The Lewis Clark Wagner Award Recognizes Outstanding Resident's Paper

Recipient: Mark C. Drakos, MD

The Effect of the Shoe–Surface Interface in the Development of Anterior Cruciate Ligament Strain


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Introduction The shoe–surface interface has been implicated as a possible risk factor for anterior cruciate ligament (ACL) injuries; however, the relationship between ACL strain and the shoe–surface interface has yet to be quantified. The purpose of this study is to develop a biomechanical cadaveric model to evaluate the effect of various shoe–surface interfaces on ACL strain. We hypothesize that there will be a significant difference in ACL strain between different shoe–surface combinations when a standardized rotational moment (a simulated cutting movement) is applied to an axially loaded lower extremity.

Materials and methods Eight fresh-frozen cadaveric lower extremities were thawed, and the femurs were potted with the knee in 30° of flexion. Each specimen was placed in a custom-made testing apparatus, which allowed axial loading and tibial rotation but prevented femoral rotation. A strain gauge (Microstrain Inc., Williston, VT, USA) was then placed in the anteromedial bundle of the ACL.

- Preliminary trials: Serial axial loads, moments, and Lachman examinations were performed to evaluate the accuracy of the testing apparatus and to determine conditions, which would allow a repeated measures test to be performed in the elastic range of the stress–strain curve of the ACL.
- Experimental trials: For each specimen, a 500-N axial load and a 1.5-N m internal rotation moment were placed on the potted lower extremity for four different shoe–surface combinations: group I (Astroturf–turf shoes), group II (FieldTurf–turf shoes), group III (FieldTurf–cleats), and group IV (natural grass–cleats). Maximum strain, initial axial force and
moment, maximum axial force, and moment were calculated by the strain gauge and a six-component force plate (Bertec Corp., Columbus, OH, USA). Five trials were performed on each interface in a repeated measures fashion. (Statistics were performed with a one-way ANOVA with significance set at $p < 0.05$)

**Results** The preliminary trials confirmed a linear relationship between strain and both the moment and the axial force for our testing configuration. The average Lachman examination produced a strain of 4.33 (SD ± 1.72). In the experimental trials, the average maximum strain was 3.90, 3.19, 3.14, and 2.16 for groups I–IV, respectively. Group IV had significantly less maximum strain ($p < 0.05$) than each of the other groups.

**Conclusion** This model can reproducibly create a detectable strain in the anteromedial bundle of the ACL in response to a given axial load and internal rotation moment. Within the elastic range of the stress–strain curve, the amount of strain in the ACL appears to be affected by the shoe–surface interface. Specifically, the natural grass and cleat combination produced less strain in the ACL than the Field Turf–cleat, FieldTurf–turf shoe, and Astroturf–turf shoe combinations for a given axial load and moment.

**Disclosures** This study was funded by the Eduardo Salvati Resident Research Grant and the HSS Surgeon in Chief’s Fund. IRB approval was obtained (IRB #27105—effective 9/10/2007–9/9/2008). There are no conflicts of interest to disclose.

The Charles L. Christian Musculoskeletal Research Award Recognizes Outstanding Fellow’s Paper

**Recipient: Neal Moskowitz, MD, PhD**

Regulation of Human Macrophage and Osteoclast Differentiation by Interaction with Extracellular Matrix

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**Introduction/background**

Osteoclasts (OC) are multinucleated cells (MNC) responsible for resorbing bone in both physiological remodeling and pathologic bone loss. OCs are derived from myeloid precursors found in the peripheral circulation, which may also give rise to diverse cell types with diverse functions in health and disease, such as tissue macrophages, foreign body giant cells, and dendritic cells, and it appears that cues from the cell’s ultimate environment have much to do with the differentiation of the cell. While much has been learned about the influence of soluble and cell-surface factors on osteoclastogenesis (e.g., RANKL, osteoprotegerin, cytokines, chemokines), we are increasingly aware that cell-substrate interactions play essential roles in this process.

Integrins are heterodimeric cell surface receptors (typically, one $\alpha$ and one $\beta$ subunit) whose interactions with ligands are important for such functions as cell adhesion, activation, and motility, including extravasation into inflamed tissues. It is known that integrin $\alpha v \beta 3$ is essential for OC differentiation and function and that integrin outside-in signaling also involves cell surface immunoreceptors such as TREM2 and DAP12, which independently have known roles in osteoclastogenesis. $\alpha v \beta 5$ is found in OC precursors but not OCs, and integrin $\beta 2$ is expressed more commonly on the surface of monocytes and macrophages (complexed with $\alpha M$, $\alpha L$, or $\alpha X$) than OCs. Furthermore, $\alpha M$/$\beta 2$ is important for fibrin interactions with leukocytes that contribute to inflammatory responses (e.g. IL-1, TNF-$\alpha$, IL-6 upregulation), including inflammatory joint disease.
in collagen-induced arthritis experiments with fibrinogen knockout mice. The studies here address the hypothesis that contact with immobilized fibrinogen (FBG) by OC precursors diverts them from the OC differentiation program by inducing an inflammatory phenotype.

**Materials and methods**

Osteoclast precursor cells were isolated from human donor buffy coats (purchased from NY Blood Center) by Ficoll gradient centrifugation and purification with CD14+ affinity magnetic beads. Cells were grown in Petri dishes with MCSF for 48 h, and then nonadherent cells were lifted and replated on various surfaces in media containing MCSF and RANKL in concentrations sufficient for osteoclast development, for periods ranging from 4 h to 7 days. Surfaces tested included tissue culture plastic alone, tissue culture wells coated with integrin ligands (FBG, fibronectin, vitronectin, osteopontin, RGD polypeptide), and osteologic discs (calcified hydroxyapatite-like matrix). RNA was isolated, and the expression of genes important for osteoclast differentiation (e.g., β3 integrin, cathepsin K, calcitonin receptor, RANK, annexin VIII) were analyzed using real-time PCR. In other experiments, protein was extracted, resolved on SDS-PAGE gels, and transferred to PVDF membranes for Western blot analysis using monoclonal antibodies to signaling molecules of interest. Parallel cultures were stained for TRAP or with FITC-phalloidin to visualize cell morphology and actin ring formation and to monitor formation of OCs (TRAP + MNCs).

**Results**

CD14+ mononuclear cells grown on immobilized FBG differentiated into OCs with delayed kinetics, manifested as fewer TRAP + MNCs, early inhibition of RANK expression, delay in peak expression of OC genes such as integrin β3 and cathepsin K, and delayed fall in the expression of β2 and β5. Western blot experiments showed that growth on FBG also delayed peak protein expression and possibly dephosphorylation of NFAT-c1, a key transcription factor, which must be dephosphorylated prior to migrating to the nucleus where it mediates transcription of numerous genes important for OC differentiation. A similar delay in MNC formation was seen on surfaces coated with RGD polypeptide, a synthetic integrin binding domain, but not with integrin ligands vitronectin nor osteopontin, suggesting a degree of selectivity. Further experiments to dissect a mechanism have shown that on FBG, there is early induction of inflammatory cytokines TNF-α and IL-6, followed by anti-inflammatory IL-10. The suppressive effects of FBG were not eliminated by treatment with the LPS inhibitor polymyxin B.

**Discussion**

These data support the hypothesis that binding to FBG induces and/or maintains an “inflammatory” phenotype in monocyte precursors, precluding, or at least delaying, their differentiation to OCs. Alterations in NFAT-c1 suggests that this may work by suppressing the canonical RANKL signaling pathway rather than via a parallel pathway acting through different transcription factors to alter OC gene expression. Further experiments will aim to confirm that this is an integrin-specific effect by using neutralizing antibodies to αMβ2. Another future aim will be to define the signaling mechanisms of inhibition by FBG, including whether it is a direct effect of integrin signaling pathways on the RANKL–RANK cascade or if the role of integrin signaling is to upregulate soluble mediators (such as IL-1, TNF-α, IL-10, chemokines), which then exert suppressive effects in an autocrine fashion. In summary, these studies provide an example of regulation of osteoclast differentiation by contact with matrix. They may also help to define one element of a complex relationship between inflammation and bone resorption, in which pro-inflammatory stimuli can either promote or inhibit OC differentiation, depending upon contextual features such as matrix, other cell types, and timing of stimuli.

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The Philip D. Wilson Award Recognizes Outstanding Fellow’s Paper

Recipient: Asheesh Bedi, MD

The Effect of Matrix Metalloproteinase Inhibition on Tendon-to-Bone Healing in a Rotator Cuff Repair Model

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Background Matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes that have a critical role in tissue repair, degradation, and extracellular matrix homeostasis. Recent studies have demonstrated a potentially critical role of MMPs and tissue inhibitors of matrix metalloproteinases in the pathophysiology of rotator cuff disease.

Hypothesis We hypothesize that MMP inhibition after surgical repair of the rotator cuff will improve healing at the tendon-to-bone surface interface. An established rat rotator cuff repair model was utilized to evaluate the biomechanical and histological differences in tendon-to-bone surface healing with inhibition of matrix metalloproteinases.

Study design The study design was a controlled laboratory study.

Materials and methods Sixty-two male Sprague–Dawley rats underwent unilateral detachment of the supraspinatus tendon from the greater tuberosity of the humerus followed by immediate repair using non-absorbable suture and bone tunnel fixation. In the control group (n = 31), the supraspinatus was repaired to its anatomical footprint. In the experimental group (n = 31), recombinant alpha-2-macroglobulin protein (A2M, 1 IU/kg; Roche Applied Science, Indianapolis, IN, USA) was applied to the tendon–bone interface after performing an identical surgical repair. A2M is an endogenous plasma glycoprotein and universal inhibitor of MMPs. Eight animals from each group were sacrificed at 2 and 4 weeks for histomorphometric and immunohistochemical analysis. Collagen fiber organization at the healing tendon-to-bone attachment site was evaluated using polarized light microscopy to measure collagen birefringence. Fifteen animals from each group were sacrificed at 4 weeks after surgery for biomechanical testing. Statistical comparisons were performed using paired t tests, and significance was set at p < 0.05.

Results All repairs were noted to be grossly intact at the time of sacrifice. The healing enthesis was highly cellular and demonstrated grossly similar morphology in the control and experimental groups. Histomorphometric analysis demonstrated a significantly larger fibrocartilaginous zone at the healing tendon–bone interface in the A2M-treated group compared to control specimens by 2 weeks (p < 0.05). Evaluation of collagen birefringence revealed significantly increased organization in the A2M-treated group compared to control animals by 4 weeks (p < 0.01). Immunofluorescence analysis using a monoclonal antibody for collagen fragments demonstrated a significant reduction in collagen degradation at the A2M-treated tendon–bone interface at 2 and 4 weeks (p < 0.05). α-SMA and factor VIII-positive cells were predominantly localized proximal to the healing enthesis, but not at the tendon–bone interface in either group. Organized blood vessels were observed by 4 weeks, although no quantitative differences were detected between control and A2M-treated specimens. Biomechanical testing at 4 weeks revealed no significant differences in stiffness or ultimate load-to-failure between treatment groups.
Discussion  A2M-mediated universal blockade of MMPs is associated with distinct histological differences in the healing tendon-to-bone surface interface after rotator cuff repair. Increased fibrocartilage interface tissue and improved collagen organization in the healing enthesis of the A2M-treated repairs may reflect enhanced tendon–bone healing. The lack of a detectable difference in the biomechanical strength of the repair between treatment groups may reflect the resilient and expeditious healing of the rotator cuff in a rodent model by 4 weeks. Further investigation at earlier timepoints or with a different animal model is necessary to characterize the potential biomechanical impact of these observed histological differences.

Clinical relevance  Modulation of MMP activity after rotator cuff repair may offer a novel biological pathway to augment tendon-to-bone healing after rotator cuff repair.

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