THE MIGRATION OF HUMAN ANTERIOR CRUCIATE LIGAMENT FIBROBLASTS INTO POROUS COLLAGEN-GAG MATRICES IN VITRO

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INTRODUCTION

The migration and contractile behaviors of fibroblasts have been found to be important determinants of the wound healing processes in many connective tissues. The migration of cells from explants of the human and animal anterior cruciate ligament (ACL) has previously been investigated in two-dimensional (2-D) culture [1, 2]. One objective of our work was to evaluate the migration of fibroblasts from explants of intact and previously ruptured human ACLs into a porous collagen-glycosaminoglycan (CG) matrix to study migration in a three-dimensional (3-D) scaffold and to assess the suitability of the material as a potential implant to facilitate regeneration *in vivo*. Another objective was to determine if one particular phenotype was more prone to migrate than another. Specifically, we investigated the presence of the α-smooth muscle actin (SMA) isoform, associated with the contractile myofibroblast phenotype [3], and previously identified in the healing lapine medial collateral ligament [4].

MATERIALS AND METHODS

Six intact anterior cruciate ligaments were obtained from women undergoing total knee arthroplasty, ages 40 to 78 years. Four ruptured ACLs were obtained from patients undergoing ACL reconstruction. Each ligament was sectioned into seven fascicles that were divided transversely in the midsubstance to make explants. One fascicle was reserved for histology, while the remaining six fascicles were split into control and experimental groups. Each intact fascicle was divided in the midsubstance and a biopsy section from each plated onto a petri dish for 2-D culture. Both experimental and control acutely transected constructs were made by suturing the fascicle lengths to silicon tubing. Experimental constructs had CG scaffolding placed in the gap between fascicle lengths, while control constructs had the ends of the fascicle reapposed. The chronically ruptured constructs were made using the ruptured ACLs. Each ACL was divided into three sections, based on previous histology which demonstrated three distinct cellular zones in the ruptured ACL. Four explants from each region were used for 2-D culture onto petri dishes and four for 3-D culture on CG matrix.

The highly porous CG matrix, composed of type I bovine hide collagen and chondroitin-6-sulfate, was prepared by freeze-drying the CG dispersion under specific freezing conditions (5). The average pore size of the CG scaffold was 100 μm. The 36 acutely transected constructs and 48 ruptured constructs were cultured in media containing Dulbecco's DMEM/ F12 with 10% fetal bovine serum, 2% penicillin streptomycin, 1% amphotericin B, 1% L-glutamine and 2% ascorbic acid. An experimental and control construct from each ligament were fixed in formalin at two, four and six weeks, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Immunohistochemistry using monoclonal antibodies to detect SMA was also performed[(6), Sigma Chem.]. Cell counts were taken at the edge of the scaffold for a cell density measure and the furthest distance traveled from the tissue/scaffold interface recorded for each sample.

RESULTS

2-D Culture Outgrowth onto the 2-D surfaces began as early as six days for the intact ligaments, and as early as 2 days for the ruptured ligaments. The average time to initial outgrowth was 10±0.5 days (n=30) for the intact ligaments and 8±2 days (n=8) for the ruptured ligaments (not statistically significantly different). The rate of outgrowth was 0.23 mm/day based on a linear regression analysis of the radial outgrowth distance vs. time (coef. of determination, 0.98; n=24) for the intact ligament explants and 0.25 mm/day for the samples from ruptured ligaments (coef. of determination, 0.92; n=5).

3-D Culture Outgrowth into the 3-D scaffolds was found for both the intact and ruptured ligaments. The intact ligaments had a cell density of 123±45 cells/mm^2 (n=6) at 2 weeks and 174±88 cells/mm^2 (n=5) at 4 wks. The ruptured ligament explants demonstrated a cell density of 93±43 cells/mm^2 at 2 weeks (n=11) and 241±96 cells/mm^2 at four wks (n=10). The explants taken from the hypercellular region of the ruptured ligaments demonstrated the highest cell density of migration into the CG scaffold with 233±120 cells/mm^2 at 2 wks (n=3) and 413±297 cells/mm^2 at four wks (n=3). Of note, 2 of the 8 remaining explants from the hypercellular region had completely degraded the CG scaffold at three wks and a third had degraded the scaffold at four wks. The cell density was least in the center of the explants at all time points. The cellularity at the edges of the explants were noted to increase at the 2 and 4 week time points. After two weeks in culture, fibroblasts in the explants began to display changes in morphology, with cells in the periphery becoming rounder.

Anterior cruciate ligament tissue examined immediately after retrieval demonstrated wide variability in the percentage of cells that stained positive for SMA (0 to 100%). In general, a greater percentage of such cells were found in the midsubstance of the intact fascicles. With time in culture, the explanted tissue gradually developed a higher percentage of SMA-positive cells at the periphery of the explant. The areas displaying the greatest number of positive cells appeared to correspond to the areas of disrupted ligament architecture. All cells that migrated into the CG scaffold stained positive for SMA.

DISCUSSION

These findings show the potential for fibroblasts to migrate from the transected surface of previously intact and ruptured human ACL into CG scaffolds. Of interest is the observation that the cells of the hypercellular zone of the ruptured ACL, that occurs adjacent to the rupture site, displayed the earliest migration from the ligament tissue and yielded the highest cell densities. Another notable finding is that all cells which successfully migrated were positive for the SMA isoform, suggesting a role for this protein in cellular migration as well as in contraction.

The ability of human ACL fibroblasts to migrate from their native extracellular matrix after transection is an important step in determining the etiology of the failure of the ACL to heal. This suggests that the fibroblasts of the human ACL are capable not only of proliferation but are capable of migration to an adjacent scaffold as well. This suggests the failure of the ACL to heal may be due to a defect in the bridging scaffold which forms after injury, or a lack of formation of such a scaffold.

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