INTRODUCTION
Guided cell migration has important implications in tissue repair and engineering. Extraarticular ligament fibroblasts have been shown to migrate faster than intraarticular ligament fibroblasts, reflecting the respective healing rates [1-3]. Substrate modification with collagen has been reported to enhance ligament cell migration [4]. Previously, we reported electric-field (EF) induced cell migration (galvanotaxis) and reorientation (galvanotropism) in chondrocytes and lateral collateral ligament (LCL) fibroblasts [5,6]. In the current study, we characterized the migration of fibroblasts from anterior cruciate and medial collateral ligaments (ACL and MCL) in an applied EF, and examined the differential migration behavior on different substrates.

METHODS
Cell culture. Primary ligament fibroblasts were harvested from explant cultures of young calf ACL, MCL, and LCL. Primary chondrocytes were isolated via enzymatic digestion from young calf cartilage from the knee and plated at high density prior to use. Electric field studies. Cells were placed in a modified parallel plate flow chamber system as previously reported [5] after one hour of plating on glass slides. To study the effect of substrate on EF-induced migration, ACL and MCL fibroblasts were also plated on 0.84 µg/cm² (0.2%) type I collagen (Collaborative Biomedical Products)-coated slides for one hour. Cells were subjected to an applied DC EF at a field strength of 6 V/cm for one hour and were digitally recorded with time-lapse microscopy. Migration distance and angle (θ) were quantified with Metamorph software (UIC) and used to calculate the migration speed, sin(θ), and the directional velocity (a measure of migration speed in a single direction relative to the positive pole, for example, a cell that moved directly to the cathode at the speed of 1 µm/min would have θ=270° and sin(θ)= -1 with the directional velocity of -1 µm/min). Statistical analyses were performed with STATISTICA (StatSoft) using one-way ANOVA and post-hoc tests with α<0.05.

RESULTS
All cell types exhibited cathodal migration and significant increases in migration speed in response to an applied EF compared to their no-field controls (Fig 1A, [5,6]). While migration speed for LCL fibroblasts (LCLF) was significantly greater than that for the other cell types without an applied field, the migration speeds of MCL and ACL fibroblasts (MCLF and ACLF) were higher than LCLF after exposure to EF (p<0.05 for MCLF, ACLF, and MCLF also showed significantly higher directional velocities than LCLF and chondrocytes (Fig 1B). Both ACLF and LCLF migration speeds were increased significantly on type I collagen with or without EF (Fig 2A). Under EF, MCLF had significantly greater migration speed than ACLF on collagen, whereas in directional velocity, there was no difference between MCLF and ACLF (Fig 2B). Polar plots of the ACLF and MCLF translocation (Fig 3) revealed that directionality of ACLF was less than that of MCLF on glass. However, on collagen, ACLF had slightly greater directionality, as evident from the sin(θ) values: -0.18±0.74 for ACLF (n=150) and -0.64±0.55 for MCLF (n=207) on glass (p<0.05) and -0.31±0.73 for ACLF (n=196) and -0.21±0.74 for MCLF (n=210) on collagen. Examination of cell morphology showed that while both ACLF and MCLF aligned perpendicular to the applied EF after one hour, ACLF were more elongated than MCLF and there was less elongation and more random orientation for both cell types on type I collagen-coated slides (Fig 4). Both cell types exhibited similar random orientation without EF (not shown).

DISCUSSION
Tissue repair involves cell migration into the wound site. The different healing rates of ACL and MCL tissues may be a reflection of differences in cell motility [1-3]. In this study, we demonstrated that an applied EF enhances the migration speed and directionality of both ACL and MCL fibroblasts, and that their motility is further improved by coating the substrate with type I collagen. The variations in directionality under the applied EF appeared to correspond with an observed disparity in cellular morphology. ACL fibroblast migration into collagen-coated scaffolds has been suggested to facilitate ligament healing and regeneration [7]. The ability to augment and direct cell migration has potential to impact strategies for addressing tissue healing/repair and for scaffold seeding applications.

REFERENCES

ACKNOWLEDGEMENT
This work was supported by the Whitaker Foundation.