Strain distribution in the anterior cruciate ligament in response to anterior drawer force to the knee

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Received: 28 October 2016; Revised: 13 December 2016; Accepted: 13 February 2017

Abstract

In the present study, an image correlation method was used to determine the site-dependent strain in the porcine anterior cruciate ligament (ACL). In particular, the strain of the ACL in the femoral and tibial attachment areas was quantified when the eight knees were subjected to a maximum of 50 N anterior tibial load that was applied using a 6-DOF robotic system. The ACL strains in the anterior, central, and posterior bundles of the medial, middle and lateral layers were determined as a function of the applied anterior load. In addition, the surface of the ACL was observed using a light microscope under no loading condition. Results revealed that the strain in the medial layer of the ACL increased gradually and almost linearly with the increase of anterior load in the mid-substance area. In contrast, the strain in the femoral and tibial attachment areas of the medial layer of the ACL increased rapidly at the beginning of anterior loading and gradually thereafter with significant differences in slope of strain-anterior load curve found in the anterior and posterior bundles. The largest strain in response to 50 N of anterior force was found in the tibial attachment area in medial and middle layers, while the maximum strain was found in the femoral attachment area in the lateral layer. Microscopic observation indicated that crimp structure was more clearly observed in the femoral and tibial attachment areas than in the mid-substance. These microstructural features of the ACL were may be attributable to the higher and load-dependent, nonlinear strain observed in the attachment areas. Our study suggested that the strain in the ACL at full extension is site- and load-dependent in a non-linear manner at the femoral and tibial attachment areas.

Key words : Anterior cruciate ligament, Strain, Knee joint, Robotic system, Image correlation method

1. Introduction

The ACL is an important ligament at the knee since it plays a crucial role in stabilizing the knee in response to external forces and moments such as an anterior tibial load and internal rotation moment (Butler et al., 1980; Fukubayashi et al., 1982; Wang et al., 1974). Since high loads are often applied to the ACL in daily activities, the incidence of ACL injury is high (Majewski et al., 2006). Therefore, the mechanical behavior of the ACL must be investigated for a better understanding its mechanical function and injury mechanisms. For this purpose, previous studies mainly focused on the in-situ forces in the ACL in response to external forces and/or moments (Fujie et al., 2011; Gabriel et al., 2004; Sakane et al., 1997), and the contribution of the ACL to knee kinematics (Butler et al., 1980; Fukubayashi et al., 1982; Wang et al., 1974). Recent anatomical and biomechanical studies indicated that the ACL consists of multiple bundles of fibers (Otsubo et al., 2012; Tantisricharoenkul et al., 2013) and the in-situ forces in the ACL are dependent on location within the ACL, knee position, and external load applied to the knee (Amis et al., 1991; Fujie et al., 2011). However, it is almost impossible to measure force distribution data in the ACL due to experimental limitations and difficulties in force

measurements for biological soft tissues. Thus, previous studies have focused on the strain or strain distribution on the ACL surface. The strain in the mid-substance of the human ACL was previously quantified *in vivo* using a differential variable reactance transducer (DVRT) and found to be $2.00 \pm 0.17\%$ during the Lachman test at 140 N of anterior tibial drawer force (Cerulli et al., 2003). A DVRT was also used to measure the strain in the mid-substance of the ACL *in vitro* and found to be 4.8% when the knee was subjected to a simulated bipedal landing (Kiapour et al., 2014). Thus, the strain in the mid-substance of the ACL has been quantified, but the strain distribution throughout the ACL could not be obtained using a DVRT.

Several studies developed new methods for measuring ACL strain distribution. For example, the strain distribution was determined in the human ACL during passive flexion using a photoelastic measurement technique (Yamamoto et al., 1998). With the ACL coated with a saline solution containing aluminum powder, the strain in the anterior region of the ACL was found to increase more than that in the posterior region as the flexion angle was increased. Ultrasound elastography was also used to characterize the strain distribution of the ACL (Spalazzi et al., 2006). Large strain of approximately 8% was observed in the insertion sites of the ACL when 3 mm of anterior displacement was applied to the knee. Although important data on the strain distribution have been obtained, physiological knee loading conditions were not simulated in those studies due to experimental limitations. Moreover, previous studies did not obtain high resolution strain data in the femoral and tibial attachment areas of the ACL.

Recently, image correlation methods have been developed for two-dimensional motion tracking of arbitrary points (Gao et al., 2015; Tyson et al., 2002). In the method, a point on an object can be identified by means of a brightness gradient between neighboring pixels, and the X-Y coordinates of the point can be tracked. Thus, it is possible to accurately quantify the strain distribution in the ACL using the method with a high resolution. Therefore, the objective of the present study was to apply the image correlation method to the determination of the strain distribution in the porcine ACL. In particular, the strains in the ACL at the femoral and tibial attachment areas were quantified when the knee was subjected to an anterior tibial load using a 6-DOF robotic system developed in our laboratory (Fujie et al., 2013).

2. Materials and Methods

A 6-DOF robotic system (FRS2010, Technology Service, Chino, Japan) was used to apply anterior load to the knee at full extension (Fujie et al., 2013). Briefly, the robotic system consists of a velocity-control custom-designed 6-DOF manipulator, servo-motor drivers (HA-800B-3A, Harmonic Drive Systems, Japan, and SGDS-01F12A, YASKAWA Electric Co, Japan), a universal force/moment sensor (UFS) (SI-660-60, ATI, USA), a high-speed motion network (Mechatrolink-II, YASKAWA Electric Co, Japan), and a control PC (Windows 7, Microsoft, USA) (Fujie et al., 2013). The manipulator consists of 3 translational axes driven by 3 AC servo-motors (SGMAS-01A2A21, SGMAS-C2A2A2C, YASKAWA Electric Co, Japan) and 3 rotational axes driven by 3 AC servo-motors (FHA-25C-160-S248-A, FHA-17C-160-S248-A, Harmonic Drive Systems, Japan). The features of the novel system are; 1) the manipulator has a unique, material-testing-machine-like arm configuration consisting of two mechanisms that allow precise and easy control; 2) the arms and clamps of the mechanisms consisted of highly stiff, hollow-body structures; 3) the control PC and servo-motor controllers are serially connected using a high-speed motion network (Mechatrolink, YASKAWA Electric Co, Japan); and 4) system control is performed using a graphical language programming environment (LabVIEW 8.5.1, National Instruments, USA) on the PC. Iteration of data acquisition, kinematic and kinetic calculation, and motion of actuator was performed at a rate of 200 Hz. The maximum clamp-to-clamp stiffness of the robotic system is 820 N/mm. A LabVIEW-based control program runs on a windows PC to control the displacement of, and forces/moments applied to the joint with respect to a knee joint coordinate system developed by Grood and Suntay (Grood and Suntay, 1983).

Fresh-frozen porcine knees obtained from a local slaughterhouse (age = 6 months; $n=11$) were used. Eight knees were dissected down to the joint capsule, stored at -25°C , and thawed for 24 hours at room temperature prior to testing. The specimens were kept moist with physiologic saline solution throughout all experimental procedures. Following a previous experimental method (Fujie et al., 2011), the femur and tibia were cut to 15 cm in length from the joint line, and soft tissues were carefully removed from the knee, leaving the joint capsule intact. The ends of the femur and tibia were potted in cylindrical molds of acrylic resin (Ostron II, GC Corp, Tokyo, Japan). The fibula was cut to 5 cm in length from the tibiofibular joint and was fixed to the tibia using acrylic resin to maintain its anatomical position. The femoral and tibial cylinders were then fixed to the clamps of the robotic system.

Maximum knee extension was defined by applying a 1.0 N-m extension moment to the knee while keeping

force/moment applied along and about the other DOFs to zero using force control. The zero load configuration (or reference configuration) for the ACL was defined at this knee position. An anterior tibial load was then applied to the knee up to 50 N while maintaining the flexion angle, and the 6-DOF kinematics were recorded. During the test, the anterior-posterior DOF was translated under displacement control at a rate of 0.1 mm/s while the other DOFs, except AP and flexion-extension DOFs, were set under force control with the prescribed force/moment at zero.

The medial condyle of the femur was then removed to allow visualization of the ACL and strain measurements. While the intact knee motion was repeated using a reproduction function of joint motion equipped with the robotic system, the medial view of the ACL surface (medial layer) was recorded using a full high-vision video camera (HDR-CX500, SONY, Japan). Then, the surface deformation of the ACL including the femoral and tibial attachment areas was quantified using a motion tracking system (VW-9000, KEYENCE, Osaka, Japan) based on the image correlation method (Fig.1). It is possible with the system to track an arbitrary point based on brightness difference of the point relative to neighbors in the movie image. Note that the system can track up to 50 arbitrary points without physical markers.

Fig.1 Schematic drawing of the porcine knee for the measurement of the ACL strain. The intact joint at extension (a), the removal of the medial femoral condyle for imaging the ACL in a lateral view (b), and a focused image of the ACL for strain measurement (c).

The ACL was divided into 25 regions based on its longitudinal axis: 1) five regions from the anterior to posterior fibers, and 2) transversely partitioned into 5 areas; 0-10%, 10-20%, 20-80%, 80-90%, 90-100% along the length from the most posterior edge of each area. The femoral attachment area, mid-substance, and tibial attachment area are roughly corresponded to 0-20%, 20-80%, and 80-100%, respectively. On the recorded image, multiple gauge markers were placed at the center in each region and the length change between the markers were calculated to analyze local 1-dimensional tensile strains along the fiber direction. ACL strain as a function of anterior load (0, 10, 20, 30, 40, and 50 N) was determined for the femoral attachment area (0-20%), the mid-substance (20-80%), and the tibial attachment area (80-100%) in the most anterior bundle (0-20%), the central (40-60%), and the most posterior bundle (80-100%).

Fig.2 Lateral image of the ACL surface (left) and the 25 regions of the ACL surface for the strain distribution analysis (right).

The anterior-posterior width of the femoral attachment area of the ACL was measured using a caliper. Then the medial layer of the ACL (approximately 1/3 of the entire ACL thickness) was peeled away, so that the femoral and tibial attachment areas of the remaining ACL were approximately 2/3 of the entire attachment area (Fig.3). This was ensured by a measurement that the width of femoral attachment of the transected medial layer was 1/3 of the original width. The middle layer of the ACL was recorded during reproduction of the intact knee motion. Strain on the middle layer of the ACL in response to 50 N of anterior load was determined through the experimental manner identical to that for the medial layer. This procedure was repeated again after the removal of the middle layer of approximately 1/3 of the entire ACL to determine the strains on the lateral layer of the ACL in response to 50 N of anterior load.

Fig.3 Sequential removal of the medial and middle layers for the measurement of 3-D strain distribution in the medial, middle, and lateral layers of the ACL. The ACL was separated into three layers so that the femoral attachment area of each layer was one third of the entire area of the ACL.

Paired t-tests were performed to compare the ACL strain in the attachment areas to that in the mid-substance in the medial layer at 10, 20, 30, 40, and 50 N of anterior load to the knee. In addition, linear regression lines were determined for the ACL strain-anterior load relationship of each specimen either between 0 N and 20 N or between 30 N and 50 N of anterior load. From shape of the lines, slope of strain-anterior load curve was analyzed during a low load (0-20 N) and a high load (30-50 N). Non-paired, t-test was performed to compare the rates for detecting the non-linearity in the ACL strain-anterior load relationship in the anterior, central, and posterior bundles. Paired t-tests were performed to statistically compare the ACL strains in the femoral and tibial attachment areas to that in the mid-substance in response to 50 N of anterior load in 25 portions. Statistical significance was set at 0.05.

In addition, the fiber structure of the ACL of 3 additional knees was observed using a light microscope (VHX-1000, KEYENCE, Osaka, Japan). The ACL with bone fragments was carefully dissected from the porcine knees, and fixed to the metal stage of the microscope so that the fiber direction of the ACL aligned along tensile direction. During 0.1 N of tensile loading was applied to the ACL, the crimp structures of the central bundle in the medial layer surface (surface of the ACL) were microscopically observed.

3. Results

In the medial layer, the ACL strain gradually and almost linearly increased with the increase of anterior load in the mid-substance area. In contrast, the ACL strain rapidly increased at the beginning of load application and gradually increased thereafter in the attachment areas in the medial layer (Fig.4). Slope of strain-anterior load curve of the ACL strain with the anterior loading was significantly higher in the low load (0-20 N) than in the high load (30-50 N) regions for most of femoral and tibial attachment areas (Table-1). Such a load-dependent slope of strain-anterior load curve of the ACL strain was found only in the anterior fibers in the mid-substance. While the anterior load was increased, the ACL strains in the femoral and tibial attachment areas were always higher than that in the mid-substance, with significant differences observed at 20 N in the anterior bundle, at 20, 30, 40, and 50 N in the central bundle, and at 10, 20, 30, 40, and 50 N in the posterior bundle.

Fig. 4 ACL strain-anterior load relationships of the (a) anterior, (b) central, and (c) posterior bundles in the medial layer.

Table 1 Rate of increase of the ACL strain (%/N) of anterior, central, and posterior bundles during a low load (0-20 N) and high load (30-50 N) in the femoral and tibial attachment areas and mid-substance area.

	Anterior fiber		Central fiber		Posterior fiber	
	Low load	High load	Low load	High load	Low load	High load
Femoral attachment area	*0.15 (0.14)	*0.03 (0.10)	0.13 (0.11)	0.07 (0.06)	*0.11 (0.06)	*0.04 (0.04)
Mid-substance area	0.05 (0.11)	0.01 (0.02)	*0.08 (0.04)	*0.03 (0.04)	0.07 (0.11)	0.01 (0.03)
Tibial attachment area	*0.22 (0.22)	*0.03 (0.06)	*0.23 (0.13)	*0.08 (0.09)	*0.25 (0.15)	*0.08 (0.07)

* Significant difference between the rate in low load and high load

Results revealed a trend that the strains in all layers of the ACL near the femoral and tibial attachments areas (0-20% and 80-100%) in response to 50 N of anterior load were larger than those in the mid-substance area (20-80%) (Fig. 5, Table 2-4). As compared with the mid-substance, significantly larger strains were obtained in 6 regions of the tibial attachment area in the medial and lateral layers, 5 regions of the tibial attachment area and 1 region of the femoral attachment area in the middle layer. The largest strain was 8.0% observed in the tibial attachment on the posterior bundles in the medial layer, 6.4% observed near the tibial attachment on the posterior bundle in the middle layer, and 7.7% observed in the femoral attachment on the anterior bundle in the lateral layer.

Fig. 5 Strain distribution in the medial, middle and lateral layers of the ACL in response to 50 N of anterior force to the knee. Each corn indicates the strain in each region.

Table 2 Strain distribution in the medial layer of the ACL in response to 50 N of anterior force to the knee. (Mean $\pm$ S.D., * $p < 0.05$ vs 20-80% of length (mid-substance))

Width						
Length		0-20%	20-40%	40-60%	60-80%	80-100%
0-10%		4.1 (5.3)	2.4 (2.3)	2.3 (2.2)	1.0 (1.2)	1.9 (2.0)
10-20%		1.7 (2.2)	2.4 (1.5)	3.6 (3.2)	3.7 (2.2)	2.8 (3.3)
20-80%		1.4 (1.6)	1.2 (1.0)	2.1 (1.8)	1.8 (1.4)	2.2 (2.4)
80-90%		*6.2 (4.9)	*7.6 (5.5)	1.5 (2.0)	4.7 (3.9)	*4.2 (2.6)
90-100%		5.1 (6.7)	5.4 (6.7)	*5.6 (3.6)	*8.0 (5.9)	*7.1 (3.7)

Table 3 Strain distribution in the middle layer of the ACL in response to 50 N of anterior force to the knee. (Mean $\pm$ S.D., * $p < 0.05$ vs 20-80% of length (mid-substance))

Length \ Width	Width				
	0-20%	20-40%	40-60%	60-80%	80-100%
0-10%	3.4 (4.2)	4.7 (4.2)	1.6 (1.5)	2.2 (2.2)	2.0 (3.0)
10-20%	2.5 (2.0)	2.0 (2.3)	*3.1 (2.4)	5.3 (6.4)	2.2 (2.0)
20-80%	1.4 (1.5)	1.5 (1.2)	1.4 (0.7)	2.5 (2.6)	3.2 (2.8)
80-90%	*4.5 (4.6)	*5.1 (4.6)	4.9 (5.7)	5.3 (3.5)	*5.8 (4.4)
90-100%	*4.2 (3.8)	3.7 (4.6)	5.1 (6.3)	*6.3 (5.5)	6.4 (4.6)

Table 4 Strain distribution in the lateral layer of the ACL in response to 50 N of anterior force to the knee. (Mean $\pm$ S.D., * $p < 0.05$ vs 20-80% of length (mid-substance))

Length \ Width	Width				
	0-20%	20-40%	40-60%	60-80%	80-100%
0-10%	4.4 (6.4)	7.7 (14.9)	1.9 (1.6)	1.2 (1.1)	2.2 (2.0)
10-20%	2.9 (4.7)	2.7 (2.6)	2.8 (2.8)	4.2 (3.8)	2.9 (2.8)
20-80%	1.3 (1.3)	1.2 (0.8)	1.8 (2.2)	2.3 (2.1)	1.6 (1.1)
80-90%	*5.1 (4.6)	*3.8 (3.6)	2.6 (4.1)	*5.4 (4.1)	*6.1 (5.0)
90-100%	*3.8 (3.1)	1.0 (1.5)	5.3 (7.8)	5.5 (5.2)	*4.9 (2.2)

Microscopic observation indicated that the periodic patterns (crimp structure) at a right angle to the fiber direction were recognized in unloaded condition in all the areas (Fig.6). The periodic pattern was more clearly observed in the femoral and tibial attachment areas as compared with in the mid-substance area.

Fig. 6 Microscopic observation of the crimp structures in the femoral attachment, the mid-substance and the tibial attachment areas of the porcine ACL.

4. Discussion

The strain distribution in the ACL in response to anterior tibial load at knee full extension was determined using an image correlation method in the present study. Results revealed that the ACL strain in the mid-substance gradually and almost linearly increased with the increase of anterior load. In contrast, in the femoral and tibial attachment areas, the strain increased rapidly at the beginning of anterior loading and gradually thereafter. Thus, our data clearly defines the load-dependent non-linearity of the ACL strain in the attachment areas.

Results also revealed that large ACL strain was observed near the tibial and femoral attachment areas in response to 50 N of anterior load in all the layers. Large strain was also previously observed near the attachment areas of the ACL (6-8% in their scale) in response to 3 mm of tensile displacement (Sparazzi et al., 2006). Although a simple comparison

is not possible between the two studies because of a difference in knee loading condition, the trend of site-dependency of the ACL strain is similar.

In the lateral layer, the ACL strain tended to increase in the femoral attachment in spite of no significant difference observed. Although there is no definite reason for the result, previous anatomical studies indicated that the femoral attachment of the ACL can be separated into two different fiber types, the direct insertion and indirect insertion (Mochizuki et al., 2014; Sasaki et al., 2012; Smigielski et al., 2015). The ACL fibers connected to the direct insertion are flexible and can respond to forces from any direction, while the ACL fibers connected to the indirect insertion are simple fibers and work like anchors (Mochizuki et al., 2014; Sasaki et al., 2012). Since the ACL fibers in the lateral layer mainly consist of the former fibers in the femoral attachment, the strain behavior in response to an anterior load may be different as compared with those in the medial and middle fibers.

Ligaments and tendons have a crimp structure at the microscopic level. The crimp structure in ligaments and tendons was found to suggest that fibrillar crimps acted as biological hinges while absorbing tensile deformation (Franchi et al., 2012). They also reported that the crimp structure was more located near both insertion sites of ligaments and tendons than in the mid-substance. A wavy crimp structure was also found in the proximal one quarter of the ACL (Weiss et al., 2012). Microscopic observation in the present study indicated that crimp structures existed in all the areas of the ACL, however, it was more clearly observed in the femoral and tibial attachment areas than in the mid-substance area. In addition, black bands were more clearly observed within the waves near the attachment areas as compared with in the mid-substance. We believe that these bands were valley of the waves in the crimp structure. It is, therefore, suggested that the wave depth of the crimp structure was larger in the attachment areas than that in the mid-substance. These microstructural features may be attributable to the load- and site-dependent ACL strain found in the present study.

In previous studies, 2-D or 3-D video tracking systems were often used to measure the deformation and/or strain in soft tissues (Lujan et al., 2005; Phatak et al., 2007). Although measurement with high resolution was possible with those systems, the position and number of markers were limited because markers must be physically attached to the surface of the tissues. It was also problematic in the previous systems that marker motion relative to tissues might affect experimental results. These problems do not exist in the present study due to the use of the new motion tracking method. Since it is possible with the method to select up to 50 arbitrary points for tracking on a recorded image, high-resolution analysis of local strain distribution on the ACL surface was possible.

One of the limitations of the present study is that the surface orientation of the ACL was assumed to remain unchanged during load application for a proper strain calculation. This assumption is appropriate for the present study because rotations at the knee were almost negligible. For example, the largest internal-external rotation was less than 2 degree. But the assumption is not reasonable for other knee flexion angles. This limitation will be addressed in future studies. Another limitation of the present study is that specimens were porcine knees. However, in previous studies, porcine knees were used for biomechanical study of the ACL as a substitute for human knee joint because the size of the joint and ACL are similar to human knee (Liu et al., 1995; Maeyama et al., 2011).

Our study suggested that the strain in the ACL at full extension is site- and load-dependent in a non-linear manner at the femoral and tibial attachment areas. This feature may be attributable to the existence of much more clear crimp structures located at these areas. Because of the unique and complex characteristics of the ACL strain behavior, the mechanical function and stability of the knee joint are probably maintained in response to various physiological conditions. Obtained results regarding the ACL strain distribution are useful for both understanding ACL biomechanics and improving the ACL reconstruction technique.

Acknowledgement

The present study was financially supported by the JSPS KAKENHI (#16J06537), the Grant-in-Aid for Scientific Research (B) (#16H03172) from the MEXT, Japan, and the Priority Research Found from Tokyo Metropolitan University.

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