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ENHANCING CELL MIGRATION IN COLLAGEN GELS BY MODULATING COLLAGEN ADHESIVITY

Gary A. Monteiro, Harini G. Sundararaghavan, Anthony V. Fernandes and David I. Shreiber

Rutgers, The State University of New Jersey
Department of Biomedical Engineering
Piscataway, NJ 08854

INTRODUCTION

The organized movement of cells is critical during tissue morphogenesis and wound healing. While different tissue cells use distinct mechanisms for migration, the underlying biophysical balance of adhesive and tractional forces for effective migration is similar. The extracellular matrix provides the structural framework through which a cell can migrate. In particular, collagen is an abundant and ubiquitous ECM protein that supports cell migration. The excellent biocompatibility and physiological relevance of collagen have made it a primary material for tissue engineered regenerative therapies and in vitro studies with tissue equivalents.

In general, there is a biphasic relationship between cell adhesion and cell migration [1-3]. If a substrate is too adhesive, cells cannot effectively release for propulsion; if the substrate is not adhesive enough, cells cannot generate the traction required for propulsion. Because of its ubiquity throughout the body, collagen has limited cell type specificity, and it is a relatively adhesive substrate [2-3]. As such, baseline migration in collagen systems is minimal, and cell migration is generally enhanced via changes in expression of integrin ligands from soluble factors or genetic reprogramming, or by blocking adhesion with soluble competitive ligands. From a biomaterials perspective, the ability to enhance cell migration through collagen gels without the addition of soluble factors or manipulation of cells would have great potential for tissue engineering applications such as wound healing. Herein, we demonstrate that the adhesivity of collagen scaffolds can be decreased by covalently grafting non-adhesive peptide sequences, and that the decrease in adhesivity causes a concomitant increase in cell migration and decrease in cell traction.

METHODS

Two peptide sequences – GRGDS, which includes the cell-binding domain RGD, and its scrambled, non-adhesive version GRDGS (RDG) were grafted onto collagen backbone using a hetero-bifunctional coupling agent, 1-ethyl-3-(3-Dimethylaminopropyl) carbodiimide (EDC). EDC activates the carboxylic group of the peptide, which in turn covalently binds to free amines along the collagen backbone via nucleophilic attack. Peptides were grafted to soluble collagen, which was then dialyzed to remove ungrafted peptides. Grafting efficiency was between 50-60% as determined by including fluorescently-tagged peptides and calibrating fluorescence to a standard curve. Rat dermal fibroblast (RDF) adhesion, matrix reorganization, and migration were assayed with three grafted concentrations of RGD or RDG – High: 0.14mg/ml, Medium: 0.07mg/ml and Low: 0.035mg/ml – and native 2.0mg/ml collagen gels using methods detailed below.

Adhesion Assay: RDFs (50K/ml) that constitutively express GFP were seeded on collagen gels and allowed to attach for 3hrs. Following rinsing, the number of remaining cells was determined by counting cells under epifluorescent microscopy.

Matrix Compaction Assay: Cell traction was indirectly assayed by measuring compaction over a period of 6 days of free-floating, disc-shaped collagen gels seeded with 50Kcells/ml. Compacting gels were imaged under bright field and disc cross-sectional area was measured every 24 hours.

Cell Migration Assay: RDF migration was assayed on and in grafted and ungrafted collagen gels in 24-well plates. Selected fields of view were imaged under epifluorescent microscopy at 10-15 min intervals for 12–14 hrs with computer-controlled microscopy using an Olympus IX81 microscope. Images were processed using MATLAB and imported into Image Pro Plus (Version 5.1 for windows, Media Cybernetics, MD) to determine two dimensional spatial cell tracks, which were verified manually. At least 50 cells in each well were tracked.

For each well, the mean squared displacement of the cells was determined and fit to a persistent random walk model in two dimensions to determine the cell migration coefficient.

RESULTS

Adhesion: Cell adhesion was assayed by recording the number of cells that remained attached to each grafted collagen condition post rinsing (Fig 1). As expected, grafting RGD to collagen significantly increased adhesivity. Conversely, grafting the RDG scrambled version negatively influenced cell adhesion. (ANOVA $p<0.001$)

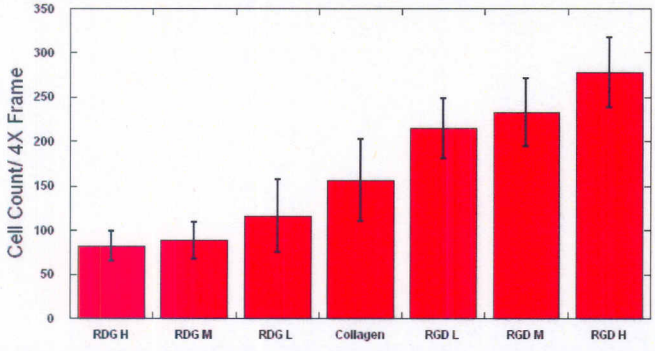

Figure 1: Grafting RGD peptides to collagen increased cell adhesion and grafting RDG peptides decreased adhesion significantly.

Matrix Compaction: The influence of cell adhesion on cell traction was assayed by examining fibroblast-mediated gel compaction over 6 days. Increasing cell adhesion via RGD-grafting increased the percent of compaction, whereas decreasing cell adhesion via RDG-grafting decreased cell compaction. (Fig 2). (ANOVA $p<0.001$)

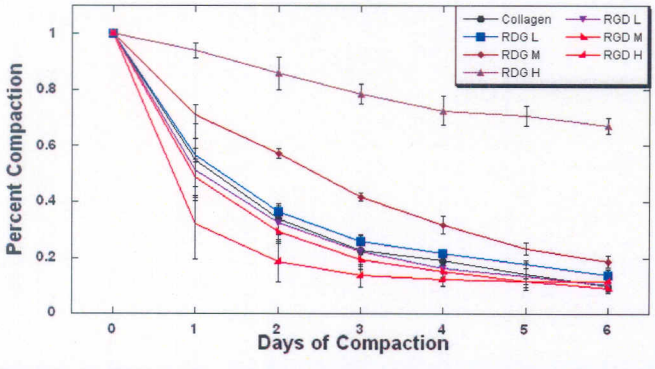

Figure 2: Collagen gel compaction decreases significantly when GRGDS peptides are grafted to collagen

Cell Migration: The motility of cells seeded on collagen and entrapped within collagen gels was quantified following time-lapse microscopy. Cell migration decreased when in (Fig 3A) and on (Fig 3B) RGD-grafted gels, and increased in and on RDG-grafted cells. Migration on gels with additional concentrations of RGD- and RDG-grafting was evaluated, which confirmed the biphasic dependence of migration on adhesion.

DISCUSSION

The ability to control cell migration in collagen biomaterials may be beneficial for a number of tissue engineering applications. For example, accelerated repopulation of engineered skin grafts by host fibroblasts, endothelial cells, and epithelial cells is often a goal for wound healing therapies. Enhancing migration of host Schwann cells into peripheral nerve grafts can provide an improved trophic environment and guidance cues for regenerating axons. Herein, we have shown that RDG, a scrambled version of the bioactive peptide sequence RGD, is capable of impeding cell adhesion to collagen matrix and enhancing cell migration. Typically, RDG is used as a non-acting control peptide for experiments with soluble RGD. However, immobilization of RDG to the collagen backbone limits the binding of integrins to the otherwise sticky collagen, which undermines natural cell-collagen interactions. This results in a weaker fastening of cells to the matrix. Indeed, if excessive scrambled peptide is grafted, cell adhesion is eliminated– and thus, all traction-mediated behavior, including gel compaction and cell migration are also eliminated. We would expect similar results for any non-adhesive peptide.

By exploiting the biphasic relationship between cell adhesion and migration from a biomaterials perspective, we can avoid inclusion of soluble factors to affect cell adhesion – which often entails complex, drug-releasing materials or transfection of cells – can be avoided. Incorporating non-adhesive peptide sequences on to collagen is a simple, yet elegant means of manipulating traction-mediated behavior.

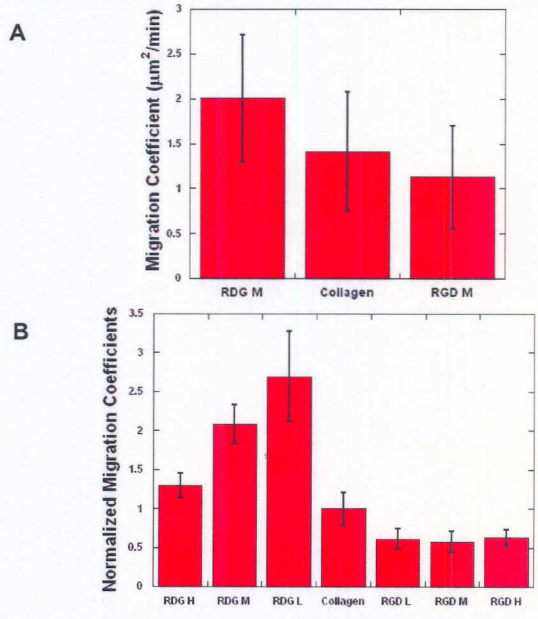

Figure 3: Decreasing cell-matrix adhesion leads to an increased cell migration within collagen matrices. (A) Cell migration is enhanced in collagen gels grafted with non-adhesive RDG and is decreased in gels grafted with adhesive RGD. (B) Cells exhibit a biphasic response to increasing adhesion strength, with highest migration coefficients at intermediate levels of extracellular matrix adhesion.

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REFERENCES

1. Gupton, S.L. and C.M. Waterman-Storer, Cell, 2006. **125**(7): p. 1361-74.
2. Palecek, S.P., et al., Nature, 1997. **385**(6616): p. 537-40.
3. Palecek, S.P., et al., J Cell Sci, 1998. **111 (Pt 7)**: p. 929-40.

Regulation of Embryoid Body Germ Layer Formation in Tunable Collagen Scaffolds.

Gary Monteiro, D.I.Shreiber

Embryoid body (EB) culture is a standard technique for differentiating embryonic stem cells in vitro. Previously, we demonstrated that the growth of EBs was significantly different in 3D collagen scaffolds vs. on that same scaffold. In this study, we investigated the role of 3D matrix stiffness on differentiation of cells within EBs into ectodermal cells, which include neurons and glia that are critical for reversing the effects of traumatic brain and spinal cord injury. EBs were entrapped in self-assembled type I collagen gels and incubated in 0, 0.1, 0.5, or 1mm genipin (a cell-tolerated crosslinking agent) for 12 hrs, which results in over an order of magnitude increase in gel shear modulus. EBs were harvested after 9 days of incubation in collagen, cryosectioned, and immunolabeled for nestin, which is a marker for ectoderm lineage cells such as pancreatic and neural progenitors. EBs cultured in collagen scaffolds cross linked with 1mM genipin had the greatest number of nestin-positive cells, which were clearly demarcated in a ring just inside the perimeter of the EB. EBs cultured in 0.5mM genipin had lower and less organized staining of nestin positive cells, while, EBs cultured in 0 and 0.1mM genipin had almost no nestin-positive cells. This study points to simple means of manipulating the culture environment to drive specific differentiation, and we intend to study the influence of stiffness on differentiation into other germ layers. Sponsored by the NJ Commission for Spinal Cord Research, the Charles and Johanna Busch Foundation, and a J&J Discovery Award.

2D Vs 3D Collagen Environment for Embryoid Body Formation

Traditional 2D tissue culture (TC) methods have demonstrated that adhesive cues from extracellular matrix (ECM) molecules significantly influence growth and differentiation of embryonic stem cells (ESC). Although they provide models for investigating aspects of ECM-cell interaction, they do not replicate the 3D environment experienced by embryoid cells, which is critical when studying embryoid body (EB) formation and differentiation. We hypothesize that a 3D ECM scaffold will present distinct adhesive and mechanical cues from a 2D, ECM-coated surface, leading to potential differences in growth rate, EB formation/cell aggregation, and ultimately differentiation of ESCs. In the present study Day 2 murine EBs, cultured by standard hanging drop techniques, were grown either on TC plastic, on TC plastic coated with type I collagen, in a 3D collagen scaffold, or maintained in the hanging drop. EBs plated on collagen-coated and uncoated surfaces flattened and increased in diameter and exhibited pronounced migration of differentiated cells into the intermediate zone. The intermediate zone was larger on uncoated plastic. In contrast, EBs grown in collagen remained spherical with negligible cell outgrowth and had a larger diameter than EBs maintained in hanging drops. These results suggest that a tissue-like environment influences cell aggregation and the time course of differentiation, and presents opportunities to engineer differentiation pathways via control of the 3D environment. Sponsored by the New Jersey Commission for Spinal Cord Research (05-2907-SCR-E-0).