An Analytical Model for Elucidating Tendon Tissue Structure and Biomechanical Function from In Vivo Cellular Confocal Microscopy Images

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Introduction: Recent technological advancements in fibered confocal fluorescence microscopy provide the unique ability to image tissues at very high spatial resolutions (5 microns), and can directly visualize single cells and cellular behavior in vivo. Using a newly introduced technique, the cells can also be used as markers of tissue deformation, from which the underlying tissue mechanical properties may be deduced [1]. Preliminary work using a murine Achilles tendon model has revealed that inter-fascicle gliding can yield complex cellular motions that can only be interpreted within the context of an appropriate anatomical model. In this study, we introduce a simple two fascicle model to interpret endo-microscopic cellular images in terms of mechanical strains within the fascicles, as well as the relative gliding between fascicles.

Materials and Methods: An in vivo image library of tendon cell displacements was created as previously described [1]. Briefly, the femora and tibiae of seven anesthetized C3H/HeN mice, aged 10 weeks were constrained as a quantified rotational moment was applied to the ankle, dorsiflexing the foot and consequently stretching the Achilles tendon. The motions of fluorescently labeled tenocytes in the tendon midsubstance were recorded at discrete loading intervals using a Cell-Vizio® endoscopic micro-imaging system (Mauna Kea Technologies, France).

Based on the in vivo image library, a physics-based model was developed to replicate 3D in vivo cellular displacements in the mouse tendon. Cells were embedded within tendon substructures, with motions dictated by the deformations of their host structures. The geometry of the model consisted of two neighboring fascicle segments, each with an independent functional axis along which the fascicle was loaded. The fascicles were discretized into a regular 3D grid with nodes orthogonally connected by springs (Fig. 1). The springs were assigned elastic properties that could be individually tuned to vary local tissue elasticity. This spring-mass system was mathematically described by a set of simultaneous differential equations that were iteratively solved to determine the displacements of the tendon model nodes under an applied boundary condition. “Cells” were uniformly distributed within this three dimensional field and a virtual imaging focal plane was made to transact the model in a such a way to mimic actual endoscopic measurements. At each incremental load step, an artificial image was created by projecting the cell outlines onto the virtual imaging plane, with intensities that were inversely proportional to their distance from the plane. A library of in silico datasets was produced by systematically varying the orientation of the individual fascicles, and the depth and angle of incidence of the imaging plane.

Finally, experimental (in vivo) cell trajectories were tracked and grouped according to directionality and magnitude. After identifying and clustering cells belonging to the same structure, and choosing the model configuration that best represented the experimental displacements, it was then possible to calculate the engineering strains within each tendon fascicle, as well as to characterize sliding between neighboring fascicles.

Results: Nearly 90% of the in vivo datasets could be effectively reproduced by three basic model cases. The first configuration (Fig. 1, case 1) was characterized by the confocal imaging plane falling within a single tendon fascicle, and mimicked approximately 25% of the in vivo image datasets. The second configuration (Fig. 1, case 2) represented simultaneous imaging of cells from two fascicles, with distinct regions of the imaging plane dedicated to each fascicle. This second case corresponded to approximately 20% of the in vivo datasets. The majority of the in vivo data (roughly 50%) were best described by a third configuration (Fig. 1, case 3) whereby cells from two fascicles were simultaneously imaged, with cells from both fascicles superimposed and distributed over the entire image field.

In the synthetic datasets, deviation of the endoscopic imaging axis from perpendicular to the functional axis of a fascicle did not affect the accuracy of strain measurement, but did compromise measurement sensitivity and precision. For instance, a 4% reference strain in the fascicle was quantified as 3.9 ± 0.8% when the angle of the imaging axis was perpendicular to the functional axis of the fascicle, while a deviation of 30° yielded a strain measurement of 3.8 ± 1.5%. The ability to accurately quantify fascicle sliding depended more heavily on the endoscope imaging angle and the anatomical location of the focal plane within the tendon. Specifically, in regions where tendon fascicles were “anchored” (e.g. at the bony insertion), relative movements between fascicles were less than in certain regions of the tendon midsubstance.

Among the twenty in vivo datasets that were assessed with regard to engineering strain in individual Achilles tendon fascicles, calculated strains ranged between 1 and 5% (3.2 ± 1.7%) at the maximum applied load. Considerable relative movements between neighboring fascicles were observed, with up to 0.6 mm (or 15% of tendon length) of relative sliding between juxtaposed fascicles. Again, the magnitude of fascicle sliding was dependent both upon the local anatomy being imaged, and the amount of applied ankle flexion.

Discussion: Fibered confocal microscopy provides a unique imaging platform for assessing skeletal tissue mechanics in vivo, and appropriate micro-anatomy based models are required to implement precise 3D cell tracking and improve the accuracy of engineering analysis. The model presented here is able to mimic, and thus explain, the relative movements of individual cells in loaded tendon, and provides a physical basis for elucidating mechanical strains within individual fascicles, as well as for quantifying inter-fascicle sliding. As has been indicated in ex vivo experiments on rat tail tendon[2], relative sliding between fascicles can be considerable, and may in fact dominate the biomechanical behavior of the tendon.


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