The Microscopic Anatomy of the Human ACL Entheses: A Quantitative Analysis

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Introduction: More than 250,000 anterior cruciate ligament (ACL) injuries occur annually in the United States [1]. A common mode of ACL injury is a tear at or near the femoral origin, or “enthesis”, of the posterior-lateral (PL) region (aka ‘bundle’). While these ruptures have been reported both in vivo and in vitro [2-3], it is unclear why they occur at that location. Our recent computer simulations have shown the presence of a strain concentration at the posterior-inferior margin of the femoral enthesis, where the PL bundle inserts at an acute angle, under loads simulating a pivot landing [4]. Whether or not injury occurs in this region will depend on its detailed microstructure, material properties, and loading history and thus, this study focuses on the first of these, the microstructure of the ACL entheses.

The ACL entheses are described as fibrocartilaginous [5], which are characterized by four zones of tissue: dense fibrous connective tissue, uncalcified fibrocartilage (UF), calcified fibrocartilage (CF), and bone [6]. The quantity of each tissue type is said to be characteristic of the mechanical loading at the enthesis: the quantity of CF supposedly relates to the tensile force applied to the bone while the quantity of UF relates to how much the angle between the ligament’s collagen fibers and the bone changes during joint rotation and/or translation [7]. Because computer simulations show larger changes in the ACL fiber orientation at the femoral than at the tibial enthesis during a simulated pivot landing [4], one might expect less UF at the latter enthesis.

The purpose of this study was to quantify the microscopic anatomical differences within the human ACL entheses. We tested the primary hypothesis (H₁) that the relative area of CF and depth of UF would be greater in the femoral enthesis than in the tibial enthesis. We also tested the secondary hypothesis (H₂) that, within the femoral enthesis, the relative area of CF and depth of UF would be greatest at the anterior-inferior margin of the enthesis [8].

Methods: Ten unembalmed human femur-ACL-tibia specimens were harvested from four male (age: 51.5 ± 4.8 years; height: 1.76 ± 0.12 m; mass: 74.1 ± 18.1 kg) and four female (age: 52.8 ± 11.9 years; height: 1.66 ± 0.05 m; mass: 66.9 ± 15.1 kg) donors, then fixed in 15° of flexion. Each bone-ACL tissue block was defatted, dehydrated, and embedded in methyl methacrylate. Per block, four thick sections (a=20%, b=40%, c=60%,d=80% of the width of the enthesis) were prepared and stained with toluidine blue. Tibial blocks were sectioned along an anterior-posterior axis; meanwhile the femoral blocks were sectioned along the longitudinal axis of the ACL (Figure 1). High resolution digital images (4000 dpi) of all sections were obtained, from which the relative area of CF was quantified by outlining this tissue using a pen display and dividing this area by the length of the enthesis. The depth of UF was measured at 500-μm intervals along the entire enthesis using a light microscope and the digital images. CF relative area and average UF depth were quantified for each quarter of the length of the enthesis (i.e., A=0-25%, B=25-50%, C=50-75%, D=75-100%). The hypotheses were statistically tested by means of a series of linear mixed-effects models with CF relative area and UF depth as the outcome variables and enthesis region, knee specimen, and knee donor as the predictor variables. Enthesis region was coded as
1=femur regions BC (mid 50%), 2=femur regions AD (outer 50%), 3=tibia regions BC (mid 50%), 4=tibia regions AD (outer 25%) to test H₁ (Figure 1 Tibia) and as 1=femur sections ab regions AB (anterior-superior), 2=femur sections ab regions CD (anterior-inferior), 3=femur sections cd regions AB (posterior-superior), 4=femur sections cd regions CD (posterior-inferior) to test H₂ (Figure 1 Femur). Entheses region was treated as a fixed effect; whereas knee specimen and knee donor were treated as random effects. An α < 0.05 indicated statistical significance.

Results: At the femoral enthesis, most of the fibrocartilage tissue was located in the middle region, with only small areas of CF and little to no UF in the regions near the inferior and superior boundaries (Figure 2). At the tibial enthesis, CF and UF tissue was present, but in small quantity, especially near the anterior and posterior boundaries (Figure 2). We accepted our primary hypothesis that more CF and UF would be present in the femoral enthesis than in the tibial enthesis. Specifically, both the relative area of CF and the average depth of UF were significantly greater in the middle 50% region of the femoral enthesis than in its outer 50% and all regions of the tibial enthesis (Figure 3A). The secondary hypothesis that the quantity of fibrocartilaginous tissue would be greatest in the anterior-inferior region was also accepted (Figure 3B). These data were variable, however, with several specimens having more CF, but only one specimen with more UF at the inferior margin of the PL bundle’s femoral origin than that of the AM bundle.

Discussion: Our results corroborate and extend previous work that the amount of CF is greatest at the anterior-inferior margin of the femoral enthesis [8], which corresponds to the inferior margin of the antero-medial (AM) bundle’s origin. The present study improved the method used to quantify CF and also quantified UF at the femoral enthesis and both UF and CF at the tibial enthesis. There was more calcified and uncalcified fibrocartilage at the femoral than the tibial enthesis. We speculate that the larger footprint of the tibial enthesis [9] and the concavity in which it sits (i.e., extends the length of the enthesis) reduces stress and thus less fibrocartilage may be required in comparison with the femoral enthesis. Furthermore, the inferior margin of the PL bundle’s femoral origin had less UF and CF in comparison with that of the AM bundle. Given the strain concentration at the inferior margin of the femoral enthesis during athletic cutting and pivoting activities that produce high internal tibial torque [4], the PL bundle may be susceptible to injury there. Further work is needed to investigate whether entheses with high AM/PL ratios of CF and UF quantity are at greater risk of injury. We conclude that the quantity of fibrocartilage varies not only between the ACL entheses, but also across the femoral enthesis.

Significance: We developed a method and used it to quantify the distribution of calcified and uncalcified fibrocartilage across the human ACL entheses. The next step would be to examine the effect of age and physical activity on these distributions.
Figure 1. Location of the four sections prepared for histological analysis per bone. ACL block (a-d). Femur: for the intra-femoral enthesis analysis, total CF relative area and average UF depth were computed for the inferior 50% (2: anterior inferior; 4: posterior inferior) and the superior 50% (1: anterior superior; 3: posterior superior) and averaged across the two most anterior sections (23) and the two most posterior sections (14), corresponding to the origin of the AM and PL bundles, respectively. Tibia: For the inter-enthesis analysis (i.e., femur vs. tibia), total CF relative area and average UF depth were computed for the middle 50% (grey cross-hatched region) and the outer 50% (gray region) of the entheses, as shown for the tibia, and averaged across all four sections. PL: posterior lateral bundle, AM: antero-medial bundle.

Figure 2. Microscopic anatomy of the ACL’s femoral and tibial entheses. Four zones of tissue characteristic of fibrocartilaginous entheses are present. More CF and UF tissue was present in the femoral entheses than the tibial entheses. The insets show these tissue types and their relative quantities. For analysis of CF area and UF depth, tissue sections were divided into four equal regions along the length of the enthesis. For the inter-enthesis analysis, fibrocartilage quantity was computed for the middle 50% and the outer 50% of the entheses, as shown, and averaged across all four sections. UF: uncalcified fibrocartilage; CF: calcified fibrocartilage.
Figure 3. Mean (SD) relative area of calcified fibrocartilage and depth of uncalcified fibrocartilage by region of the ACL enthesis. (A) Results of the inter-enthesis analysis show greater CF area and CF depth in the femoral enthesis, specifically in its middle region. (B) Results of the intra-enthesis enthesis analysis show greatest CF area and CF depth in the anterior inferior region of the femoral enthesis, corresponding to the inferior margin of AM bundle origin. *significantly greater than all other regions in the graph, p = 0.000; **significantly greater than all other regions in the graph, p = 0.000-0.015.

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