Cell adhesion peptides alter smooth muscle cell adhesion, proliferation, migration, and matrix protein synthesis on modified surfaces and in polymer scaffolds

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Abstract: The effects of cell adhesion peptides (RGDS, KQAGDV, VAPG) on vascular smooth muscle cells grown on modified surfaces and in tissue-engineering scaffolds were examined. Cells were more strongly adhered to surfaces modified with adhesive ligands than to control surfaces (no ligand or a nonadhesive ligand). Cell migration was higher on surfaces with 0.2 nmol/cm^2 of adhesive ligand than on control surfaces, but it was lower on surfaces with 2.0 nmol/cm^2 of adhesive ligand than it was on control surfaces. Further, cell proliferation was lower on adhesive surfaces than it was on control surfaces, and it decreased as the ligand density increased. Similarly, in the peptide-grafted hydrogel scaffolds, cell proliferation was lower in scaffolds containing the adhesive peptides than it was in control scaffolds. After 7 days of culture, more collagen per cell was produced in control scaffolds than in scaffolds containing adhesive peptides. In addition, collagen production decreased in the scaffolds as the ligand concentration increased. While modification of a surface or scaffold material with adhesive ligands initially increases cell attachment, it may be necessary to optimize cell adhesion simultaneously with proliferation, migration, and matrix production. © 2002 John Wiley & Sons, Inc., J Biomed Mater Res 60: 86–93, 2002

Key words: tissue engineering; adhesion peptide; extracellular matrix; migration; polyethylene glycol

INTRODUCTION

In tissue engineering and wound-healing applications, scaffold materials are utilized to provide a mechanical support for cell growth and tissue formation. These scaffolds can be modified such that they also provide specific biologic signals to cells in order to control or facilitate tissue formation or regeneration. For example, bioactive ligands such as peptides or polysaccharides can be covalently grafted to many scaffold materials.[1] Cell adhesion peptides have been incorporated into scaffolds to enhance cell adhesion or to allow biospecific cell adhesion. RGD, an integrin ligand, has been the most extensively studied cell adhesion peptide and has been covalently bound to surfaces and scaffolds. RGD peptides grafted to semi-interpenetrating polymer networks of polyethylene glycol-co-acrylic acid have been found to promote spreading of fibroblasts while similar polymer films containing control peptides RDG or RGE did not.[2] Similarly, hydrogels formed from polyethylene glycol (PEG) with RGD grafted by a PEG spacer arm also promoted spreading of fibroblasts while PEG films containing RDG did not.[3] RGD also has been covalently coupled to collagen/glycosaminoglycan matrices used for dermal repair.[4] These peptide-coupled matrices supported attachment of fibroblasts, endothelial cells, and keratinocytes while matrices without the RGD peptide only supported attachment of fibroblasts. Spreading of endothelial cells was increased on thin polymer films of poly(lactic acid-co-lysine) (PLAL) grafted with RGD over PLAL films with no peptide or grafted with RDG.[5]

In some cases, it is desirable or even necessary to create a surface that is preferentially adhesive for a particular cell type. REDV, a peptide derived from fibronectin, has been shown to allow specific adhesion of endothelial cells but not fibroblasts, smooth muscle cells, or platelets.[6] This suggests that this ligand may be useful for modification of vascular graft surfaces. While there have been numerous studies investigating the effects of adhesive peptides on cell attachment and spreading, the effects of these peptides on other as-

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pects of cell behavior, such as migration, proliferation, and matrix protein production, have received relatively little attention.

A more complete understanding of cellular interactions with bioactive materials and how these interactions may affect tissue formation is needed in order to allow the design of a material that will evoke appropriate cellular responses. For example, migration of cells into scaffolds from surrounding tissue or within the scaffolds themselves may be important in wound healing and tissue engineering applications.[7–10] Similarly, proliferation of cells seeded into scaffolds generally is required to generate a tissue-like structure.[7–9,11] In addition, the production of extracellular matrix components is an important aspect of tissue formation, largely determining the mechanical properties of the new tissue.

We have previously shown that cells grown on surfaces modified with cell adhesion peptides produce fewer extracellular matrix proteins than cells grown on control surfaces, either modified with the nonadhesive peptide RGES or unmodified.[12] Differences also were found in extracellular matrix production, depending on the affinity of the cell type for the various cell adhesion ligands; for example, modification of the surfaces with the most adhesive peptide for vascular smooth muscle cells, KQAGDV, resulted in the least extracellular matrix production by vascular smooth muscle cells. We further found that increasing the ligand density on the surface resulted in decreased matrix protein production by the cells. This reduced matrix protein production could compromise the performance of an engineered tissue, particularly as the scaffold material used for temporary support degrades.

In the current study, we have expanded our study of the effects of adhesive peptides on cell behavior by evaluating migration and proliferation of smooth muscle cells grown on peptide-modified surfaces. We also have evaluated proliferation and extracellular matrix production by smooth muscle cells grown in hydrogel scaffolds grafted with cell adhesion peptides as a function of bioactive ligand affinity and density.

MATERIALS AND METHODS

Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated.

Cell maintenance

Smooth muscle cells from the thoracic aorta of Wistar-Kyoto rats (WKY-SMCs) or Sprague-Dawley rats (SD-SMCs) were isolated and characterized as previously described.[13] Human aortic smooth muscle cells (HASMCs) were obtained from Clonetics (San Diego, CA). Cells were used between passages 4 and 8. Cells were maintained on minimal essential medium Eagle (MEM) supplemented with 10% fetal bovine serum (FBS; BioWhittaker, Walkersville, MD), 2 m*M* L-glutamine, 500 units penicillin, and 100 mg/L streptomycin. Cells were incubated at 37°C in a 5% CO_2 environment.

Preparation of peptide-modified surfaces

Peptides used in this study were RGDS, VAPG, and KQAGDV (Research Genetics, Huntsville, AL). RGES was used as a nonadhesive control peptide. The peptides were acetylated by reaction with acetic anhydride and coupled to aminophase glass slides as previously described.[12] Briefly, aminophase slides were prepared by incubating glass slides with 3-aminopropyltriethoxysilane in dry acetone at 37°C overnight. Acetylated peptides then were coupled to the slides using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) chemistry.[12] Peptide surface density was determined using the ninhydrin assay as previously described.[12] Slides were sterilized under UV light overnight prior to use.

Attachment of cells to peptide-modified surfaces

Suspensions of WKY-SMCs were prepared in MEM with 10% FBS at a concentration of 10,000 cells/mL. Glass slides grafted with peptides at 2.0 nmol/cm^2 were attached to MicroFlexiPerm strips in QuadriPerm cell culture vessels (Heraeus, Osterode am Harz, Germany) to create 12 wells (0.635 × 0.79 cm) on each slide. Two hundred μL of the cell suspension were placed in each well. At 4 h, the medium was removed, the wells were rinsed with phosphate-buffered saline (PBS), and new medium was added. The remaining attached cells then were counted by viewing under a phase contrast microscope (Nikon, model TMS-F, Melville, NY) fitted with an eyepiece grid at 200× magnification.

Resistance of adherent cells to shear stress

Suspensions of HASMCs were prepared in MEM at a concentration of 12,000 cells/mL. Glass slides grafted with peptides at 0.5 nmol/cm^2 were attached to MicroFlexiPerm strips in QuadriPerm Cell Culture Vessels. Two hundred μL of the cell suspension were placed in each well. For each experiment, six glass slides were used for each treatment, three that would serve as a stationary control while the other three were exposed to shear stress. The slides were incubated at 37°C/5% CO_2 for 24 h. The MicroFlexiPerm strips were removed and the slides were placed in centrifuge tubes filled with MEM. The slides then were centrifuged at 2100× g, corresponding to a shear stress of 25 dyn/cm^2,[14] for 15

min. The centrifuged and stationary surfaces then were rinsed twice in PBS to remove any nonadherent cells. The number of cells on each slide was determined by removing the cells with trypsin/EDTA and counting samples on a Coulter counter (Multisizer #0646, Coulter Electronics, Hialeah, FL).

Migration of cells on peptide-modified surfaces

A fence-style migration assay was utilized to evaluate cell migration on modified surfaces. Suspensions of SD-SMCs were prepared in MEM at a concentration of 100,000 cells/mL. Glass slides grafted with peptides at densities of 0.2 or 2.0 nmol/cm^2 were attached to MicroFlexiPerm strips in QuadriPerm cell culture vessels. Two hundred μL of the cell suspension were placed in four of the wells, and the slides were incubated at 37°C/5% CO_2 for 16 h. The MicroFlexiPerms then were removed and the slides incubated in MEM at 37°C/5% CO_2 for 24 h. The distance that cells had migrated beyond the original boundary of the MicroFlexiPerm wells was determined under phase contrast microscopy. Cell locations were tracked by placing grid slides (Fisher Scientific, Pittsburgh, PA) under the glass slides.

Proliferation of cells on peptide-modified surfaces

Suspensions of HASMCs were prepared in MEM at a concentration of 40,000 cells/mL. Glass slides grafted with peptides at densities of 0.2 or 2.0 nmol/cm^2 were attached to FlexiPerm strips in QuadriPerm Cell Culture Vessels to create 8 wells (1.11 × 0.79 × 0.79 cm) on each slide. Five hundred μL of the cell suspension were placed in each well. The slides were incubated at 37°C/5% CO_2 for 48 h. The wells were rinsed with phosphate-buffered saline (PBS) and the cells were fixed with 10% buffered formalin. Immunohistochemical staining for proliferating cell nuclear antigen (PCNA) was used as an indicator of cell proliferation. Cells were permeabilized with methanol, incubated with mouse anti-PCNA IgG (Dako, Carpinteria, CA), incubated with HRP-conjugated anti-mouse IgG (Dako), incubated with 3-amino-9-ethylcarbazole (AEC) substrate-chromogen (Dako), and, finally, counterstained with Mayer's hematoxylin. The numbers of proliferating (red) and nonproliferating (blue) cells in each section were counted under light microscopy (Axiovert 135, Carl Zeiss, Thornwood, NY).

Preparation of PEG-diacrylate

PEG-diacrylate was prepared by combining 0.1 mmol/mL dry PEG (6000 Da; Fluka, Milwaukee, WI), 0.4 mmol/mL acryloyl chloride, and 0.2 mmol/mL triethylamine in anhydrous dichloromethane and stirring under argon overnight. The resulting PEG-diacrylate then was precipitated with ether, filtered, and dried *in vacuo*. Addition of acrylate groups was confirmed via proton NMR (Bruker Avance 400 MHz, $CDCl_3$ as solvent).

Preparation of acryloyl–PEG peptides

Peptides were conjugated to polyethylene glycol (PEG) by reacting the peptide with acryloyl–PEG-n-hydroxysuccinimide (acryloyl–PEG–NHS, 3400 Da; Shearwater Polymers, Huntsville, AL) in 50 m*M* Tris buffer (pH 8.5) for 2 h.[3] The mixture then was lyophilized and stored frozen. Gel permeation chromatography equipped with UV/vis (260 nm) and evaporative light-scattering detectors was used to analyze the resulting acryloyl–PEG peptides and PEG standards (Polymer Laboratories, Amherst, MA). Acryloyl–PEG peptides were sterilized by filtration (0.8 μm prefilter and 0.2 μm filter) prior to use.

Photopolymerization of hydrogels

Hydrogels were prepared by combining 0.4 g/mL PEG-diacrylate in 10 m*M* HEPES-buffered saline (pH 7.4; HBS). This aqueous polymer solution was sterilized by filtration (0.8 μm of prefilter and 0.2 μm of filter) and added to an equal volume of a suspension of SD-SMCs at 2 × 10^6 cells/mL, such that the resulting cell–polymer solution contained 1 × 10^6 cells/mL. Sterile acryloyl–PEG peptide was added to achieve peptide densities of 0, 0.14, 0.7, 2.8, or 7 μmol/mL. Forty μL /mL of the long wavelength ultraviolet photoinitiator 2,2-dimethyl-2-phenyl-acetophenone in N-vinylpyrrolidone (600 mg/mL) then were added to the polymer solution, and 0.25 mL of the polymer solution were placed in a disk-shaped mold (20 mm diameter, 2 mm thickness). This was exposed to UV light (365 nm, 10 MW/cm^2) for 20 s to convert the liquid polymer–cell mixture to a hydrogel with homogeneously seeded cells. Hydrogels were incubated in MEM containing 10% FBS for 7 days in a 37°C/5% CO_2 environment. The medium was changed every 3 days.

Proliferation of cells in hydrogels

After 7 days of culture, hydrogels were frozen in tissue-freezing medium (TBS, Fisher Scientific) and sectioned to a thickness of 20 μm using a cryostat (HM505E, Carl Zeiss). The sections were fixed with 10% buffered formalin and stained for PCNA to assess cell proliferation, as described above.

DNA and hydroxyproline measurement in hydrogels

After 7 days of culture, hydrogels were removed from the culture media, weighed, and digested with 0.1*N* NaOH

overnight at 37°C. Digested hydrogels then were neutralized with 0.1*N* HCl. The DNA content of the digested, neutralized hydrogels was determined using a fluorescent DNA binding dye, Hoechst 33258 (Molecular Probes, Eugene, OR). The fluorescence of the samples was determined on a fluorometer (VersaFluor, Bio-Rad Laboratories, Hercules, CA), with the excitation filter at 360 nm and the emission filter at 460 nm, and compared to the fluorescence of calf thymus DNA standards. Hydroxyproline concentration was determined by oxidation with chloramine T (ICN Biomedicals, Aurora, OH) and development with p-dimethylaminobenzaldehyde (ICN Biomedicals).[15] Hydroxyproline is a marker for collagen production.[15]

Statistical analysis

Data sets were compared using two-tailed, unpaired *t*-tests. *P* values less than 0.05 were considered to be significant. All data reported are means ± standard deviation.

RESULTS

Cell adhesion to peptide-modified surfaces

SMCs were seeded onto peptide-modified surfaces and the number of adherent cells determined after 4 h. The peptides examined in the study were RGDS, VAPG, and KQAGDV. These peptides were chosen based on prior studies examining SMC adhesion on peptide-modified surfaces.[12] RGES was used as a non-adhesive control peptide. There was a greater amount of early cell attachment to surfaces modified with the three adhesive peptides than there was to the control surfaces, with KQAGDV-modified surfaces displaying the greatest number of adherent cells [Fig. 1(a)].

In order to examine the strength of adhesion of SMCs to these peptide-modified surfaces, cells were seeded onto the surfaces and cultured for 24 h. The surfaces then were subjected to a shear stress of 25 dyne/cm^2 for 15 min. The number of cells remaining on the surfaces was determined and compared to stationary control surfaces (surfaces modified with the same peptides but not exposed to shear stress). As seen in Figure 1(b), greater than 85% of the cells remained adherent to all of the peptide-modified surfaces after exposure to shear, which was significantly higher than the percentage of cells remaining on the control surfaces, either RGES-modified or unmodified aminophase glass.

Cell migration on peptide-modified surfaces

Migration of SMCs on the peptide-modified surfaces was determined using a fence-style assay. On surfaces with 0.2 nmol/cm^2 of peptide, more cells migrated beyond the fence perimeter on surfaces with cell adhesion ligands than they did on the control surface, RGES-modified glass (Fig. 2). However, on surfaces with 2 nmol/cm^2 peptide, fewer cells migrated beyond the fence perimeter on the adhesive ligand surfaces than on the control surface. Further, there was no difference in the number of cells that migrated on the RGES control surfaces at the two different concentrations of peptide used here.

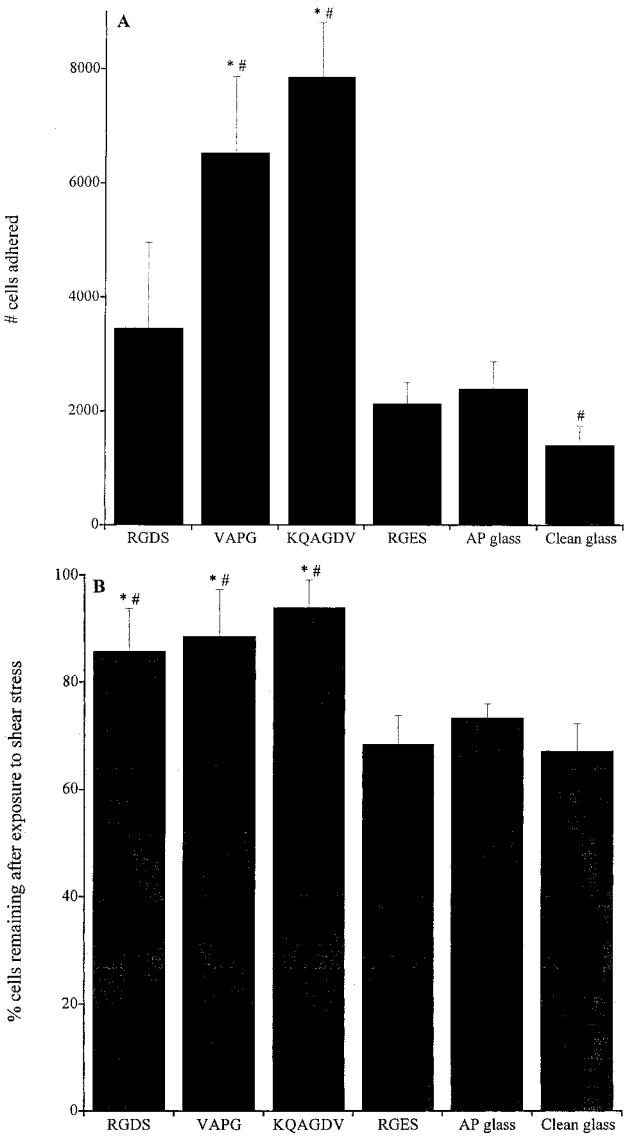

Figure 1. (A) Adhesion of SMCs at 4 h to glass covalently grafted with cell adhesion ligands (RGDS, VAPG, KQAGDV) at 2.0 nmol/cm^2. (B) Percentage of SMCs remaining on peptide-modified surfaces (0.5 nmol/cm^2) following exposure to a shear stress of 25 dyn/cm^2 for 15 min. RGES was used as a control peptide for comparison. Data represent the mean of three experiments ± standard deviation. (*) $P < 0.05$ compared to RGES; (#) $P < 0.05$ compared to AP glass.

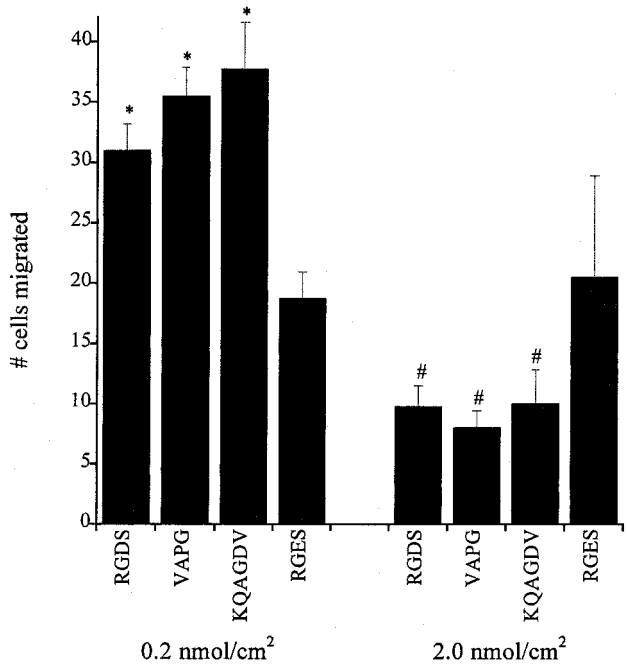

Figure 2. Migration of SMCs at 24 h on glass surfaces covalently grafted with cell adhesion ligands (RGDS, VAPG, KQAGDV) and the control peptide RGES at either 0.2 or 2.0 nmol/cm^2. Data represent the mean of four experiments ± standard deviation. (*) $P < 0.001$ compared to RGES at the same concentration; (#) $P < 0.001$ compared to the same peptide at 0.2 nmol/cm^2.

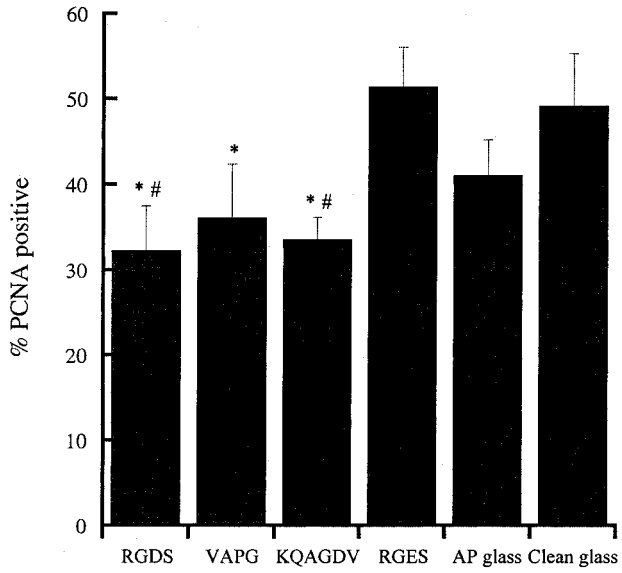

Figure 3. Proliferation of SMCs at 48 h on glass surfaces covalently grafted with cell adhesion ligands (RGDS, VAPG, KQAGDV) at 2.0 nmol/cm^2. RGES was used as a control peptide. Cells were stained for PCNA and counterstained with hematoxylin. Data represent the mean of three experiments ± standard deviation. (*) $P < 0.001$ compared to RGES; (#) $P < 0.05$ compared to AP glass.

Cell proliferation on peptide-modified surfaces

Proliferation of SMCs growing on peptide-modified surfaces was examined by staining cells for proliferating cell nuclear antigen (PCNA, indicates the S-phase of the cell cycle) after 48 h of culture. The presence of cell adhesion ligands on the surface resulted in a decrease in the percentage of PCNA-positive cells as compared to control surfaces (Fig. 3). We also examined the effect of ligand density on proliferation of SMCs. As shown in Figure 4, an increase in ligand density on the surface results in a decrease in the number of proliferating cells.

Cell proliferation in peptide-grafted scaffolds

We examined the effect of cell adhesion ligands on the proliferation of SMCs growing in PEG hydrogel scaffolds. Peptide-grafted hydrogels were prepared with SMCs homogeneously seeded within them. After 7 days of culture, sections of the hydrogels were stained for PCNA. As in the previous studies on peptide-modified surfaces, the peptides examined were RGDS, VAPG, and KQAGDV (all at a density of 7 μmol/mL). RGES, a peptide that does not interact with cell-surface receptors, was grafted into hydrogels as a control material. The presence of cell adhesion ligands resulted in a statistically significant decrease in the number of proliferating cells compared to control hydrogels (Fig. 5). The effect of peptide density on

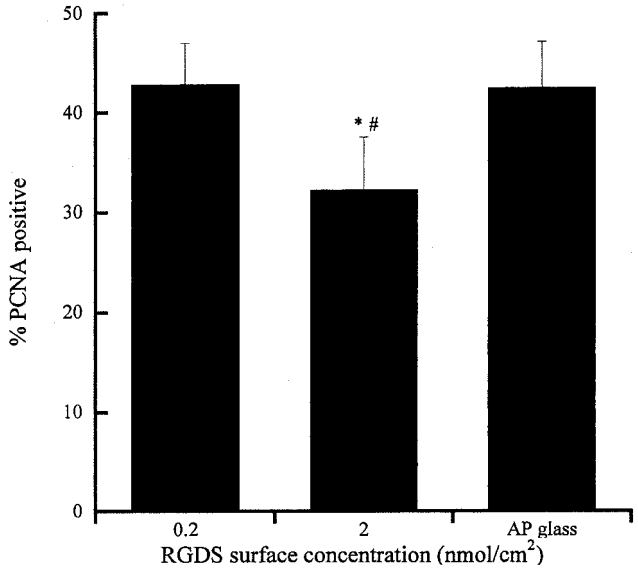

Figure 4. Proliferation of SMCs at 48 h on RGDS-modified glass surfaces at 0.2 and 2 nmol/cm^2 and on AP glass at 2 nmol/cm^2. Cells were stained for PCNA and counterstained with hematoxylin. Data represent the mean of three experiments ± standard deviation. (*) $P < 0.005$ compared to AP; (#) $P < 0.002$ compared to RGDS at 0.2 nmol/cm^2.

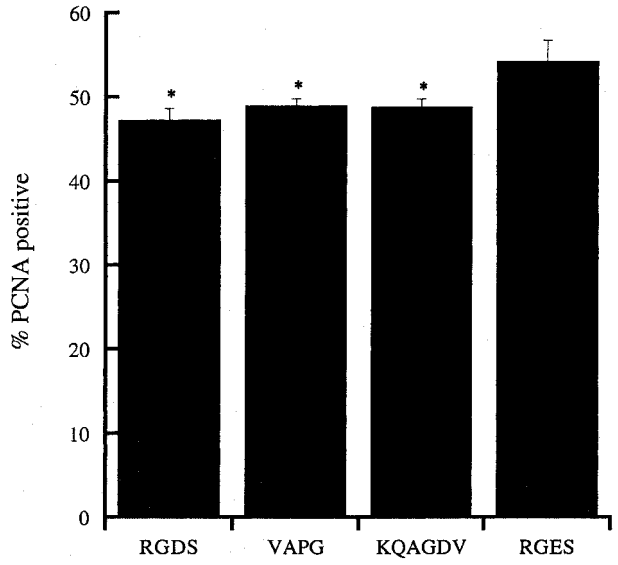

Figure 5. Proliferation of SMCs at 7 days grown in PEG hydrogels grafted with 7 μmol/mL acryloyl–PEG peptide. Cells were stained for PCNA and counterstained with hematoxylin. Data represent the mean of three experiments ± standard deviation. (*) $P < 0.002$ compared to RGES.

proliferation also was examined. RGDS was incorporated at 0.14, 2.8, or 7 μmol/mL. There was no difference in the number of proliferating cells in hydrogels containing either 0.14 or 2.8 μmol/mL RGDS (Fig. 6). However, as the concentration of RGDS was increased to 7 μmol/mL, the percentage of proliferating cells significantly decreased (Fig. 6).

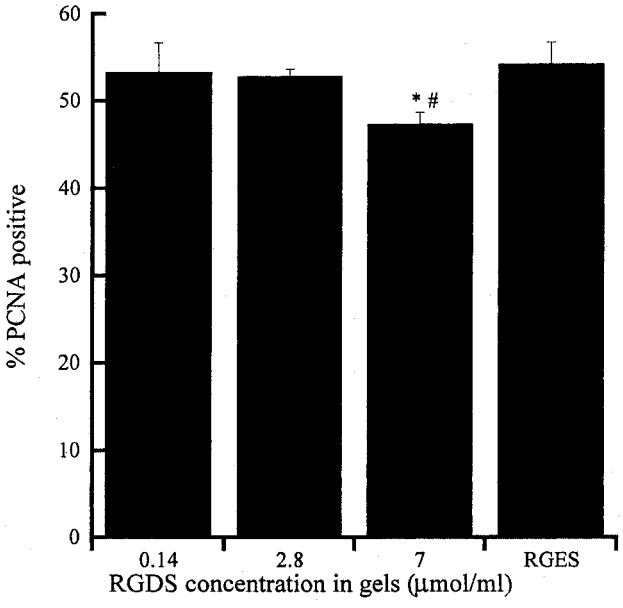

Figure 6. Proliferation of SMCs at 7 days grown in PEG hydrogels grafted with acryloyl–PEG–RGDS at concentrations of 0.14, 2.8, and 7 μmol/mL. PEG hydrogels grafted with 7 μmol/mL acryloyl–PEG–RGES were used as controls. Cells were stained for PCNA and counterstained with hematoxylin. Data represent the mean of three experiments ± standard deviation. (*) $P < 0.005$ compared to 2.8 μmol/mL; (#) $P < 0.002$ compared to RGES.

Collagen production in peptide-grafted hydrogels

In order to examine the effect of cell adhesion ligands on extracellular matrix production by SMCs in the hydrogels, peptide-grafted hydrogels were prepared with SMCs homogeneously seeded within them. After 7 days of culture, the amounts of hydroxyproline and DNA within the hydrogel scaffolds were determined. Since hydroxyproline is a marker for collagen,[15] it is an indication of how much extracellular matrix has been produced by the cells. The peptides were incorporated at a concentration of 7 μmol/mL. The amount of hydroxyproline produced per ng of DNA, indicating the amount of collagen produced per cell, was lower in hydrogels containing the three adhesive peptides than in either of the nonadhesive controls (Fig. 7). The effect of ligand density on extracellular matrix production also was examined. KQAGDV was incorporated into PEG hydrogels at concentrations of 0.14, 1.4, 7, and 14 μmol/mL of cell-polymer solution. Hydroxyproline production per cell decreased as the concentration of KQAGDV incorporated into the scaffold increased (Fig. 8). These results are similar to our prior observations of matrix protein production by smooth muscle cells grown on peptide-modified surfaces.[12]

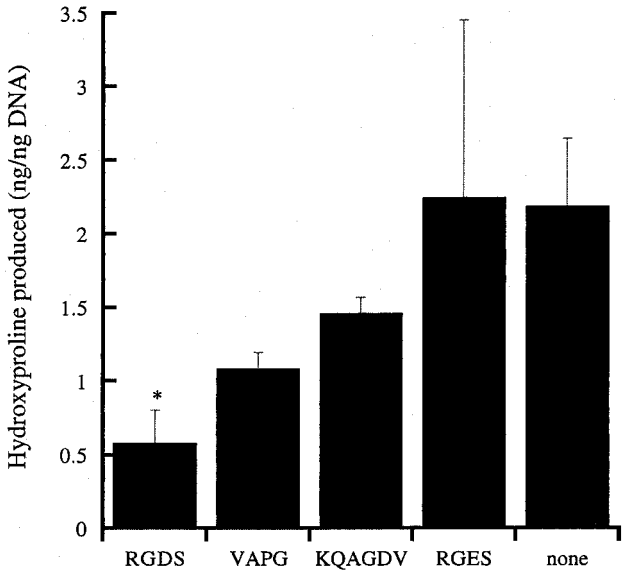

Figure 7. Hydroxyproline production by SMCs grown in PEG hydrogels grafted with 7 μmol/mL acryloyl–PEG peptide. The peptides evaluated were RGDS, VAPG, and KQAGDV. Hydrogels containing either the peptide RGES or no peptide were used as nonadhesive controls. Data represent the mean of three experiments ± standard deviation. (*) $P < 0.05$ compared to hydrogels with no peptide.

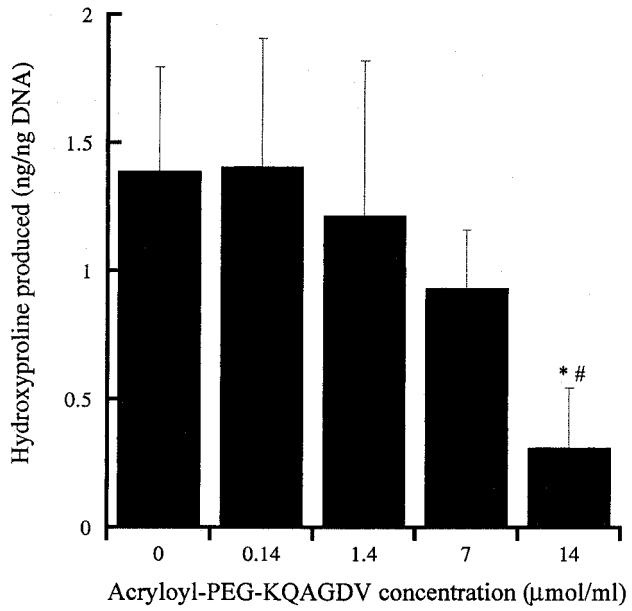

Figure 8. Hydroxyproline production by SMCs grown in PEG hydrogels grafted with acryloyl–PEG–KQAGDV at concentrations of 0, 0.14, 1.4, 7, and 14 μmol/mL. Data represent the mean of three experiments ± standard deviation. (*) $P < 0.005$ compared to 0 μmol/mL; (#) $P < 0.01$ compared to 7 μmol/mL.

DISCUSSION

Creating bioactive materials that provide signaling to cells may lead to improved tissue formation and thus improved results in tissue engineering and wound healing applications. However, in order to determine which signals may be best for a specific application and at what levels they should be incorporated, we need to have a more complete understanding of how the signals may affect various aspects of cell behavior.

Cell adhesion ligands provide one type of signaling that can be incorporated into bioactive materials. We previously found that increasing cell attachment and spreading by covalently grafting cell adhesion ligands to a surface leads to a decrease in extracellular matrix production by cells grown on these modified surfaces.[12] Here, we have extended the study of vascular smooth muscle cell (SMC) behavior on peptide-modified surfaces to include strength of adhesion, migration, and proliferation. Further, we have examined proliferation and extracellular matrix production of SMCs growing in polyethylene glycol (PEG)-based hydrogel scaffolds grafted with cell adhesion ligands.

As expected, not only did more SMCs initially attach to surfaces grafted with cell adhesion ligands, but also the cells became more strongly adherent than did those on control surfaces (RGES modified or unmodified). We also observed that with a low ligand density on the surface, more cells migrated on surfaces with the cell adhesion ligands than they did on control surfaces. However, with a high ligand density, fewer cells migrated on adhesive surfaces than on control surfaces, indicating that a low concentration of cell adhesion ligand may aid cell migration while a higher concentration may impede cell migration. These results reflect the fact that cells require cell adhesion ligands for migration,[16,17] but they also indicate that there may be an optimal ligand concentration for migration. Previously, fibroblasts were shown to migrate more slowly on surfaces grafted with a high concentration (on the order of 10 nmol/cm^2) of RGDS as compared to RDGS.[18] Recently, Burgess et al.[10] examined the migration of murine melanoma cells in RGD-grafted collagen gels and found that migration was highest for an intermediate level of adhesiveness. DiMilla et al.[19] also have shown that maximal migration of SMCs on surfaces coated with matrix proteins, such as fibronectin and collagen IV, occurs at an intermediate level of cell–substratum adhesiveness.

The number of PCNA-positive cells (in the S-phase of the cell cycle, an indicator of proliferation) was lower on surfaces with the cell adhesion ligands than on control surfaces. Further, as the ligand density on the surface increased, the number of proliferating cells decreased. Neff et al.[20] found a maximum proliferation of fibroblasts at an intermediate RGDS concentration (~1.33 pmol/cm^2), indicating that above a certain level of peptide on the surface, proliferation decreases with increasing peptide concentration. While we did not see the initial increase in proliferation with increasing peptide concentration that Neff et al. observed, we may not have used a low enough concentration to observe this phenomenon. Our results and those of Neff et al. are in contrast to results seen with endothelial cells (slight increase in proliferation observed) and osteoblasts (no difference in proliferation observed) grown on surfaces modified with RGDS compared to RDGS[21]; however, the surface concentration of peptide used in these studies was not reported. These differences may be due to differences in cell type used or may be due to differences in the concentration of peptide on the surface.

When SMCs were seeded homogeneously in PEG-based hydrogel scaffolds, fewer proliferating cells were observed in scaffolds grafted with cell adhesive ligands than in nonadhesive control scaffolds, and proliferation increased as the ligand concentration decreased, reflecting results observed on peptide-modified surfaces. However, there was no change in the number of proliferating cells at the two lowest ligand concentrations, indicating that it may be possible to add low concentrations of cell adhesive ligand to the scaffold without affecting the proliferation of the cells.

Additionally, the amount of hydroxyproline pro-

duced per ng of DNA, indicating collagen production per cell, was lower in hydrogels containing the adhesive peptides than in nonadhesive controls, indicating that the cells produce less matrix in the presence of adhesive peptides. Further, hydroxyproline production decreased as the ligand concentration in the scaffold increased. These results are similar to those found when cells were grown on peptide-grafted surfaces.[12] This decrease in matrix production by cells grown on peptide-modified scaffolds may have detrimental effects in tissue engineering applications, where the scaffold is expected to degrade over time and be replaced by new ECM. In some applications, too little matrix production may result in failure of the tissue. As with proliferation and migration, however, an intermediate concentration of adhesive ligand may allow sufficient matrix production while enhancing adhesion. In these photopolymerized PEG hydrogel scaffolds homogeneously seeded with vascular smooth muscle cells, an adhesive peptide concentration between 2.8 and 7 μmol/mL appears to be optimal for promoting cell attachment and spreading while also promoting migration, proliferation, and extracellular matrix production by the cells.

CONCLUSIONS

While the addition of cell adhesion peptides to materials has been shown to increase cell attachment and spreading, we have found that the changes in cell signaling induced by these bioactive peptides also can lead to altered migration, proliferation, and matrix production of cells grown on peptide-modified surfaces or in peptide-grafted scaffolds. These changes in migration, proliferation, and extracellular matrix production could, in turn, affect the formation rate and mechanical integrity of new tissue. An optimal concentration of cell adhesive ligand would need to be found for a given application to achieve appropriate adhesion, proliferation, migration, and matrix production.

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