Connective Tissue Research, 48:188–197, 2007

ISSN: 0300-8207 print / 1521-0456 online
DOI: 10.1080/03008200701424451

informa
healthcare

Effects of Applied DC Electric Field on Ligament Fibroblast Migration and Wound Healing

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Applied electric fields (static and pulsing) are widely used in orthopedic practices to treat nonunions and spine fusions and have been shown to improve ligament healing in vivo. Few studies, however, have addressed the effect of electric fields (EFs) on ligament fibroblast migration and biosynthesis. In the current study, we applied static and pulsing direct current (DC) EFs to calf anterior cruciate ligament (ACL) fibroblasts. ACL fibroblasts demonstrated enhanced migration speed and perpendicular alignment to the applied EFs. The motility of ligament fibroblasts was further modulated on type I collagen. In addition, type I collagen expression increased in ACL fibroblasts after exposure to pulsing EFs. In vitro wound-healing studies showed inhibitory effects of static EFs, which were alleviated with a pulsing EF. Our results demonstrate that applied EFs augment ACL fibroblast migration and biosynthesis and provide potential mechanisms by which EFs may be used for enhancing ligament healing and repair.

Keywords ACL Fibroblast, Collagen Synthesis, Galvanotaxis, Wound Healing

INTRODUCTION

The phenomena of galvanotaxis and galvanotropism (electric fields [EF]-induced cell migration and morphological change, respectively) have been studied to provide a greater understanding of development and wound-healing processes [1–3]. Many cell types have been shown to demonstrate a galvanotactic response, including embryonic somatic fibroblasts, corneal fibroblasts, keratinocytes, and neural crest cells [2–6]. An electrical gradient of 1–5 V/cm can be found in embryonic tissue or wound sites [2, 7–9]. Disruption of this gradient in embryos results in failed development [10, 11].

Applied EFs have been shown to improve healing of diabetic foot ulcers and lapine ligaments in vivo [12, 13] as well as in vitro wound models of cornea epithelial cells and 3T3 fibroblasts [14, 15]. Manipulation of endogenous EFs has been shown to regulate the rate of cornea epithelial wound healing [14]. Most studies demonstrating in vivo healing capabilities of applied EFs have used pulsing frequencies, however, few studies have examined effects of pulsing EFs on cell migration in vitro [16, 17].

Applied electric and electromagnetic fields are used widely in orthopedic practices to treat fracture nonunions and spinal fusions [18–23], despite the fact that their mechanisms of action are not understood. Several studies have shown that applied EFs stimulate cell proliferation and differentiation, as well as synthesis of growth factors and matrix proteins [24–27]. The potential of electrical stimulation for soft tissues also has been explored in cartilage and ligaments. Lippielo and co-workers [28] have shown that an applied pulsing direct current (DC) EF enhances the repair of osteochondral defects. EF application also has been reported to yield beneficial results in rabbit and rat ligament healing [13, 29, 30].

In the present study, we investigated the migratory response of fibroblasts derived from the ACL to an EF (E = 0–6V/cm, [31]), applied in a static or pulsing manner. The ACL is an intra-articular ligament that is often torn in sports-related injuries and is critical in stabilization of the knee joint [29, 32]. We have chosen to study the ACL because it exhibits poor healing capacity after injury [29, 32, 33]. To evaluate the effects of substrate, which influences cell motility [15, 34–38], we performed studies on cell adhesion and mobility

Received 1 February 2007; accepted 27 April 2007.
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with ACL fibroblasts cultured on varying concentrations of type I collagen, a major matrix constituent of these tissues. As such, the relationship between cell migratory behavior (speed and directionality) with EF application and cell attachment was investigated. We also assessed cell alignment, cytoskeletal reorganization, and the wound-healing response in vitro to applied EFs. Our findings may aid efforts in ligament tissue engineering and may lead to the development of new strategies for ligament repair.

METHODS AND MATERIALS

Cell Culture

ACLs were harvested from freshly killed 4–6-month-old calves. The response of ACL fibroblasts were compared with those of articular chondrocytes, which have been shown to demonstrate directed migration in applied EF [31]. ACLs were dissected into small cubes $\sim$1 mm^3 and incubated in Dulbecco's Modified Essential Medium (DMEM, Mediatech), supplemented with 10% FBS, until the fibroblasts had grown out to confluence. Prior to experiments, cells were released and plated at 2.7×10^4 cells/cm^2 on a glass slide for 1 hr for *fresh* and 2.7×10^5 cells/cm^2 overnight for *wound* studies. A wound was created by scratching a cell-free area ($\sim$150 μm wide) into a confluent monolayer [39].

Collagen Substrate Coating and Cell Culture

To study the effect of a physiologically relevant extracellular matrix molecule on attachment strength and migration, cells were cultured on a native substrate of type I collagen [40]. Sterile glass microscope slides were incubated with type I collagen (Collaborative Biomedical) for 1 hr at 0–8.4 μg/cm^2. Coating was confirmed with immunofluorescence staining with a monoclonal type I collagen antibody (Rockland) and imaged with fluorescence microscopy. Fluorescence intensity was uniform within the field of view and correlated with collagen coating concentration (Figure 2C, $R = 0.977$, $p = 0.022$, $n = 3$ for each concentration).

Electric Field Studies

Cells were placed in a modified parallel-plate flow chamber system as previously reported [31]. In brief, the glass slide was placed in the device with a silicon spacer to form the enclosed chamber (length: 5×10^{-2} m, width: 1.3×10^{-2} m, and height: 2.5×10^{-4} m). The well-defined chamber geometry permits a uniform EF and calculation of field strength (E) and current density. The galvanotaxis chamber was connected to phosphate buffered saline (PBS) reservoirs via Ag-AgCl electrodes and a pair of agarose salt bridges, then placed on an inverted microscope (Olympus CK40). A power supply (Keithley) was used to apply a DC current at 0–0.3 mA, resulting in E = 0–6 V/cm. Cells were subjected to the field strengths of 0, 2, and 6 V/cm for 1 hr for subconfluent cells and 3 hr for wound studies (perpendicular to wound) at 37°C. Effects of pulsing field were also studied at 0, 10, 20, 40, and 60-sec pulsing (on/off) intervals with E = 2 V/cm. The range of applied field strengths was adapted from previous studies [4, 6, 31, 41]. Approximately 30–50 cells were observed in a given field of view, which was digitally recorded every 10 min using MetaView (Molecular Devices).

Migration Analysis

Migration distances of cells were analyzed by manually selecting the centroid of each cell and tracking its position. The average migration speed was calculated as {net migration distance} / {duration of migration}. The migration direction was quantified as percentage of cells migrating toward the cathode at the end of the experiment, which was the ratio of {cells in the quadrant facing the cathode} / {total number of cells}, where the cathode-facing quadrant is between 225° and 315°, represented by the shaded region in Figure 1C. A randomly migrating population would have 25% of the cells in this region. For wound-healing models, images were processed with a custom Matlab program to track the edges of wounds for calculating the wound-closing speed, which accounts for both sides of the wound. To facilitate analysis, images in wound experiments were evenly subdivided perpendicularly into 5 regions ($\sim$4–5 cells/region) where average gap distances in each region were tracked.

Fluorescence Imaging

To investigate cytoskeletal changes in response to the different substrates and applied fields, we examined cytoskeletal and cell surface markers. After 2 hr of experiment, Texas Red-conjugated concanavalin A (Invitrogen) was used for labeling cell surface glycoproteins, and Oregon Green 488-conjugated phalloidin was used for actin. An Olympus IX-70 microscope with a FluoView confocal system was used with a 60X objective.

Adhesion Strength Assay

Forty-five min after cell plating, the slide was placed into a parallel plate flow chamber as described above. The chamber permits the shear stress acting at the chamber wall (and cells) to be calculated as a function of the laminar flow rate. Flow rate was controlled by a pulse-free pump (Scilog ACCU MP-30, Fisher). Flow rate from 0 to 2 mL/s with 0.1 mL/s increments were applied with the corresponding shear stress calculated to be 0 to 800 dyn/cm^2 with 40 dyn/cm^2 increments. PBS containing Ca^{2+} and Mg^{2+} was used as the flow medium; the chamber and medium were maintained at 37°C. Cells on the slide were viewed using the microscopy system described above and images acquired at the end of each shear interval. In this manner, the percentage of detached cells as a function of shear

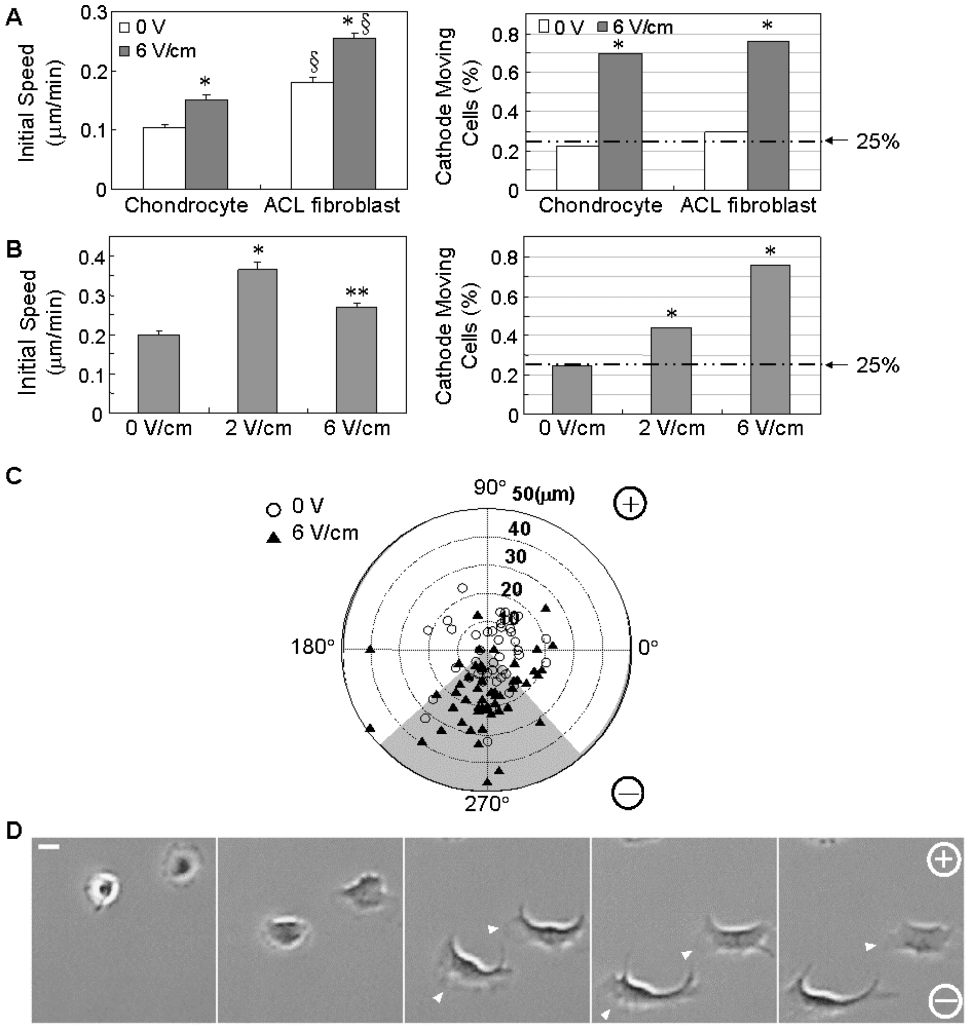

FIG. 1. (**A**) Initial migration speed and directionality of primary chondrocytes and anterior cruciate ligament (ACL) fibroblasts migrate in response to EF. Left: Average initial migration speed was calculated for the first hour of migration using cell centroid displacement (cell diameter ~10 μm). Right: For cathode-migrating cells, dotted line indicates 25%, i.e., random migration ($^{*}p < 0.01$ vs. respective 0 V/cm no-field control group, $^{\S}p < 0.05$ vs. other corresponding groups, $n =$ 151–238 cells). (**B**) ACL fibroblast migration in response to applied EF strengths of 0–6 V/cm. ($^{*}p < 0.001$ vs. 0 V/cm no-field control group, $^{\S}p < 0.01$ vs. 2V/cm group, $n =$ 149–219 cells, error bars in all graphs represent standard error of the mean). (**C**) Polar plots of representative ACL fibroblast translocation with shaded areas represent cathode migrating cells. Cells that do not move over the duration of the experiment remain at the center (radius = 0, angle:0). (**D**) Representative time-lapse image of ACL fibroblasts migrating in 6V/cm EF for 2 hr, Scale bar = 15 μm.

stress was obtained. Cell adhesion strength was determined from the shear stress level at which half of the cells detached.

Gene Expression Studies

ACL fibroblasts were subjected to EFs as described above and harvested with TRIzol reagent (Invitrogen). Total RNA from each experiment was reverse transcribed with SuperScript III (Invitrogen). GAPDH (accession no. **U85042**) and type I collagen (accession no. **AB008683**) levels were quantified using an iCycler real-time PCR system and SYBR green supermix (Bio-Rad). Primers were designed with Beacon Designer (Premier Biosoft) or Primer3 [42]. Duplicates of 20 μL reactions were performed for each sample. Type I collagen mRNA levels were calculated using the -$\Delta\Delta C_T$ method [43] and normalized to GAPDH and control levels (no EF).

Statistical Analysis

Statistical analysis were performed with Statistica (StatSoft) using ANOVA and LSD *post-hoc* tests or χ^2 test when appropriate ($\alpha = 0.05$). Error bars represented standard errors of the mean.

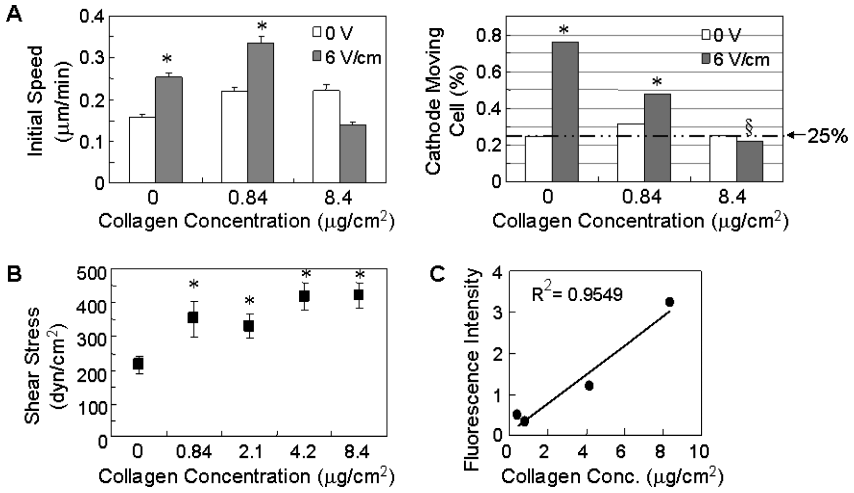

FIG. 2. (**A**) Initial migration speed and directionality of ACL fibroblasts on type I collagen-coated substrates. (*$p < 0.001$ vs. respective 0 V/cm no-field control group, §$p < 0.001$ vs. 0 μg/cm^2 collagen group, $n = 149$–325 cells). (**B**) ACL fibroblast attachment strength, as a function of substrate collagen coating concentration (*$p < 0.02$ vs. 0 μg/cm^2 group, $n = 3$–7 slides). (**C**) Scatter plot of type I collagen immunostaining intensity, as a function of substrate collagen coating concentration (R = 0.977, $p = 0.022$, $n = 3$ for each concentration).

RESULTS

Chondrocyte and ACL Fibroblast Migrate and Align in EFs

We found that with or without application of DC EF, the average initial speed (for the first hour of EF application) of the ligament fibroblasts exceeded that of chondrocytes. Figure 1A shows that in the presence of EF strength = 6 V/cm, motility of ACL fibroblasts was significantly enhanced compared with respective no-field controls (i.e., 0 V/cm) and chondrocytes ($n = 76$–325). When EF was applied, most fibroblasts migrated in the direction of the cathode, establishing pseudopodia and lamellipodia at their leading edges (Figure 1D, white triangles), similar to that observed for chondrocytes (see Figure 2 in [31]). After ~30 min, ACL fibroblasts started to elongate and align perpendicular to the EF. Alignment in response to the applied field was most prominent after 1 hr (Figure 1D, 3), faster than that of chondrocytes (see Figure 3 in [31]). ACL fibroblasts and chondrocytes demonstrated similar directionality in the applied field as indicated by the percentage of cells migrating toward the cathode (Figure 1B).

Type I Collagen Substrates Alters Migration and Adhesion of ACL Fibroblasts

To study the effect of substrates on ACL fibroblast migration, type I collagen was used to coat glass slides, at concentrations of 0, 0.84, and 8.4 μg/cm^2. ACL fibroblasts showed increased motility on both 0.84 and 8.4 μg/cm^2 collagen (Figure 2A). Under the influence of applied EFs, ACL fibroblasts migrated most rapidly on substrates coated with 0.84 μg/cm^2 collagen ($p < 0.001$ versus other groups, $n = 149$–325). However, EF-migration speed of ACL fibroblasts on the highest concentration of collagen (8.4 μg/cm^2) was slower than that of controls (0 V/cm or 6 V/cm on uncoated substrate). Directionality for the ligament fibroblasts was significantly reduced with both concentrations of collagen substrate. ACL fibroblasts showed reduced motility (as seen by lower migration speed compared with 0 V and no collagen 6 V/cm groups) and no directionality on 8.4 μg/cm^2 collagen substrates (Figure 2A). To test the hypothesis that the migration of ligament fibroblasts on collagen substrates is related to adhesion to the substrate, we measured adhesion strength directly, using a cell detachment assay in which we applied shear forces under conditions similar to those used in the migration studies.

As seen in Figure 2B, detachment strengths of ACL fibroblasts on glass and collagen substrates were measured between 191.6 to 447.0 dynes/cm^2, similar to values reported by

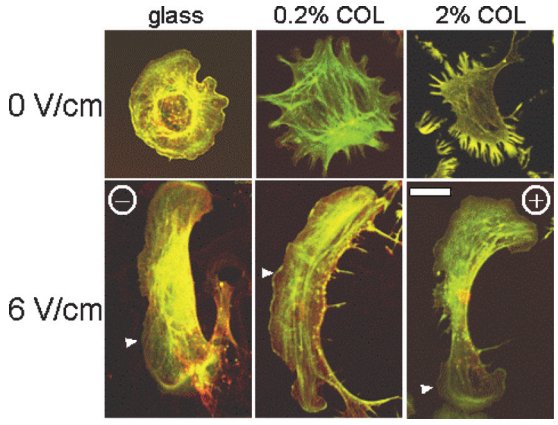

FIG. 3. Actin cytoskeletal organization (green) and alignment of ACL fibroblasts on glass and type I collagen-coated substrata after 1 hr motility with and without applied EF (0 and 6 V/cm). Concanavalin A staining (red) for cell surface glycoprotein distribution. Arrows indicate lamellipodia present at the leading edge of the cell. The cathode is to the left in all panels. Scale bar = 10 μm.

Yang et al. [36] using micropipette aspiration. ACL fibroblasts demonstrated a trend of increasing attachment, with increasing collagen concentrations, whereas adhesion to all collagen-coated surfaces was significantly greater than that on glass (Figure 2B).

Applied EF and Collagen Substrates Alter Cell Morphology

As shown in Figure 3, more actin structural organization was evident for the ACL fibroblasts plated on collagen substrates without applied field. In the presence of the EF, ACL fibroblasts aligned perpendicular to the direction of the field, as did their actin fibers. Concanavalin A staining revealed no polarized distribution of surface glycoprotein, as indicated by the red staining in Figure 3.

Applied Pulsing EF on Cell Migration and Type I Collagen Expression

To study the effect of an applied pulsing EF on migration, we subjected ACL fibroblasts to DC EFs with frequencies from 0 (static) to 0.008 Hz. Maximum migration speed in a pulsing EF was achieved at 0.025 Hz whereas no significant differences were found for directionality at any frequency (Figure 4). Compared with ACL fibroblasts exposed to static EFs, those exposed to pulsing EFs exhibited slower migration speed and less directionality and alignment (Figures 4B and 4C). To test the effect of EF on the phenotype, gene expression of type I collagen, a major ligament matrix component, was assayed using real-time PCR. Gene expression of ACL fibroblasts was altered by the applied EF. Type I collagen expression appeared to be slightly enhanced with applied static EF; after exposure to a pulsing EF at 0.025 Hz, type I collagen was significantly increased (Figure 5, $p < 0.05$).

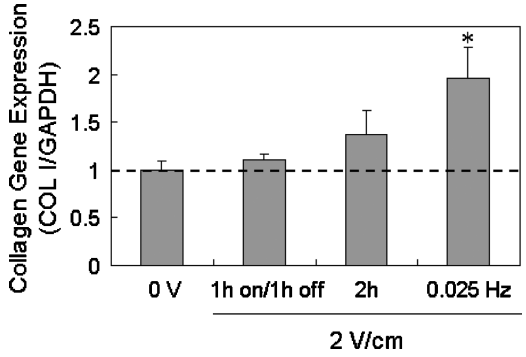

FIG. 5. Gene expression in ACL fibroblast after exposure to a EF for 2 hr at 2 V/cm. Type 1 collagen expression was normalized to constitutive expression of GAPDH (Col I/GAPDH). ($^*p < 0.05$ vs. 0 V/cm no-field group, $n = 10$–19 slides)

Applied Electric Fields Alter Wound Healing of Ligament Fibroblasts

Application of DC EFs to a wound-healing model in vitro resulted in reduced wound closing speed with applied EF (Figures 6 and 7). The pulsing fields group of 2 V/cm improved motility compared with constant fields. These cells did move into the wound but still migrated more slowly than no-field controls. Preliminary studies using 6 V/cm showed detrimental effects on cells; they detached from each other and/or the substrate, as previously observed by Finkelstein et al. [15]. In contrast, no such effects were observed for subconfluent cells plated overnight under the same condition (data not shown).

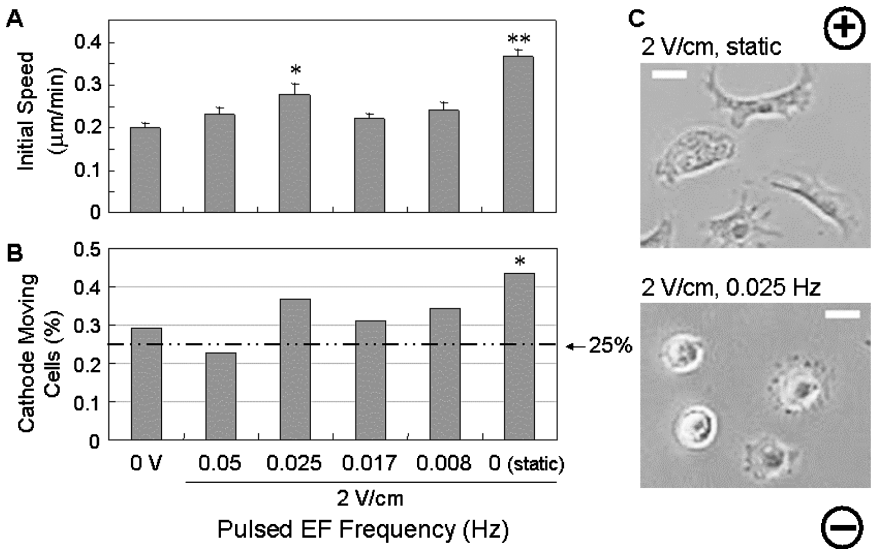

FIG. 4. ACL fibroblast migration induced by pulsing DC EF (2 V/cm). (**A**) Initial speed and (**B**) Percentage of cells moving toward the cathode are shown. ($^*p < 0.05$ vs. 0V group or as indicated, $^{**}p < 0.05$ vs. all other groups, $n = 149$–219 cells). (**C**) Representative images of ACL fibroblast morphology in static and pulsing EFs. Scale bar = 10 μm.

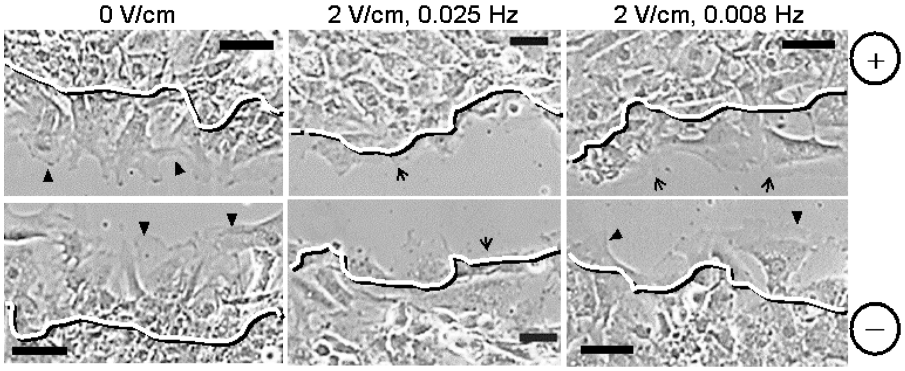

FIG. 6. Phase contrast images of ACL fibroblasts migrating in a wound-healing model for 3 hr. Shadowed line indicates each edge of the original wound edge at t = 0. EFs of 0–2 V/cm, at the indicated frequencies, were applied perpendicular to the wound. Triangles indicate cells migrating with perpendicular alignment relative to the wound (i.e., parallel to the applied EF); arrows indicate cells migrating with parallel alignment relative to the wound (i.e., perpendicular to the applied EF). Scale bar = 20 μm.

As shown in Figure 6, wound-edge cells in the control group (0 V/cm) extended into the wound site (triangles); that is, they were aligned perpendicular to the wound direction. In the 0.008 Hz group, while the anode-facing edge extended into the wound site, aligning perpendicular to the wound (parallel to the direction of the EF, triangles in Figure 6), the cathode-facing edge aligned parallel to the wound direction (perpendicular to the EF, arrows in Figure 6). In the 0.025 Hz group, no parallel alignment was observed for the anode-facing edge. Since alignment of dividing cells has been observed on EF exposure [14], it is important to note that no apparent cell division was observed for the duration of the experiment (3 hr).

DISCUSSION

ACL injuries are one of the most common knee joint problems, and they are complicated by poor healing of the ligament [29, 32]. The current study, in which we investigated the migratory response of ACL fibroblasts to applied DC EFs at E = 0–6 V/cm, bears on this issue. The range of EFs examined is similar to those in epithelial wounds that have been shown to elicit short term responses in chondrocytes [1, 31]. We found that ACL fibroblasts exhibited enhanced migration; their migration speed with and without application of EF was twice as fast as that of chondrocytes. In addition, ACL fibroblasts exhibited enhanced migration in pulsing EFs at 0.025 Hz, although pulsing EFs were not as effective as static EFs in inducing ACL fibroblast migration. Interestingly, maximal migration speed was achieved at the field strength of 2 V/cm for ACL fibroblasts. EF-induced cell displacements of nearly 1 cell diameter per hour were observed for ACL fibroblasts, similar to the EF-induced migration speed of other cell types including NIH3T3 fibroblasts and human keratinocytes (0.4–0.9 μm/min, [3, 15]). We also found that ACL fibroblasts aligned perpendicular to the direction of the applied EF, corresponding with the alignment of their actin. Our methodology provides single cell measurements with no complication from cell division given the short experimental times. The system is amenable to performing population experiments of cell migration using theoretical statistical analysis (i.e., Alt's Law [44]).

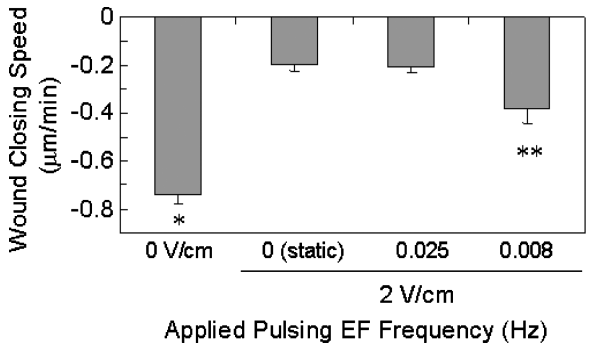

FIG. 7. Wound-closing speed of ACL fibroblasts in response to applied EFs (0–2 V/cm, frequency as indicated). (*$p < 0.05$ with others, **$p < 0.05$ with 0.025 Hz, n = 15–25 from 3–5 slides)

The galvanotactic response of various cell types is known to be modulated by the substrate [45], as well as the strength of the applied EF. In fact, we did find that type I collagen, a major ligament matrix protein, modulated EF-migration of ACL fibroblasts. Maximal migration speed was found at low collagen concentration, which corresponded to an intermediate level of measured cell attachment strength. This finding is in accord with previous reports in which moderate cell attachment strength favored cell migration due to integrin-substrate interactions [46–48]. Moreover, higher ligand densities have been shown to reduce directionality and migration speed [49], which may explain the reduced migratory response of ACL fibroblasts on substrates with higher collagen concentration. ACL fibroblast migration into collagen-coated scaffolds has been studied as a potential method to facilitate ligament healing [50, 51].

We also investigated the effects of applying pulsing (sequential on-off DC) EFs. ACL fibroblasts were increased in migration speed but unchanged in directionality at 0.025, perhaps indicating a frequency-dependent response. Furthermore, application of EFs at 0.025 Hz increased type I collagen gene expression in ACL fibroblasts, a result reminiscent of increases in matrix biosynthesis observed for other connective tissues subjected to pulsing EFs [24, 26, 52]. ACL fibroblasts also exhibited frequency dependence in the wound healing model, in which a significantly higher rate of wound closure was observed at 0.008 Hz (Figure 7). Either static or pulsing EFs have been utilized in orthopedic procedures, using surgically implanted electrodes or capacitive or inductive coupling [22, 23]. While the mechanisms mediating the beneficial effects of EFs in vivo are largely unclear [53], applied static EFs have been shown in vitro to reorient cell surface molecules [16, 54, 55] and induce or enhance migration. However, few studies have examined the effects of pulsing EFs on mammalian cell migration. For example, Franke and Gruler [56] reported that granulocytes exhibit directed movement in pulsing EFs, and their migration was enhanced when the pulsing interval was 8 sec (i.e., 0.125 Hz). Interestingly, pulsing DC EFs also have been shown to resonate with, and increase the amplitude of, nicotinamide adenine dinucleotide phosphate (NADPH) oscillations that promote neutrophil extension and locomotion [57]. Thus, it is possible that EF pulsing frequencies can be optimized to promote single cell migration or wound healing of ligament fibroblasts.

Wound healing involves a series of complex cell-matrix interactions, including migration of cells into the wound site, production of new extracellular matrix, and during the maturation phase, remodeling of matrix [58]. Common experimental models of cell migration include migration to a chemical gradient (chemotaxis) or migration into an artificially inflicted wound/scratch in a monolayer of cells. Insufficient cell migration has been implicated in delayed or poor healing ability such as in diabetes [59]; in vitro migration models might be useful to investigate the cause of insufficient motility. In our in vitro wound-healing experiments, EFs applied at 2 and 6 V/cm proved to be inhibitory to cell migration, compared with cells in the absence of EFs, in contrast to sparse single cells that were enhanced motility by the same applied EF. Furthermore, when EF was applied at 6 V/cm, cells near the wound edge would separate from each other and retract from the wound edge (data not shown), while sparse cells were enhanced in migration by this EF strength. Thus, we can conclude that ligament fibroblasts behave differently when subjected to EF as a confluent monolayer or as single cells.

Zhao and coworkers [60] have utilized corneal epithelium wounds with real-time imaging to reveal that cells migrate into a wound as a unified unit to heal a stratified epithelium. This phenomenon may be due to the monolayer of cells "sensing" the EF as a continuous single unit, owing to tight or gap junctions between cells that establish polarity and electrical connectivity that results in a voltage drop across the cell/monolayer [60]. The differences between single cell and wound-edge migration also may arise via other possible mechanisms. Previously, our group showed in 3T3 fibroblasts that application of weak EFs enhanced migration of randomly oriented cells in the wound-healing model and curtailed migration of preoriented cells [15]. While polarization of detyrosinated microtubules (Glu MT) was required for cell migration in the wound-healing model, inhibition of Glu MT was found to enhance EF-induced motility. This suggests that EFs mediate fibroblast migration via participation of microtubules and adhesive components, but that their involvement is different during motility in a wound environment. The differential alignment of the cells at the cathode-facing wound edges may support this notion although more studies are needed to delineate these associations.

We speculate that applied DC electric fields can serve as the basis for a treatment modality to improve healing of injured ligaments (as was more recently suggested for general tissue healing in medicine by Huttenlocher and Horwitz [61]) or indirectly as a tool that can be incorporated in strategies for ligament tissue regeneration. Time-varying (e.g., sinusoidal waveforms) electric and electromagnetic fields (of magnitudes generally much smaller magnitude and for longer periods than those described in in vitro galvanotaxis studies) are more commonly described in reports of clinical studies, as they have been reported to elicit frequency-dependent alterations in cell activities, including cell proliferation and biosynthesis [22, 62]. Direct current applications have been described in clinical studies as a means of promoting osteogenesis and healing of nonunion fractures and spinal fusions [22]. There is limited information available, primarily derived from culture and animal studies, regarding the effects of electric field stimulation on tendons and ligament healing [13, 30].

In the current studies, we did not identify pulsing EF conditions that caused cells to migrate more rapidly. However, we did find that cells increased their production of collagen and that cells migrated more rapidly when collagen was present. Thus, a possible mechanism by which electric fields exert in vivo effects on motility speed might be attributable to an interplay of these two effects. In addition, we found that electric fields altered cell morphology; since ligament fibroblasts are aligned parallel to each other (with the fiber structure) in situ, the ability to confer an elongated cell morphology using electric fields is particularly relevant for ligament engineering [63]. The ability to direct ligament fibroblast migration using DC electric fields may provide a means of in vitro cell seeding and spatial manipulation of cells within scaffold systems that are designed for engineering of ligament replacement tissues. The application of DC electric fields may serve as an adjunct to micropatterning techniques aimed at directing cell migration and alignment via contact guidance [64].

In principle, electric field application may prove to be more effective than chemotactic gradients or haptotactic gradients incorporated to tissue scaffolds in controlling spatial and

temporal characteristics of cells seeded into tissue scaffolds. For example, we envisaged that cells could be guided spatially and then the field conditions could be modified to initiate changes in cell shape/alignment (rather than migration). Our preliminary studies indicate potential avenues to optimize cell migration or cell orientation, including the application of pulsed rather than constant fields, and the variation of substrate concentration or type. A relationship between migration speed and attachment strength has been described and modeled, and this may provide at least a partial explanation for decreased migration speed at the higher levels of collagen substrate examined in the current study [37, 46].

Cell migration plays an important role in wound healing. Electrical potentials are believed to arise at the wound site from leaky cell membranes or ion pumps [2, 9]. In cultured cells, researchers hypothesized that the EF creates an electro-osmosis force to recruit membrane proteins to polarize and trigger a series of signaling events that, in turn, cause directed migration of cells [54, 65–69]. The rate of wound healing in vivo is closely correlated with changes in the electrical current generated from the wound site [70]. Our laboratory previously reported chondrocyte cathodal migration and alignment to applied DC EFs [31]. Like the ACL, articular cartilage also exhibits a poor healing capacity [71]. The ACL fibroblasts demonstrated greater migration potential when compared with the chondrocytes. Thus, our results on the effects of applied static and pulsing DC EFs on ACL fibroblast cell migration, morphology, gene expression, and wound-closure suggest that EFs may be used to guide biosynthesis and migration, giving rise to potentially new strategies for promoting ligament repair and tissue regeneration [50, 51, 72].

ACKNOWLEDGEMENT

Funding of this work was provided in part by the National Institutes of Health (CTH-AR48791, SBN-AR051056), the National Science Foundation (MCB-0423475), and the Whitaker Foundation.

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