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MODULATING THE ADHESIVE PROPERTIES OF COLLAGEN TISSUE EQUIVALENTS

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INTRODUCTION

Type I collagen-based 'tissue equivalents' comprising tissue cells entrapped in a collagen fiber network have proven to be vital analogs for studying aspects of cell-tissue interactions in controlled, 3D, in vitro culture systems [1, 2], and form the basis for many bioartifical tissues, including Apligraf™, which was the first 'living' tissue engineered product to receive FDA approval. The mechanical properties and cell adhesion profile of collagen, in 2D and in 3D, combine to dictate phenotypic behavior of cells, such as migration, traction, proliferation, and apoptosis through mechanotransduction mechanisms [3, 4]. The adhesion density and stiffness act together to affect the force balance that exists between an adherent cell and the surrounding substrate [5]. The functionality of these collagen-based engineered tissues can be enhanced by controlling the density and type of cell adhesion. By spatially controlling the adhesiveness of the biomaterial, we can exercise fine control over the distribution and types of cells that attach, and therefore regulate functions such as attachment, migration, differentiation, traction, and apoptosis.

Herein, we report on efforts to alter collagen gels to suppress cell adhesion. As a natural and ubiquitous ECM molecule, collagen supports adhesion of most tissue cell types, making it difficult to control adhesion-mediated behavior without genetic modification of cells or continuous addition of soluble factors that alter the nature, density, and distribution of integrin receptors. For example, decreasing cell adhesion by competitive inhibition of RGD binding sites with soluble peptide in collagen gels increases cell migration [6]. Our goal is to achieve the same effect via modulation of the collagen molecule. Specifically, we are interested in altering the adhesivity of collagen without affecting its ability to self-assemble into a fibrous network by grafting molecules to the collagen backbone prior to self-assembly, so that the same general procedure for forming a tissue equivalent can be followed [6]. As such, we will not alter the structure of the gel, and therefore influence mechanotransductive pathways only through the nature and density of cell adhesion. We have previously shown that we can increase cell adhesion without influencing mechanical properties by grafting RGD peptides to the collagen [7]. We present results for albumin, a serum protein known to limit cell adhesion, and a scrambled version of the RGD (G*RDG*S) bioactive peptide sequence.

MATERIALS AND METHODS

Collagen gels: Collagen gels were prepared by mixing 20 µl 1M Hepes buffer, 175 µl 0.1N NaOH, 100µl of 10X MEM, 17 µl of M199, 1 µl of pen-strep, 10µl of L-glutamine, and 677 µl of 3mg/ml Type I Collagen (BP Biomedicals) in order to make a 2.0 mg/ml collagen solution. The collagen solution self-assembles into a gel upon incubation at 37°C.

Modifying adhesion of collagen gels: Peptides and proteins were grafted to type I collagen in solution using a carbodiimide heterobifunctional linker, 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) (Sigma). A 1:1 mixture of 1M EDC and grafting molecule was incubated at room temperature overnight. The EDC/peptide/protein solution is added to the normal collagen recipe above, replacing M199 so as to maintain a final collagen concentration of 2.0mg/ml. The final concentration of the grafting molecules is 25µM for RGD or GRGDS and 125µM for albumin. This stock solution of grafted, type I collagen was mixed in different ratios with untreated, 'native' type I collagen solutions to establish solutions with intermediate levels of grafted molecules. The structure of the gels was evaluated with scanning electron microscopy (SEM).

Adhesion assay: Grafted/ungrafted collagen solutions (250µl) were pipetted into wells of a 24-well plate and allowed to self-assemble. L929 fibroblasts were seeded on the gels and allowed to settle and attach for 5 hours. Each well was washed twice with PBS. The re-

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maining fibroblasts were stained with Calcein AM and counted spectrofluorimetrically.

Compaction Study: The influence of cell adhesion on gel compaction was evaluated in 150µl free-floating, disc-shaped tissue equivalents formed by entrapping rat dermal fibroblasts (100,000 cells/ml) in collagen gels in a poly(dimethyl siloxane) (PDMS) annulus (9.5mm ID, 4.5mm height). The collagen does not adhere to the PDMS and is easily removed after self-assembly to create a free-floating gel. Disc area was measured periodically during incubation in 300µL of medium (DMEM, 10% FBS, 1% pen-strep and 1% L-glutamine) for 6 days.

RESULTS

SEM images indicated that peptide-grafted gels (RGD or RDG) are similar to untreated collagen gels, but albumin-grafted gels demonstrate decreased fiber thickness and increased porosity (Fig 1). Grafting either albumin or RDG decreased cell adhesion significantly (ANOVA, $P<0.05$). RDG decreased cell adhesion in a dose-response manner, while the response to albumin began to recover at higher concentrations (Fig 2). Gel compaction decreased in RDG-grafted collagen (ANOVA, $P<0.05$), and was virtually eliminated for the 12.5µM GRDGS gels, but was unaffected in albumin-grafted collagen (Fig 3).

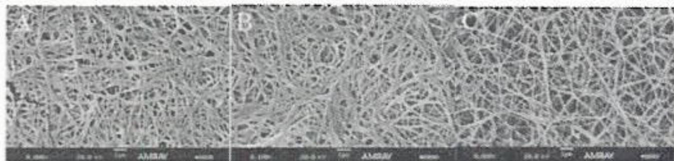

Figure 1: SEM images of collagen (A), 25µM RGD-grafted collagen (B), and 125µM albumin-grafted collagen (C)

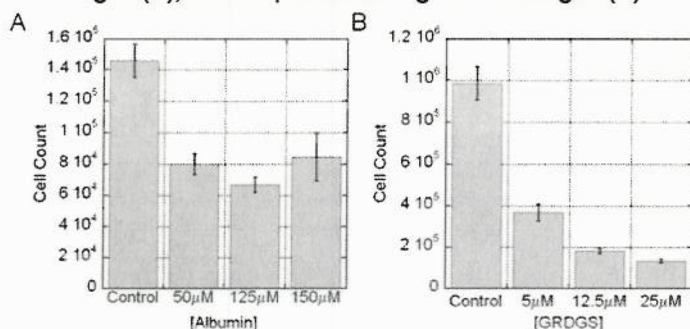

Figure 2. Effects of Albumin (A) and RDG (B) grafting to collagen on fibroblast adhesion

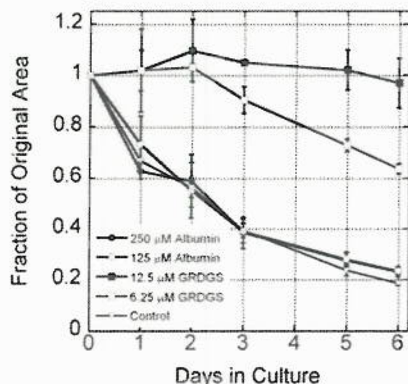

Figure 3. Effects of Albumin and RDG grafting to collagen on free-floating collagen gel compaction

DISCUSSION

As expected, grafting molecules that do not support cell attachment to a soluble collagen backbone significantly decreased cell adhesion to the collagen following self-assembly. The RDG decreased cell adhesion in a dose-response fashion, but cell adhesion recovered in albumin-grafted gels at high concentrations of albumin. Increased cell adhesion is generally associated with an increased ability for the cell to exert traction on its surroundings, in this case a free-floating collagen gel. The results for RDG-grafted collagen, where increasing the concentration of immobilized RDG decreased the gel compaction, are consistent with this hypothesis. However, compaction of albumin-grafted gels was unchanged compared to controls, despite the decrease in cell adhesion to these gels.

Compared to the peptide fragments (<1kD), albumin is a relatively large molecule (~70kD). The large size increased the likelihood that higher concentrations of albumin would interfere with self-assembly of the collagen network, as is suggested by the SEM images. The decrease in fibril size and associated increase in porosity permits passive penetration of cells into the gel. These cells are not easily washed away during rinses, which would effectively increase the measured number of attached cells in the adhesion assay. The altered gel microstructure also likely has a profound effect on the rheology, decreasing the effective stiffness of the gel. Decreasing cell adhesion will decrease cell traction; thus, for the same gel microstructure and mechanical properties, decreasing cell adhesion will decrease gel compaction, as was seen with the RDG-grafted collagen. However, exerting the same amount of traction in a more compliant gel will result in greater compaction. Thus, the decrease in cell adhesion and altered microstructure in the albumin-grafted collagen may effectively offset each other to result in compaction similar to control cases.

We now maintain the ability to selectively modulate positively and negatively the adhesion of cells within a 3D collagen gel, which greatly enhances our capacity to tune these gels for specific tissue engineering applications. We are combining these techniques with our microfluidic technology to spatially pattern of permissive and non-permissive regions in a 3D collagen gel for guided nerve regeneration [7]. We believe the techniques described herein have great potential for other tissue engineering applications, such as improved dermal replacements, where migration of several cell types into the wound site is critical.

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