperature of 23 °C to avoid drifts in frequency and dissipation (f and D). Immediately prior to use, chambers were rinsed with 20 mL 2% sodium dodecyl sulfate (SDS) solution, followed by 30 mL of ultrapure water, then dried with clean nitrogen gas. For each measurement, phosphate buffered saline (PBS) was first loaded into the liquid cell until a stable baseline was obtained. 2.5 µg/mL **PS-biotin** in PBS was then introduced into the liquid cell to replace the PBS buffer. When adsorption reached equilibrium, fresh PBS was introduced into the liquid cell to wash off weakly bound **PS-biotin**. Changes in f and D corresponding to IFBM adsorption were monitored and recorded in real-time. QTools software (Q-Sense AB; Gothenburg, Sweden) was used to analyze the QCM-D data and extract quantitative parameters of **PS-biotin** adsorption on polystyrene.

2.3. Atomic Force Microscopy (AFM) Measurements

A fresh solution of 0.1 mg/mL **PS-biotin** was prepared in 10 mM Dulbecco's phosphate buffered saline (DPBS, pH 7.4). 1 mL of the **PS-biotin** solution or a control PBS solution was added to sterile 60 mm polystyrene dishes, and incubated for 2 hours at room temperature. Dishes were rinsed three times with DPBS and three times with MilliQ water (Millipore Corp.; Burlington, MA) to remove unbound peptide, and dried with nitrogen gas before imaging. Samples were imaged using a Nanoscope IIIa AFM (Digital Instruments; Santa Barbara, CA) in tapping mode. Surfaces were scanned in air using a silicon nitride NSC15 cantilever (MikroMasch Inc.; Wilsonville, OR) at a scan rate of 1-2 Hz. Topographic data was obtained for a 1 µm x 1 µm scan size. Images were flattened using a first or second order fit to correct for piezo bow and/or sample tilt during analysis.

2.4. Matrix Assisted Laser Desorption/Ionization Time-of-flight Mass Spectrometry (MALDI-TOF-MS)

2 µL aliquots of the peptides in table 1 dissolved in nanopure water were combined with 20 µL of an α -cyano-4-hydroxycinnamic acid (CHCA) matrix solution. The MALDI matrix was made by dissolving CHCA (Fluka; St. Louis, MO) in 500 µL of acetonitrile (ACN) and 500 µL of water containing 0.1 % trifluoroacetic acid (TFA). The samples were then spotted on a plate and analyzed using a AXIMA CFR MALDI-TOF-MS (Shimadzu Biotech; Kyoto, Japan).

2.5. Contact Angle Measurements

Using a handheld rotary tool, small squares of plastic were cut from sterile PS plates. The squares were kept clean and sprayed with air before use to remove any small particles. 50 µL of either a **PS-RGD** solution (0.1 mg/mL in DPBS) or plain DPBS were each placed onto the squares and allowed to interact for 1 hour, RT. The solutions were removed and the plates were washed with excess DPBS and dried. The contact angle of 7 µL distilled water droplets placed on the surfaces was determined on a CAM-Micro contact angle meter (Tantec A/S; Lunderskov, Denmark) by measuring the angle of the projected shadow. Angles were measured by two independent observers and the readings were averaged for each sample (n=6).

2.6. Cell Culture

Human umbilical vein endothelial cells (HUVEC) were purchased from Cambrex and used between passages 3 and 6 for all experiments. Unless otherwise noted, endothelial growth medium-2 (EGM-2) (Cambrex Corp.; East Rutherford, NJ) was used for cell maintenance

and testing. The medium system contained 2% fetal bovine serum and a variety of growth factors. Cells were maintained in a humidified incubator at 37 °C with 5% CO₂.

2.7. Cell Adhesion Measurements

For the imaging experiments, wells of a 6-well polystyrene plate were treated for 2 hours with a 0.1 mg/mL solution of **PS-RGD**, or DPBS (2 mL). The wells were washed 3x with 2 mL DPBS, and then 2 mL of a 1% bovine serum albumin (BSA) solution was added for an additional 2 hours, followed by 3x washing with 2 mL DPBS. 150,000 HUVEC were added in 3 mL medium and allowed to interact for 2 hours. Images were taken to show the attachment process. After 2 hours, the slides were washed 3x with 2 mL PBS, and 1 mL of a fixative, 4% glutaraldehyde solution, was added at RT for 20 minutes. The slides were again washed 3x with 2 mL PBS-T (0.5% PBS:Tween-20). A 1 mL solution of 0.5% Triton X-100 was then added to permeabilize the cells for 20 minutes at RT. The slides were again washed 3x with 2 mL PBS-T. A 1% BSA solution was used to block non-specific sites for 1 hour at 37 °C, 5% CO₂. The slides were washed 3x with 2 mL 1% BSA solution. 1 mL of a 1 µg/mL solution of phalloidin-tetramethylrhodamine isothiocyanate conjugate (Sigma-Aldrich Corp.; St. Louis, MO) was added and the slides were kept in the dark at RT for 2 hours. All unbound phalloidin was washed off using a 3x 2 mL 1% BSA wash and images were taken using epifluorescence on an inverted IX70 microscope (Olympus America Inc.; Center Valley, PA) with attached Spot Diagnostics 11.2 Color Mosaic CCD camera (Diagnostic Instruments Inc.; Sterling Heights, MI). The images in Figure 4A show a small portion of the entire image, but to calculate the histogram data (Figure 4B) the entire micrograph was used.

Full versions of the images were measured and labeled using ImageJ software (NIH; Bethesda, MD) by drawing a freeform shape around the cell periphery. A total of 118, 124, 153, and 71 cells were used in the determination of the surfaces areas for the 60 minute **PS-RGD** coated, 60 minute uncoated, 120 minute **PS-RGD** coated, and 120 minute uncoated images respectively (Figures 4A, 4B).

2.8. Cell Apoptosis Measurements

For the constant peptide with varied cocktail experiments, wells of a 96-well PS plate were treated for 2 hours with either 0.1 mg/mL **PS-RGD**, or water. After a 3x wash with DPBS, 10,000 HUVEC cells were seeded in medium and incubated for 24 hours. The media was removed and replenished with low-serum medium (EGM-2 with 0.5% FBS and no growth factors) and varied amounts of tumor necrosis factor- α (TNF α) (R&D Systems; Minneapolis, MN) and actinomycin D (AMD) (Sigma-Aldrich) were added and incubated for 24 hours. A bolus of fluorescein diacetate (FDA) and ethidium bromide (EtBr) was added to reach a final FDA and EtBr concentrations of 2 µg/mL and 10 µg/mL, respectively. The results were imaged on the Olympus IX70 microscope using the appropriate filters (fluorescein isothiocyanate (FITC): 488 nm/519 nm; EtBr: 510 nm/595 nm).

For the constant apoptotic cocktail experiment, the above experiment was repeated, however, the plate was treated with logarithmically spaced amounts of **PS-RGD**, washed, and then 10,000 HUVEC were added. After 24 hours all the wells were challenged with an identical 1 ng/mL TNF α and 50 ng/mL AMD low-serum medium mix and incubated for a further 24 hours.

2.9. IFBM and Cell patterning

PS-biotin and **PS-FITC** were made up to a final concentration of 0.1 mg/mL in a 40% glycerol/DPBS solution. 20 µL of each IFBM was loaded into a 384-well plate and loaded onto an OmniGrid Accent™ automated pin-microarrayer (Genomic Solutions Inc.; Ann

Arbor, MI). Spots 100 μm in diameter were printed in alternating columns on a native PS slide to yield a 3×5 array of IFBMs. Following a 2 hour incubation, the slide was rinsed 3x with ultrapure water and dried using argon gas. The slide was imaged using the Olympus IX70 microscope with the appropriate filters for FITC. PS-biotin patterns were subsequently probed for streptavidin binding. Streptavidin-conjugated Alexa Fluor® 594 (4 $\mu\text{g/mL}$; Molecular Probes; Eugene, OR) solution was incubated for 1 hour over the patterned slide, then rinsed 3x with ultrapure water and dried using argon gas before imaging with the appropriate filters (FITC: 488 nm/519 nm; AF594: 589 nm/615 nm).

For the selective patterning of cells, **PS-RGD** spots 2 mm in diameter were printed onto the surface of a 35 mm PS dish using a micropipette and left to interact for 2 hours at RT. The patterned dish was blocked with 1% BSA in DPBS for 1 hour before seeding with HUVECs (10^4 cells/cm²). After 2 hours incubation at 37 °C, dishes were washed once to remove unbound cells before replacing with fresh EGM-2 media. Adhered cells were then cultured for up to 7 days to study *in vitro* cell proliferation and migration on the patterned surface. Media was replaced every 2 days. Phase contrast images of cell growth on patterned dishes were taken at several time points ranging from 2 hours to 1 week.

3. Results and discussion

3.1. Characterization of Polystyrene Binding Peptides

We previously reported a collection of peptides isolated by panning phage display libraries over untreated native polystyrene [30]. Polystyrene was chosen as a suitable material with which to demonstrate proof-of-concept due to its availability, good optical qualities, and its widespread use in, for example, cell culture, diagnostics and as a surrogate for the poly(styrene-*block*-isobutylene-*block*-styrene) (SIBS) coating used on drug eluting stents. It is important to note that polystyrene plates available from laboratory suppliers come in two main types: regular native polystyrene (PS), and tissue culture polystyrene (TCPS), the latter of which has been treated with a high energy process to create a more polar surface conducive to cellular attachment. All panning experiments were performed on regular PS plates. As a result, the selected PS-binding peptide, NH₂-FFSFFFPASAWGS-COOH, is expected to possess a higher affinity for native polystyrene over the TCPS analog. The candidate peptide was synthesized with a C-terminal biotin and subjected to an enzyme-linked immunosorbent assay (ELISA) to determine the adsorption strength of the peptide-polystyrene interaction. The biotinylated peptide, FFSFFFPASAWGSSGSSGK-biotin (**PS-biotin**), has an affinity constant of $7.3\times 10^6 \text{ M}^{-1}$ on regular PS. Affinity constants for many natural integrin-ligand interactions are 1-2 orders-of-magnitude lower than this value, exemplifying the strength of the adsorption for these phage identified peptides. When the same ELISA experiment is conducted on TCPS, a 24-fold reduction in affinity is observed, thus demonstrating the ability of the panning process to yield binders with both affinity and selectivity for a specific target surface even among closely related analogs.

A variety of surface characterization techniques were employed to investigate the physical characteristics of the peptide, and to quantify the affinity, kinetics and overall performance as a surface coating. Quartz crystal microbalance with dissipation monitoring (QCM-D) was used to study the real-time adsorption behavior of PS-binding peptides on a PS substrate, Figure 2 shows the resulting changes in frequency (f) and dissipation (D) upon adsorption of **PS-biotin** from a 2.5 $\mu\text{g/mL}$ (1 μM) solution onto a PS-coated QCM sensor. The peptide solution was introduced into the QCM-D system until equilibrium was observed at approximately -14 Hz . A slight loss of material, most likely due to the removal of non-specific peptide-peptide interactions, was observed with a phosphate buffered saline (PBS) wash, but a majority of the signal remained with the system eventually stabilizing at $\Delta f = -9.7 \text{ Hz}$. The data showed that even at this low-solution concentration, coating equilibrium

was reached in as few as 15 minutes exemplifying how powerful a non-covalent surface modification strategy can be for modifying devices without prior surface pre-treatment. The corresponding adsorbed mass was calculated using the Sauerbrey equation [34] and found to reach a final equilibrium of $\sim 180 \text{ ng/cm}^2$. In comparison, mass loadings reported in the literature for fibronectin (FN), a common extracellular matrix constituent with a molecular weight of about 450 kDa, are between 500 and 900 ng/cm^2 [35-37]. These values correspond to a surface coverage of 3.28×10^{13} and between 6.69×10^{11} and 1.20×10^{12} molecules/ cm^2 for **PS-biotin** and FN coated surfaces, respectively. The approximately 30-fold increase in surface density observed with the IFBMs translates to amplified surface functionalization over what is currently attainable with traditional large ECM proteins.

As we have seen from the QCM-D experiments, the peptides adsorb on the surface in a relatively short amount of time. To assess whether the resultant surface packing was uniform, atomic force microscopy (AFM) was used to examine the profile of coated and uncoated polystyrene substrates. PS 60 mm dishes were treated with 0.1 mg/mL **PS-biotin**, washed 3x with PBS and ultrapure water, then dried under clean nitrogen gas before imaging. The control uncoated PS surface showed a slightly uneven overall topography with a fairly flat and uniform microstructure at a 1 μm scan size (Figure 3A). Surfaces coated with **PS-biotin** revealed considerable changes in the fine microstructure (Figure 3B), where the adsorbed peptides were found to form a densely packed layer on the PS substrate with a height of 1-2 nm. RMS roughness values for the uncoated and coated surfaces were 0.277 nm and 0.522 nm respectively verifying the surface modification. Peptide coverage was uniform and no uncoated regions were evident, demonstrating the ability of IFBMs to form a complete surface coating on the target material without the need for prior surface activation and functionalization.

The candidate polystyrene-binding peptide was then combined with a bioactive cell-binding sequence, arginine-glycine-aspartic acid (RGD), to yield a bifunctional interfacial biomaterial, FFSFFFPASAWGSSGSSGRGD (**PS-RGD**). The RGD motif is a well-studied cell integrin binder and serves as a prototypical biofunctional group [38-41]. A control IFBM was synthesized containing a terminal trimer of RGE, FFSFFFPASAWGSSGSSGRGE (**PS-RGE**), which although similar in physical characteristics to RGD, does not bind cell surface integrins.[42] Table 1 summarizes the peptides synthesized for use in this study, their expected molecular weights and the experimental values calculated using MALDI-TOF-MS, which were in good agreement.

3.2. IFBM-Mediated Cell Adhesion

We next employed contact angle analysis to demonstrate how the individual peptides affect the characteristics of the PS surface at a cellular size-scale. Untreated native polystyrene is a highly hydrophobic surface with a contact angle of $79.1 \pm 3.9^\circ$. Coating with 0.1 mg/mL **PS-RGD**, which contains a number of charged residues, followed by extensive washing with water results in a decrease in the contact angle to $36.3 \pm 5.5^\circ$ due to the increased hydrophilicity of the modified PS surface. Notably, surfaces coated with soluble RGD alone did not produce a change in contact angles, exemplifying the importance of the underlying PS-binding sequence for successful and sustained surface modification. Both the biological and material domains need to be present and functional for the treatment to impart the appropriate activity to the surface.

The RGD motif is known to bind about 8-12 of the two dozen known mammalian integrins (e.g., $\alpha_v\beta_3$, $\alpha_5\beta_1$) [38, 43] and has been shown to mediate cell attachment on a number of material surfaces [31, 33, 44-53]. A coating of **PS-RGD** should result in a surface that is highly receptive to cellularization, thereby transforming a bioinert material into a bioactive one. Native PS surfaces were coated with 0.1 mg/mL of **PS-RGD** or **PS-RGE**, followed by

a wash and treatment with a 1% BSA block to reduce background binding, before seeding with human umbilical vein endothelial cells (HUVECs) (5×10^3 cells/well) for 2 hours in serum-containing medium. Pronounced results have been obtained using serum-free medium as well, however, the inclusion of serum produces a more physiologically relevant condition and all subsequent experiments will use this more stringent condition. An MTS proliferation assay was used to evaluate the number of adhered cells on each surface. The results from the assay showed **PS-RGD** coated wells had the greatest retention of cells at 25% of the original cell seeding density, at least a 9-fold increase ($n=6$; $p<0.0001$) compared to an untreated control PS (2% retention) and **PS-RGE** control (3% retention). No significant difference was observed between the untreated and **PS-RGE** surfaces ($n=6$; $p=0.49$). These results demonstrate that IFBMs can provide biologically appropriate attachment sites on an otherwise inert material surface. In other words, the **PS-RGD** IFBM acts as a surrogate extracellular matrix by displaying the critical RGD ligand typically found within much larger matrix proteins such as fibronectin, vitronectin, and laminin [39, 54, 55].

3.3. IFBM-Mediated Cell Spreading

Next, to investigate the biological response of cells on IFBM-modified surfaces, we evaluated whether IFBMs could emulate the ability of matrix proteins to promote cell spreading through integrin receptor-ligand interactions [56, 57]. Again, native PS surfaces were coated with **PS-RGD**, blocked with BSA, then seeded with HUVECs. The interactions of cells with surfaces were monitored over 2 hours. Cells attach weakly on untreated PS, with very few cells remaining following a wash (Figure 4 top, bottom panels). On the other hand, **PS-RGD** coated surfaces support the rapid and efficient capture of HUVEC (Figure 4 top, top panels), with a significant proportion of the cells adhered by one hour. In addition, HUVECs on **PS-RGD** coated surfaces exhibited a time-dependent spreading, as evidenced by the continually increasing cell surface areas over two hours of incubation (Figure 4 bottom). Staining of the cytoskeleton showed extensive membrane spreading of bound cells. Untreated PS surfaces and surfaces coated with the control IFBM, **PS-RGE**, showed negligible cell spreading over a similar time scale, confirming the importance of the RGD motif in cell attachment and spreading. Cells placed onto a TCPS surface, a material conducive to cell adherence, had cell surface areas only 73.4 % ($p < 0.005$) as large as those on the **PS-RGD** coated PS, again verifying the efficacy of the IFBM technology. Coating the TCPS material with **PS-RGD** improves the adhesion to this surface as well, decreasing the surface area difference between the coated PS and the coated TCPS to only 8 % ($p = 0.28$), an effect we believe is due to the 24-fold weaker binding of the peptide to TCPS as opposed to PS. These results all suggest that the IFBMs mediate their matrix-like effects through the binding of integrin receptors on the cell surface.

3.4. IFBM-Mediated Cell Survival

Most cells that become dissociated from the extracellular matrix will undergo caspase-8 activation and cell death in an apoptotic process called anoikis (Greek for “homelessness”) as a consequence of the loss of integrin receptor binding [58-61]. However, a cell is also capable of apoptosing when adhered to a surface if there is insufficient surface bound ligands to ligate a critical number of expressed integrins of a particular type [62-64]. For example, collagen lacks the capacity to interact with the $\alpha_V\beta_3$ and $\alpha_5\beta_1$ integrins preeminent in RGD-cell interactions, but still adheres cells through interactions with integrins from the β_1 family (e.g., $\alpha_2\beta_1$) [65]. Cells cultured in a purely collagenous environment would be capable of adhering to the substrate but might initiate apoptosis due to the presence of the unligated RGD binding integrins. In this regard, Stupack et al found that T24E cells cultured in a collagen 3D matrix will still apoptose through caspase activation due to the absence of $\alpha_V\beta_3$ ligation, but that cells of the same phenotype that had reduced amounts of this integrin exhibited increased survival on the same surface [62]. Similarly, Kim et al found that

HUVEC on vitronectin would remain adhered to the substrate while apoptosing when a soluble $\alpha_5\beta_1$ antagonist was added to the culture [64]. A clear differentiation therefore exists between surface adhesion and biologically appropriate adherence. In particular, apoptosis has been correlated with the lack of strong mechanical ECM ligation or soluble antagonist ligation of the $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins, [66-68] both known RGD interactors [43]. It follows that the induction of integrin binding by an RGD-terminated IFBM should similarly promote resistance to cell death induced by caspase-8 activators such as tumor necrosis factors (TNF) if we are able to ligate critical integrins in sufficient number.

We examined the dose response relationship between exposure to apoptotic agents and cell death on an IFBM-treated surface. HUVECs were seeded onto PS surfaces pre-coated with 0.1 mg/mL **PS-RGD** and left to interact for 24 hours. Cells were subsequently exposed to a drug cocktail consisting of TNF- α and actinomycin D (AMD) over a logarithmic range of concentrations for an additional 24 hours (1:50 ratio of TNF- α :AMD). These two compounds have been shown to synergistically promote apoptosis in mammalian cells [69, 70]. Wells were imaged using a viability stain, or assayed with a commercially available caspase detection kit. Representative images of the plated HUVEC cells (Figure 5) showed an abrupt change in cell mortality at a TNF- α concentration between 100 and 1 ng/mL on the **PS-RGD** coated surfaces. These values correspond well to the threshold concentration identified using the caspase assay (data not shown). As can be seen, cell attachment is observed, even at the maximum cocktail concentration of 100 ng/mL. Even though these cells are apoptosed, the coating provides sufficient integrin binding to retain them on the surface. Importantly, in the absence of any surface IFBMs, apoptosis occurs at TNF- α concentrations as low as 0.1 pg/mL, demonstrating the ability of the IFBM coating to increase cellular apoptotic resistance. This result is expected because as noted earlier there is little to no adherence on untreated PS.

Next, to examine the effect of integrin binding on cell survival, increasing concentrations of **PS-RGD** logarithmically spaced between 1 ng/mL and 1 mg/mL were applied to PS surfaces. HUVECs were seeded and allowed to proliferate on these surfaces before being challenged with a constant apoptotic cocktail concentration found to cause apoptosis in our previous experiment (TNF- α : 1 ng/mL, AMD: 50 ng/mL). Figure 6A shows images of plated HUVECs with the living cells stained green and dead cells stained red. Plated cell cultures were not washed prior to imaging. Cell viability increases between 0.1 and 10 μ g/mL until a concentration of ~46 μ g/mL is reached. At this concentration, the majority of cells remain viable, even in the presence of 1 ng/mL of TNF- α . Measurement of the live/dead ratio as a function of IFBM concentration confirms this threshold effect (Figure 6B) and suggests an optimal coating concentration of greater than 10 μ g/mL is necessary to achieve the maximum viability.

These data show that IFBMs replicate the two main biological roles of the integrin-ligand interaction: 1) the coating promotes cell adhesion and spreading by providing mechanical anchors and 2) the IFBM provides external signals that directly influence cell function. A modest IFBM coating concentration is sufficient to promote cell attachment to a cytophobic surface. However, too low a concentration provides insufficient integrin binding to overcome TNF activation of cell death pathways. At higher IFBM surface densities, cells become highly resistant to TNF-mediated cell death, a result of binding a critical number of integrin receptors. As exemplified by the experiments above, the IFBM coating provides a versatile *in vitro* platform on which to examine relationships between cell survival, local drug concentrations, and integrin mechanical attachment. More importantly, these findings highlight the difference between simple cell attachment versus the downstream consequences of attachment, such as apoptosis resistance, that depends to a great extent on the manner and magnitude of integrin receptor-ligand interactions.

3.5. Patterning of IFBM coatings

In addition to mediating cell adhesion and survivability on biomaterial surfaces, the controlled assembly of cells and biomolecules on a device is also crucial for performance. Moreover, the spatial organization of cells is essential for developing the precisely defined cellular architectures necessary for many tissue engineering applications. The IFBM coating platform potentially affords a simple yet robust method to create functional patterns, or arrays, on a target surface under mild aqueous conditions at room temperature. This coating method has three advantages: 1) it is amenable to a variety of sensitive biologics because extreme temperatures, organic solvents, surface pre-treatments, or extended processing times are not required; 2) it is adaptable to a number of coating technologies including dip-coating, spraying, and “ink-jet” printing; and 3) the coating can be patterned on a surface to afford more complex structures facilitating its use in future biomaterial devices. To evaluate the applicability of the IFBM platform to biomolecule patterning, the PS-binding peptide was functionalized with fluorescein isothiocyanate (**PS-FITC**) to yield a fluorescent IFBM. **PS-FITC** and **PS-biotin** spots 100 μm in diameter were printed in alternating columns on untreated PS slides using a pin-microarrayer. The slide was rinsed 3x with ultrapure water and imaged for fluorescence using the appropriate filters for FITC. The **PS-FITC** pattern is observed as defined spots with uniform fluorescence intensity (Figure 7). **PS-biotin** patterned regions register only weak fluorescence in the images.

To confirm the continued functionality of the adsorbed IFBMs, the patterned surfaces were subsequently probed for streptavidin binding. Surfaces were incubated with a solution of streptavidin-conjugated Alexa Fluor® 594 (SAv-AF594) for 1 hour and then imaged with the appropriate filters. Spatially resolved patterns of fluorescently-labeled streptavidin on PS-biotin areas were observed and non-specific SAv-AF594 binding to **PS-FITC** or untreated PS was minimal, confirming the specific molecular recognition of streptavidin for the patterned biotin (Figure 7). IFBMs possess sufficient peptide-substrate affinity to retain their functionality upon adsorption, even after multiple immersions and washing procedures. The successful patterning with minimal background shows the applicability of the approach to biomolecule organization and potentially high-throughput assay screening.

Next, microarray printing was employed to selectively pattern endothelial cells on polystyrene surfaces. **PS-RGD** spots 2 mm in diameter were printed onto the surface of a 35 mm PS dish and left to interact for 2 hours at RT. Subsequently, the patterned dish was blocked with 1% BSA for 1 hour before seeding with HUVECs. After 2 hours of incubation, cells were found to adhere to the coated regions of the plate with little to no binding observed on the untreated PS regions. The adhered cells were then cultured for 7 days to study *in vitro* cell proliferation and migration on the patterned surface. Phase contrast micrographs of the plates were taken after 24 hours and 1 week of culture (Figure 8). At all stages, normal cell growth and proliferation was observed within the patterned region, with little to no cell migration outside the original confines of the IFBM treatment. Such a coating on a PS surface would enable creation of more geometrically diverse patterns that entrap and retain adhered cells, while adjacent regions would remain cell free. Two or more IFBMs with different bioactive functionalities could also then be used to create more complex co-culture coatings and patterns to impart additional biological functions.

4. Conclusions

These peptide-based interfacial biomaterial (IFBM) coatings have been engineered and characterized for their ability to modify untreated polystyrene and direct the subsequent biological responses on coated surfaces. IFBMs spontaneously adsorb from aqueous solution to form a surface coating on PS without the need for surface activation or harsh chemical treatments. Experiments with endothelial cells show that PS-RGD acts as an ECM mimic,

and is capable of imparting biological activity to a previously bioinert surface. Cells on coated surfaces interact with the incorporated signaling cues, as evidenced by a substantial improvement in cell attachment and spreading. In addition, higher IFBM surface concentrations result in increased viability under a constant apoptotic challenge, demonstrating that cell-IFBM interactions are not limited to simple adhesion, but are in fact capable of directing important downstream biological events such as cell survival through appropriate integrin ligations. Furthermore, ligand densities achievable with these short chimeric peptides are an order-of-magnitude greater than those obtainable with common large extracellular matrix (ECM) proteins such as fibronectin. Finally, the coatings can be spatially and geometrically confined to create patterns that resist washing and submersion in buffer while retaining their biofunctionality, which allows for a range of biological patterning applications. Continued research in this area has the potential to create the next generation of implants that perform multiple roles *in vivo* with improved efficacy.

Acknowledgments

MWG and DJK thank the National Institutes of Health for funding through grants CA-77042 (DJK), CA-81088 (DJK), and EB-000501 (MWG). Xin Huang was supported by a North Carolina Biotechnology Center Collaborative Funding Grant.

References

- [1]. Ratner, BD.; Hoffman, AS.; Schoen, FJ.; Lemons, JE. Biomaterials science: An introduction to materials in medicine. Academic Press; San Diego: 2000.
- [2]. Williams DF. On the mechanisms of biocompatibility. Biomaterials. 2008; 29(20):2941–2953. [PubMed: 18440630]
- [3]. Hofmann GO. Biodegradable implants in orthopedic surgery—a review on the state-of-the-art. Clin Mater. 1992; 10(1-2):75–80. [PubMed: 10171206]
- [4]. Jacobs JJ, Skipor AK, Patterson LM, Hallab NJ, Paprosky WG, Black J, et al. Metal release in patients who have had a primary total hip arthroplasty. A prospective, controlled, longitudinal study. J Bone Joint Surg Am. 1998; 80(10):1447–1458. [PubMed: 9801213]
- [5]. Dunne WM Jr. Bacterial adhesion: Seen any good biofilms lately? Clin Microbiol Rev. 2002; 15(2):155–166. [PubMed: 11932228]
- [6]. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: A common cause of persistent infections. Science. 1999; 284(5418):1318–1322. [PubMed: 10334980]
- [7]. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: From the natural environment to infectious diseases. Nat Rev Microbiol. 2004; 2(2):95–108. [PubMed: 15040259]
- [8]. Marrie TJ, Nelligan J, Costerton JW. A scanning and transmission electron microscopic study of an infected endocardial pacemaker lead. Circulation. 1982; 66(6):1339–1341. [PubMed: 7139907]
- [9]. Davey ME, O’toole GA. Microbial biofilms: From ecology to molecular genetics. Microbiol Mol Biol Rev. 2000; 64(4):847–867. [PubMed: 11104821]
- [10]. Ratner BD. Reducing capsular thickness and enhancing angiogenesis around implant drug release systems. J Control Release. 2002; 78(1-3):211–218. [PubMed: 11772462]
- [11]. Kirkpatrick CJ, Krump-Konvalinkova V, Unger RE, Bittinger F, Otto M, Peters K. Tissue response and biomaterial integration: The efficacy of *in vitro* methods. Biomol Eng. 2002; 19(2-6):211–217. [PubMed: 12202185]
- [12]. Horbett TA. The role of adsorbed adhesion proteins in cellular recognition of biomaterials. BMES Bull. 1999; 23(2):5–9.
- [13]. Anderson JM. Biological responses to materials. Annu Rev Mater Res. 2001; 31:81–110.
- [14]. Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. Semin Immunol. 2008; 20(2):86–100. [PubMed: 18162407]

- [15]. Wisniewski N, Klitzman B, Miller B, Reichert WM. Decreased analyte transport through implanted membranes: Differentiation of biofouling from tissue effects. *J Biomed Mater Res*. 2001; 57(4):513–521. [PubMed: 11553881]
- [16]. Voskerician G, Shive MS, Shawgo RS, von Recum H, Anderson JM, Cima MJ, et al. Biocompatibility and biofouling of mems drug delivery devices. *Biomaterials*. 2003; 24(11):1959–1967. [PubMed: 12615486]
- [17]. Gifford R, Kehoe JJ, Barnes SL, Kornilayev BA, Alterman MA, Wilson GS. Protein interactions with subcutaneously implanted biosensors. *Biomaterials*. 2006; 27(12):2587–2598. [PubMed: 16364432]
- [18]. Staples M, Daniel K, Cima MJ, Langer R. Application of micro- and nano-electromechanical devices to drug delivery. *Pharm Res*. 2006; 23(5):847–863. [PubMed: 16715375]
- [19]. Slottow TL, Waksman R. Drug-eluting stent safety. *Am J Cardiol*. 2007; 100(8B):S10–S17.
- [20]. Van Belle E, Susen S, Jude B, Bertrand ME. Drug-eluting stents: Trading restenosis for thrombosis? *J Thromb Haemost*. 2007; 5(s1):238–245. [PubMed: 17635732]
- [21]. Sheiban I, Carrieri L, Catuzzo B, Destefanis P, Oliaro E, Moretti C, et al. Drug-eluting stent: The emerging technique for the prevention of restenosis. *Minerva Cardioangiol*. 2002; 50(5):443–453. [PubMed: 12384626]
- [22]. Hoess RH. Protein design and phage display. *Chem Rev*. 2001; 101(10):3205–3218. [PubMed: 11710069]
- [23]. Smith GP, Petrenko VA. Phage display. *Chem Rev*. 1997; 97(2):391–410. [PubMed: 11848876]
- [24]. Kay, BK.; Winter, J.; McCafferty, J. Phage display of peptides and proteins. Academic Press; San Diego: 1996.
- [25]. Burritt JB, Bond CW, Doss KW, Jesaitis AJ. Filamentous phage display of oligopeptide libraries. *Anal Biochem*. 1996; 238(1):1–13. [PubMed: 8660577]
- [26]. Dwyer MA, Lu W, Dwyer JL, Kossiakoff AA. Biosynthetic phage display: A novel protein engineering tool combining chemical and genetic diversity. *Chem Biol*. 2000; 7(4):263–274. [PubMed: 10780926]
- [27]. Sidhu, S. Phage display in biotechnology and drug discovery. CRC Press; Boca Raton: 2005.
- [28]. Adey NB, Mataragnon AH, Rider JE, Carter JM, Kay BK. Characterization of phage that bind plastic from phage-displayed random peptide libraries. *Gene*. 1995; 156(1):27–31. [PubMed: 7737512]
- [29]. Middleton, C.; Walsh, EB.; Kenan, DJ.; Grinstaff, MW. Interfacial biomaterial coatings for polystyrene. *Trans 28th Biomat Ann Meeting*; 2002. p. 213
- [30]. Kenan DJ, Walsh EB, Meyers SR, O'Toole GA, Carruthers EG, Lee WK, et al. Peptide-peg amphiphiles as cytophobic coatings for mammalian and bacterial cells. *Chem Biol*. 2006; 13(7):695–700. [PubMed: 16873017]
- [31]. Meyers SR, Hamilton PT, Walsh EB, Kenan DJ, Grinstaff MW. Endothelialization of titanium surfaces. *Adv Mater*. 2007; 19(18):2492–2498.
- [32]. Huang, X.; Zauscher, S.; Klitzman, B.; Truskey, GA.; Reichert, WM.; Grinstaff, MW., et al. Peptide interfacial biomaterials improve endothelial cell adhesion and biological function on synthetic polyglycolic acid materials. submitted
- [33]. Sanghvi AB, Miller KPH, Belcher AM, Schmidt CE. Biomaterials functionalization using a novel peptide that selectively binds to a conducting polymer. *Nat Mater*. 2005; 4(6):496–502. [PubMed: 15895095]
- [34]. Sauerbrey G. Verwendung von schwingquarzen zur wägung dünner schichten und zur mikrowägung. *Z Phys*. 1959; 155(2):206–222.
- [35]. Underwood PA, Steele JG, Dalton BA. Effects of polystyrene surface chemistry on the biological activity of solid phase fibronectin and vitronectin, analysed with monoclonal antibodies. *J Cell Sci*. 1993; 104(3):793–803. [PubMed: 7686170]
- [36]. Lhoest JB, Detrait E, van den Bosch de Aguilar P, Bertrand P. Fibronectin adsorption, conformation, and orientation on polystyrene substrates studied by radiolabeling, xps, and tof sims. *J Biomed Mater Res*. 1998; 41(1):95–103. [PubMed: 9641629]

- [37]. Garcia AJ, Vega MD, Boettiger D. Modulation of cell proliferation and differentiation through substrate-dependent changes in fibronectin conformation. *Mol Biol Cell*. 1999; 10(3):785–798. [PubMed: 10069818]
- [38]. Ruoslahti E. Rgd and other recognition sequences for integrins. *Annu Rev Cell Dev Biol*. 1996; 12:697–715. [PubMed: 8970741]
- [39]. Pierschbacher MD, Ruoslahti E. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature*. 1984; 309(5963):30–33. [PubMed: 6325925]
- [40]. Gardner JM, Hynes RO. Interaction of fibronectin with its receptor on platelets. *Cell*. 1985; 42(2):439–448. [PubMed: 2411420]
- [41]. Yamada KM, Kennedy DW. Dualistic nature of adhesive protein function: Fibronectin and its biologically active peptide fragments can autoinhibit fibronectin function. *J Cell Biol*. 1984; 99(1):29–36. [PubMed: 6736130]
- [42]. Harbers, GM.; Grainger, DW. Cell-material interactions: Fundamental design issues for tissue engineering and clinical considerations. In: Guelcher, SA.; Hollinger, JO., editors. *An introduction to biomaterials*. CRC Press; Boca Raton: 2006. p. 15-45.
- [43]. Hynes RO. Integrins: Bidirectional, allosteric signaling machines. *Cell*. 2002; 110(6):673–687. [PubMed: 12297042]
- [44]. Gauvreau V, Laroche G. Micropattern printing of adhesion, spreading, and migration peptides on poly(tetrafluoroethylene) films to promote endothelialization. *Bioconjugate Chem*. 2005; 16(5):1088–1097.
- [45]. Santiago LY, Nowak RW, Peter Rubin J, Marra KG. Peptide-surface modification of poly(caprolactone) with laminin-derived sequences for adipose-derived stem cell applications. *Biomaterials*. 2006; 27(15):2962–2969. [PubMed: 16445976]
- [46]. Elmengaard B, Bechtold JE, Soballe K. In vivo study of the effect of rgd treatment on bone ongrowth on press-fit titanium alloy implants. *Biomaterials*. 2005; 26(17):3521–3526. [PubMed: 15621242]
- [47]. Larsen CC, Kligman F, Kottke-Marchant K, Marchant RE. The effect of rgd fluorosurfactant polymer modification of eptfe on endothelial cell adhesion, growth, and function. *Biomaterials*. 2006; 27(28):4846–4855. [PubMed: 16762410]
- [48]. Tugulu S, Silacci P, Stergiopoulos N, Klok HA. Rgd-functionalized polymer brushes as substrates for the integrin specific adhesion of human umbilical vein endothelial cells. *Biomaterials*. 2007; 28(16):2536–2546. [PubMed: 17321591]
- [49]. Schuler M, Owen GR, Hamilton DW, de Wild M, Textor M, Brunette DM, et al. Biomimetic modification of titanium dental implant model surfaces using the rgdsp-peptide sequence: A cell morphology study. *Biomaterials*. 2006; 27(21):4003–4015. [PubMed: 16574219]
- [50]. Harbers GM, Healy KE. The effect of ligand type and density on osteoblast adhesion, proliferation, and matrix mineralization. *J Biomed Mater Res A*. 2005; 75(4):855–869. [PubMed: 16121356]
- [51]. Benoit DS, Anseth KS. The effect on osteoblast function of colocalized rgd and phsrn epitopes on peg surfaces. *Biomaterials*. 2005; 26(25):5209–5220. [PubMed: 15792548]
- [52]. Fittkau MH, Zilla P, Bezuidenhout D, Lutolf MP, Human P, Hubbell JA, et al. The selective modulation of endothelial cell mobility on rgd peptide containing surfaces by yigsr peptides. *Biomaterials*. 2005; 26(2):167–174. [PubMed: 15207463]
- [53]. Groll J, Fiedler J, Engelhard E, Ameringer T, Tugulu S, Klok HA, et al. A novel star peg-derived surface coating for specific cell adhesion. *J Biomed Mater Res A*. 2005; 74(4):607–617. [PubMed: 16035061]
- [54]. Yamada KM, Kennedy DW. Peptide inhibitors of fibronectin, laminin, and other adhesion molecules: Unique and shared features. *J Cell Physiol*. 1987; 130(1):21–28. [PubMed: 3805128]
- [55]. Suzuki S, Oldberg A, Hayman EG, Pierschbacher MD, Ruoslahti E. Complete amino acid sequence of human vitronectin deduced from cDNA. Similarity of cell attachment sites in vitronectin and fibronectin. *EMBO J*. 1985; 4(10):2519–2524. [PubMed: 2414098]
- [56]. Arnaout MA, Goodman SL, Xiong JP. Coming to grips with integrin binding to ligands. *Curr Opin Cell Biol*. 2002; 14(5):641–651. [PubMed: 12231361]
- [57]. Yamada KM. Integrin signaling. *Matrix Biol*. 1997; 16(4):137–141. [PubMed: 9402002]

- [58]. Giancotti FG, Ruoslahti E. Integrin signaling. *Science*. 1999; 285(5430):1028–1032. [PubMed: 10446041]
- [59]. Frisch SM, Francis H. Disruption of epithelial cell-matrix interactions induces apoptosis. *J Cell Biol*. 1994; 124(4):619–626. [PubMed: 8106557]
- [60]. Meredith JE Jr, Fazeli B, Schwartz MA. The extracellular matrix as a cell survival factor. *Mol Biol Cell*. 1993; 4(9):953–961. [PubMed: 8257797]
- [61]. Ruoslahti E, Reed JC. Anchorage dependence, integrins, and apoptosis. *Cell*. 1994; 77(4):477–478. [PubMed: 8187171]
- [62]. Stupack DG, Puente XS, Boutsaboualoy S, Storgard CM, Cheresch DA. Apoptosis of adherent cells by recruitment of caspase-8 to unligated integrins. *J Cell Biol*. 2001; 155(3):459–470. [PubMed: 11684710]
- [63]. Zhao H, Ross FP, Teitelbaum SL. Unoccupied alpha(v)beta3 integrin regulates osteoclast apoptosis by transmitting a positive death signal. *Mol Endocrinol*. 2005; 19(3):771–780. [PubMed: 15591537]
- [64]. Kim S, Bakre M, Yin H, Varner JA. Inhibition of endothelial cell survival and angiogenesis by protein kinase a. *J Clin Invest*. 2002; 110(7):933–941. [PubMed: 12370271]
- [65]. Orr AW, Ginsberg MH, Shattil SJ, Deckmyn H, Schwartz MA. Matrix-specific suppression of integrin activation in shear stress signaling. *Mol Biol Cell*. 2006; 17(11):4686–4697. [PubMed: 16928957]
- [66]. Brooks PC, Montgomery AM, Rosenfeld M, Reisfeld RA, Hu T, Klier G, et al. Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell*. 1994; 79(7):1157–1164. [PubMed: 7528107]
- [67]. Kim S, Bell K, Mousa SA, Varner JA. Regulation of angiogenesis in vivo by ligation of integrin alpha5beta1 with the central cell-binding domain of fibronectin. *Am J Pathol*. 2000; 156(4): 1345–1362. [PubMed: 10751360]
- [68]. Friedlander M, Brooks PC, Shaffer RW, Kincaid CM, Varner JA, Cheresch DA. Definition of two angiogenic pathways by distinct alpha v integrins. *Science*. 1995; 270(5241):1500–1502. [PubMed: 7491498]
- [69]. Kim HS, Loughran PA, Kim PK, Billiar TR, Zuckerbraun BS. Carbon monoxide protects hepatocytes from tnfr-alpha/actinomycin d by inhibition of the caspase-8-mediated apoptotic pathway. *Biochem Bioph Res Co*. 2006; 344(4):1172–1178.
- [70]. Polunovsky VA, Wendt CH, Ingbar DH, Peterson MS, Bitterman PB. Induction of endothelial cell apoptosis by tnfr alpha: Modulation by inhibitors of protein synthesis. *Exp Cell Res*. 1994; 214(2):584–594. [PubMed: 7925652]
- [71]. Cell portion of the schematic adapted from monocyte SEM released into the public domain by Microscope Service of Universitat Autònoma de Barcelona.

Figure 1. Schematic of an IFBM at the interface between the biologic and material realms. (not to scale) [69]

Figure 2.
Real-time changes in frequency (blue), dissipation (red).

Figure 3. AFM surface profiles of A) uncoated and B) PS-biotin coated polystyrene surfaces. A dense layer of adsorbed molecules were observed on the coated substrates. ($1\ \mu\text{m}$ by $1\ \mu\text{m}$)

Figure 4.

(top) Micrographs of HUVEC interacting with **PS-RGD** treated PS (top row) and untreated PS (bottom row) over 2 hours followed by washing and immunostaining. (bottom) Cell surface area calculations for all of the cells in the micrographs at 1 and 2 hours in both surface treatment conditions.

Figure 5.
Viability stained HUVEC on PS treated with 0.1 mg/mL **PS-RGD**.

Figure 6.

(top) Viability stain of HUVEC apoptosis on varied **PS-RGD** surface coating, after exposure to a constant apoptotic cocktail. (bottom) Ratio of the live to dead pixels in each image with additional concentrations not shown in the micrographs. Solid line represents the ratio in a condition with no apoptotic cocktail added, dashed line is a 4-parameter sigmoidal model fit to the data.

Figure 7.
(left) Alternating columns of **PS-FITC** and **PS-biotin** spots printed on a PS slide. (left) **PS-FITC** spots show higher fluorescence intensity. (right) Upon exposure, streptavidin-Alexa Fluor 594 bound only to **PS-biotin** patterned regions.

Figure 8. HUVEC adhesion and proliferation on **PS-RGD** patterned spots after A) 24 hours and B) 1 week in culture. Scale bar = 100 μm

Table 1

Polystyrene-binding peptides and resultant IFBMs with MALDI-TOF-MS calculated weights.

Nomenclature	Peptide Sequence	Mw Theo.	Mw Calc.
PS-biotin	FFSFFFPASAWGSSGSSGK(biotin)	2227.6	2227.5
PS-RGD	FFSFFFPASAWGSSGSSGRGD	2201.2	2201.4
PS-RGE	FFSFFFPASAWGSSGSSGRGE	2215.4	2214.9