Cell migration through defined, synthetic ECM analogs[1]

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SPECIFIC AIMS

The objective of this study was to develop a biomimetic polymer system that can be used as an in vitro model system to evaluate cell migration. The substrate consisted of a photopolymerizable hydrogel based on acrylate-terminated derivatives of polyethylene glycol (PEG) with proteolytically degradable peptides targeted for degradation by specific enzymes involved in cell migration, incorporated into the backbone, and grafted cell adhesion peptides. An aim was to evaluate the effects of adhesion ligand density on cell migration in this hydrogel system.

PRINCIPAL FINDINGS

1. Cell morphology and migration through collagenase-sensitive hydrogels with varying concentrations of RGDS

A modified Boyden chamber assay was used to investigate cell morphology on and migration through biomimetic hydrogels. After a 7 day incubation period, phase contrast micrographs were taken of the fibroblasts on the surface of hydrogels, which contained varying concentrations of arginine-glycine-aspartic acid-serine (RGDS) (0.0, 1.4, 2.8, 3.5, 5.0 μmol/ml), to assess cell spreading. At the lowest RGDS concentration (1.4 μmol/ml), cells on the top of the hydrogel layer were found to be small with relatively little extension of lamellae. At the highest RGDS concentration observed (5.0 μmol/ml), cells were more fully spread with multiple lamellae extended in all directions. Cell areas at 1.4 and 5.0 μmol/ml were found to be statistically different from the control, which contained no RGDS (**Fig. 1**). A significant difference in cell area was observed between 1.4 and 5.0 μmol/ml RGDS.

Human dermal fibroblasts were found to migrate through the collagenase-sensitive hydrogels. These hydrogels contained the sequence LGPA, which is targeted for degradation by collagenase, and the sequence RGDS for cell adhesion. No cell migration was seen unless the adhesive and the degradable sequences were both present. However, varying concentrations of RGDS (1.4, 2.8, 3.5, 5.0 μmol/ml) were found to affect migration. It was observed that an intermediate level of adhesiveness (~3.5 μmol/ml) could be used to obtain optimal migration.

2. Cell migration through plasmin-sensitive hydrogels with varying concentrations of RGDS

HDF migration was also evaluated through plasmin-sensitive hydrogels, which contained the degradable peptide sequence NRV and varying concentrations of the adhesive ligand RGDS (0.0, 1.4, 2.8, 3.5, 5.0 μmol/ml). A fine balance was found between adhesive level and migration. Optimal migration index was observed at ~ 2.8 μmol/ml of RGDS.

3. Smooth muscle cell morphology on and migration through elastase-sensitive hydrogels with varying concentrations of RGDS

Smooth muscle cell morphology on and migration through an elastase-sensitive hydrogel containing the polyalanine peptide sequence was assessed. After the incubation period, cell area distribution was similar to the collagenase-sensitive hydrogel study: cell area increased with increasing concentration of the adhesion ligand RGDS. Migration of SMCs was also affected by varying levels of adhesiveness (**Fig. 2**). At low and high concentrations of RGDS, the SMC migrated less as a population than at an intermediate level (~3.5 μmol/ml).

CONCLUSIONS AND SIGNIFICANCE

Cell migration is essential to processes such as embryonic development, wound healing, angiogenesis, and cancer metastasis. Migration involves a complex integration of cellular adhesion to the substratum, proteolysis and remodeling of surrounding extracellular matrix (ECM), and activation and regulation of chemical signaling by growth factors and other chemotactic cues. These cooperative mechanisms enable a cell to reach its target and perform its function, whether to

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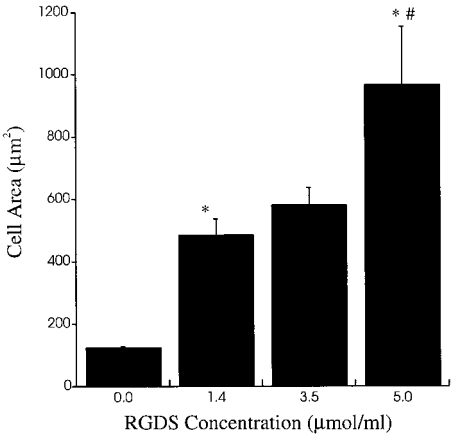

Figure 1. Average area of fibroblasts on the surface of collagenase-sensitive hydrogels with varying concentrations of the adhesion ligand RGDS. $*P < 0.01$ relative to 0.0 μmol/ml RGDS; $\#P < 0.05$ relative to 1.4 μmol/ml RGDS.

repair injured tissue, fight infections, or build new blood vessels. The balance between adhesion and detachment largely governs the rate of migration. Palecek et al. demonstrated in 1997 that changes in migration speed depend on 2-dimensional substratum adhesive ligand density, cellular integrin expression levels, and integrin-ligand binding affinities. Maximal migration speed was observed at intermediate levels of receptor occupancy, where cells were found to be moderately spread and moved by extending lamellae in different directions. At low ligand concentrations, cells were rounded with smaller and shorter lamellae. At high ligand concentrations, cells were widely spread and exhibited no movement, presumably due to their inability to detach. Similar results have been found on surfaces with immobilized cell adhesion peptides.

The study of cell migration over 2-dimensional surfaces has revealed much detail on the mechanisms of cell migration, such as the biphasic dependence on substrate adhesiveness. However, the ECM provides a 3-dimensional adhesive substrate with multivalent, interconnected ligands. The ECM also imposes a spatial barrier or biophysical resistance to cells. For migration, the cell must not only interact with matrix adhesive ligands for force generation, but also develop strategies to overcome biomechanical resistance imposed by the matrix. A change in cell morphology is one such strategy to overcome extracellular matrix resistance, where the cell adapts to the ECM environment and uses the path of least resistance. Another strategy is proteolysis of the ECM to create pathways for migration. Proteases such as collagenase, elastase, or plasmin can be secreted or activated by cells, thus removing the ECM barriers. It has been observed that this proteolytic activity is localized and focused at the cell's leading edge. In addition, proteolysis may enhance migration by favoring cell detachment.

Various 3-D models have been used to investigate cell migration including reconstituted basement membrane, fibrin gels, or collagen gels. These model systems allow investigators to mimic the biochemical and biophysical architecture of interstitial tissues, providing in vitro systems for detailed analysis of migration behavior. In 2000, Burgess et al. investigated cell migration through 3-dimensional collagen structures, focusing on migration as a function of adhesiveness. This was accomplished by grafting the adhesive peptide sequence RGDS to collagen at varying concentrations. Burgess et al. found similarities to studies performed on 2-dimensional substrata, such as optimal levels of adhesive ligands for the duration and distance cells persisting in the same direction. However, there were discrepancies in the cell speed between 2- and 3-dimensional studies; this may be due to the mechanical barrier imposed by the collagen gel. The collagen matrix provides biochemical signals and adhesion ligands in addition to the grafted RGD peptides, which complicates analysis of the mechanisms of cell migration.

To study cell migration in vitro in three dimensions, one needs to place cells in an environment that resembles the extracellular matrix. These environments can be created using natural materials as discussed above. However, these materials usually incorporate a multitude of factors, which makes deciphering the actual cause and effect of the behavior difficult. These systems only allow manipulation of limited experimental parameters. Another approach is to design and use biomimetic systems that can provide the mechanical support, adhesion ligands, degradation sequences, and other signals, so that a cell can migrate. Such systems should provide tight control over many experimental parameters and should minimize nonspecific cell–material interactions. Hence, an aim of such a system is to stimulate active interaction between the synthetic polymer and the biological environment.

We have developed a biomimetic hydrogel system that allows us to control the availability and identity of

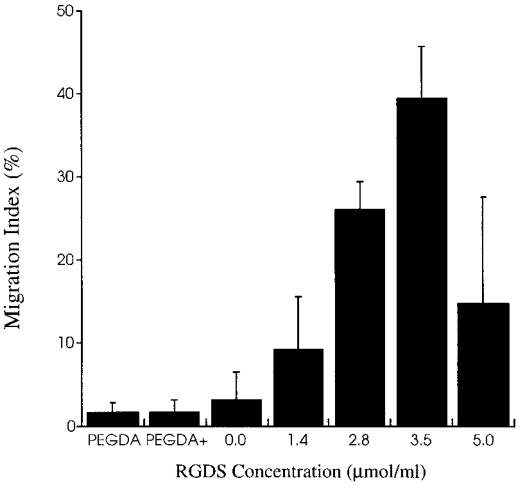

Figure 2. Smooth muscle cell migration through elastase-sensitive, RGD-modified hydrogels. Hydrogels contained the polyalanine sequence, which is targeted for degradation by elastase secreted by the cells. PEGDA: PEG diacrylate; PEGDA+: PEG diacrylate with 1.4 μmol/ml RGDS. Migration was optimal at ~3.5 μmol/ml RGDS.

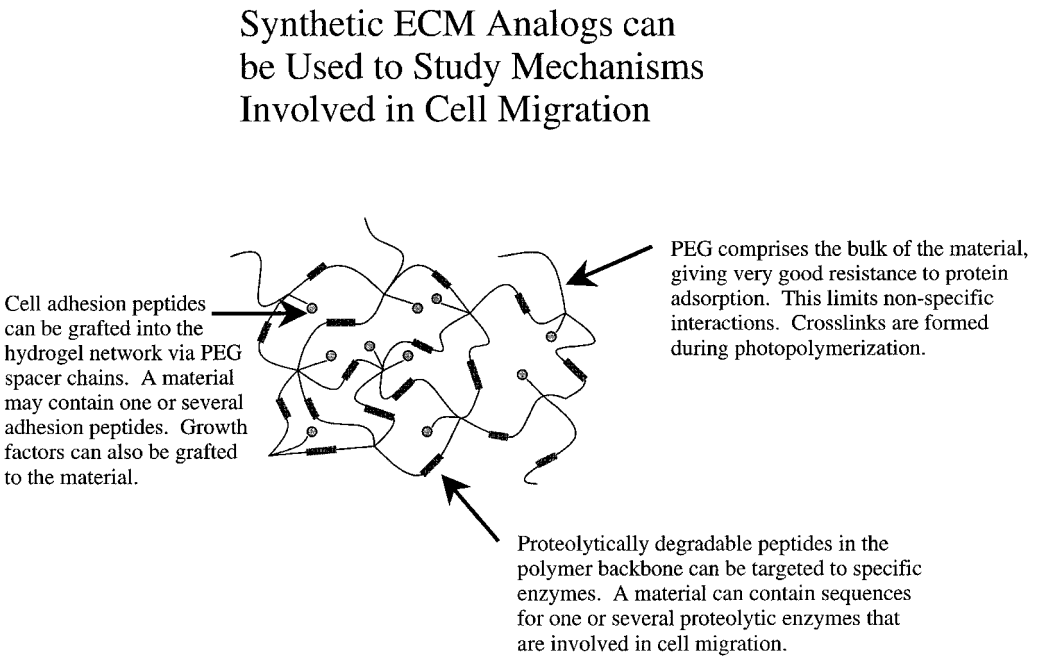

Figure 3. Schematic diagram depicting the structure of the hydrogel materials used in these studies as ECM analogs

adhesive and proteolytically degradable sequences to systematically examine their effects on cell migration. These materials are photopolymerizable hydrogels based on acrylated derivatives of polyethylene glycol. Polyethylene glycol was selected as the base material due to its excellent biocompatibility and resistance to protein adsorption, which minimizes nonspecific interactions with the material. These materials contain proteolytically degradable peptide sequences, targeted for degradation by specific enzymes involved in cell migration, in the polymer backbone. Degradable peptide sequences chosen for this study are a collagenase-sensitive sequence, GGLGPAGGK, a plasmin-sensitive sequence, GGVRNGGK, and an elastase-sensitive sequence, AAAAAAAAAK. It has been reported that when incorporated into the backbone of the polymer, these sequences are not only specific to the appropriate protease, but that degradation is dependent on the amount of protease present. Cell adhesion peptides such as RGD can also be grafted into these hydrogels during photopolymerization to promote interaction with specific cell surface receptors. A single hydrogel material can contain several different proteolytically sensitive segments and many cell adhesion ligands, thus allowing one to mimic many properties of the ECM in a synthetic material. For this report, we investigated the effects of material adhesiveness of the hydrogels on cell migration.

We had earlier reported that fibroblast migration through collagenase-sensitive hydrogels with grafted adhesive peptide RGDS is similar to that through a collagen layer of the same thickness. In the current study, we have demonstrated that the amount of RGDS peptide incorporated into the material dramatically affected migration. The migration index had a biphasic dependence on substratum adhesiveness. Furthermore, adhesiveness had a remarkable effect on cell spreading area and morphology. On weakly adhesive substrata, cells were found to spread poorly and presumably were unable to form sufficient adhesions to support contractile forces during migration. On strongly adhesive surfaces, cells were well spread and unable to disrupt the adhesion sites. At intermediate levels of adhesiveness, there is a balance between adhesion and force to enable cells to migrate. These biomimetic materials allow us to examine the effects of adhesiveness on cell migration; in the future, other bioactive molecules can be incorporated to assess their effects on cell migration. FJ