The Macro-to-micro-to-nano Scale Structure Of The ACL And Its Enthesis.

Lei Zhao, Neil Broom, PhD, Ashvin Thambyah.
The University of Auckland, Auckland, New Zealand.

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Introduction: Despite both the large number of studies into ACL injury prevention and the relative success of reconstructive techniques in being able to restore knee function following an ACL rupture there is ongoing interest in improving our understanding of ACL structure and function. The hope is that new and improved methods can be developed, that would incorporate more accurately the anatomy of the full ligament-bone system. One major challenge for the ligament researcher is to achieve a greater understanding of the complexities of the tissue structure across the macro, micro and nano scalar levels. Previous studies have mostly been confined to the structure of a single scale level of the ACL [1-4]; the structural features across multi-scale levels and their relationships between each level having been largely ignored. Further, there have been very few studies focused on the structure of the ligament enthesis and the anchorage mechanism of ligament to bone despite this region being an important anatomical structure in both the morphogenesis and mechanical function of this compliant-rigid tissue system. This new study employed a series of novel experimental methods to investigate in detail the macro-to-micro-to-nano scale structure of the ACL and its enthesis.

Methods: Twenty-two porcine knee joints were cleared of their musculature in order to expose the intact ACL following which ligament-bone samples were obtained. The samples were fixed in formalin followed by decalcification with formic acid. Thin sections containing the ligament insertion into the tibia were then obtained by cryosectioning and analysed using differential interference contrast (DIC) optical microscopy and scanning electron microscopy (SEM).

Results: At the micro-level, the anteromedial (AM) bundle insertion at the tibia displayed a significant deep-rooted interdigitation into bone (Fig 1), while for the posterolateral (PL) bundle the fibre insertions were less distributed and more focal (Fig 2). Three sub-types of enthesis were identified in the ACL and related to (a) bundle type, (b) the positional aspect within the insertion, and (c) specific bundle function.

At the nano-level, the fibrils of the AM bundle were significantly larger than those in the PL bundle (Fig 3). The modes by which the AM and PL fibrils merged with the bone matrix fibrils were significantly different (Fig 4 and 5).

Discussion: The present study describes in detail the multiscale structural make-up of the ACL tibial enthesis, revealing the micro-to-nano scale insertion of the AM and PL bundles into the tibia. The PL bundle insertion with its different zonal changes in structure is typical of a direct enthesis. The AM bundle with its deep rooted fibres may be mistakenly identified as an indirect type enthesis containing ‘Sharpey’s fibers’. However, Sharpey’s fibers are the deeply embedded extensions from the periosteum and thus cannot accurately describe the deep ligamentous fibres buried in bone and seen continuous with the AM bundle as demonstrated in this study. Further, the deep-rooted fibrils of the AM bundle are shown to interweave with those of the bone matrix collagen (Fig 4) and highlights the degree of mechanical complexity involved in attempting to relate the ligament-bone attachment’s nano-scale structure to the macro-level joint mechanics. The relatively shallow interdigitation of the PL bundle into bone compared to that of the AM bundle may be a microstructural reflection of macro-level differences in biomechanical function.

Significance: With the advent of tissue engineering as a promising future technology for many orthopaedic procedures, it is important that our current knowledge on the structures of the musculoskeletal tissues, and particularly those spanning the biomechanically critical soft-hard junction, be enhanced. This new study provides new information that may assist in future ligament designs, serving as blueprint data for tissue engineered ligament replacements. Further, the study provides new insights into how this complex tissue system adapts structurally to its loading environment at the macro-to-micro-to-nano scales and is thus relevant to our understanding of ligament development, injury, repair and healing.

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Figure 1. Sagittal view of the AM bundle insertion showing the intense interdigitation of ligament fibres with its rigid substrate (see the boxed region). The vertical white line indicates the depth of fibre rooting. Optical image obtained by top-illumination of the fully hydrated section.
Figure 2. Lateral-most sagittal views of (A) the AM bundle and (B) the PL bundle showing the irregular profile of the cement line (solid line) with respect to the approximate plane of the tibial plateau (see dotted lines). The AM bundle in (A) shows a sharp turn of the ligament fibres (black arrow) into the fibrocartilage-bone substrate, and also deep fibre rooting (black vertical line). Conversely, the PL bundle in (B) shows a significantly reduced depth of ligament fibre rooting.

Figure 3. SEM images, taken at the same magnification, (A) the AM bundle, (B) the PL bundle, and (C) the bone, showing collagen fibrils that are largest in AM bundle and smallest in bone.
Figure 4. SEM of AM bundle interdigitation into bone, showing fibrillar-level integration with bone. Note also the presence of near-transversely organized collagen fibrils forming well-defined ‘nodal’ points along the ligament-bone interface (see arrows).
Figure 5. SEM image of PL bundle insertion. Unlike that of the AM bundle (refer to earlier figure 4), here in the PL insertion, the ligamentous fibrils end in a shallow bone socket.