Electrical Stimulation Waveforms from Physical Therapy Stimulate ACL Fibroblast Migration

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Introduction
Numerous approaches have been proposed to enhance the healing capability of the anterior cruciate ligament (ACL). Electrical stimulation is widely used for the treatment of pain and to promote wound healing [1]. In orthopaedic practices, applied electric fields (EFs) have been used clinically to promote bone healing [2]. EF has also been shown to improve lapine ligament repair in vivo [3]. We have previously demonstrated that applied EF enhanced ligament fibroblast migration and collagen production, depending on the applied EF parameters [4]. In the current study, several stimulation waveforms used in physical therapy for promoting tissue repair were adapted to examine their effects on ACL fibroblast migration and morphology.

Materials and Methods
Cell Culture: Porcine ACLs were harvested within 24 hours of death and fibroblasts were collected from explant cultures. The explant and cells were cultured in DMEM supplemented with 10% FBS (Invitrogen). Prior to experiments, the ACL fibroblasts were trypsinized and seeded on glass slides at 2x10^5 cells/cm² for 2 hours.

EF Stimulation: A customized electrical stimulation chamber as used as previously described [4]. Constant direct current (DC) EF was applied at either 30 V or 0.19 V across the chamber using a Keithley SourceMeter. Other waveforms that were applied using a custom stimulator (Dynaprog, MingQuo, Taiwan) are illustrated in Figure 1. All waveforms and the constant 0.19 V group were controlled to have the same amount of total current flow (37.58As). Faradic current (Far) represents a graduation series of triangular waves peaking at 2.8 V at 50 Hz. High-voltage pulsed galvanic stimulation (HVPGS) consists of monophasic, twin-spike pulses that have a fixed pulse duration of 100 μs and max intensity at 300 V with a frequency of 2 Hz. Sinusoidal waves (sin) have a peak intensity of 60 V at 50 Hz and the diadynamic waves (diadyn) are rectified monophasic sinusoidal waves.

Outcome Measures: Images were taken every 15 minutes during the one-hour stimulation and cell migration speed and directionality were analyzed from the displacement of the centroid locations of each cell. Directional velocity represents cell migration speed in the direction of the applied field. Cell morphology was analyzed by manually tracing the major and minor axes of the cell to find the aspect ratio.

Statistics: Statistical analysis were performed using Statistica (α=0.05, StatSoft).

Result
Under electrical stimulation, ACL fibroblasts exhibited significantly different responses in migration and orientation (Figure 2). All groups except for the HVPGS group exhibited enhanced migration speed. When compared with the groups subjected to constant DC EFs, all the other groups were slower except for the diadynamic group. In terms of directional velocity, interestingly, no directionality was found with the constant 0.19 V EF group even though it has similarly enhanced migration speed with the 30 V group. Morphological examination revealed differential cell shape responses. In the constant 30 V group, ACL fibroblasts exhibited significant elongation and aligned perpendicular to the constant applied EF groups (Figure 3). All other groups did not exhibit any significant elongation or orientation.

Discussion
We investigated the effects of physical therapy electrical stimulation on ligament cell migration and morphology. Most of the waveforms we tested resulted in enhanced fibroblast migration speed, while their effects on cell migration directionality were noticeably different. As we have previously shown, bovine ACL fibroblast and chondrocytes both exhibit different EF threshold response in motility and directionality [4-5]. Furthermore, ACL fibroblast exhibit frequency dependent migration behaviors [4]. Using different waveform and field strengths, we discovered a decoupling of cell shape and directionality, which may suggest disparate mechanisms in the two responses [6]. In the current study, faradic and diadynamic stimulation promoted the most significant response in both speed and directionality. Faradic stimulation has been shown to promote collagen organization while diadynamic stimulation promotes skin wound healing [7-8]. Interestingly, while HVPGS has been demonstrated to increase proliferation, collagen synthesis and skin wound healing, no significant effect of increased cell migration is shown here [9]. Future studies will examine the effects of electrical stimulation on both intra- and extra-cellular polarization as well as biosynthesis and proliferation.

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References