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Dehydration rates of meniscus and articular cartilage in vitro using a fast and accurate laser-based coordinate digitizing system

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Abstract

When used in in vitro studies, soft tissues such as the meniscus and articular cartilage are susceptible to dehydration and its effects, such as changes in size and shape as well as changes in structural and material properties. To quantify the effect of dehydration on the meniscus and articular cartilage, the first two objectives of this study were to (1) determine the percent change in meniscal dimensions over time due to dehydration, and (2) determine the percent change in articular cartilage thickness due to dehydration. To satisfy these two objectives, the third objective was to develop a new laser-based three-dimensional coordinate digitizing system (3-DCDS II) that can scan either the meniscus or articular cartilage surface within a time such that there is less than a 5% change in measurements due to dehydration. The new instrument was used to measure changes in meniscal and articular cartilage dimensions of six cadaveric specimens, which were exposed to air for 120 and 130 min, respectively. While there was no change in meniscal width, meniscal height decreased linearly by 4.5% per hour. Articular cartilage thickness decreased nonlinearly at a rate of 6% per hour after 10 min, and at a rate of 16% per hour after 130 min. The system bias and precision of the new instrument at 0° slope of the surface being scanned were 0.0 and 2.6 μm, respectively, while at 45° slope the bias and precision were 31.1 and 22.6 μm, respectively. The resolution ranged between 200 and 500 μm. Scanning an area of 60 × 80 mm (approximately the depth and width of a human tibial plateau) took 8 min and a complete scan of all five sides of a meniscus took 24 min. Thus, the 3-DCDS II can scan an entire meniscus with less than 2% change in dimensions due to dehydration and articular cartilage with less than 0.4% change. This study provides new information on the amount of time that meniscal tissue and articular cartilage can be exposed to air before marked changes in size and shape, and possibly biomechanical, structural and material properties, occur. The new 3-DCDS II designed for this study provides fast and accurate dimensional measurements of both soft and hard tissues.

Keywords: Meniscus; Articular cartilage; Laser; Dehydration; Thickness; Knee; Size; Coordinate digitizing system

1. Introduction

When studying hydrated tissues, such as the meniscus or articular cartilage, it is important to understand the effects of dehydration on the tissues. Due to the large water content of menisci and articular cartilage (between 60% and 80% by weight), these musculoskeletal tissues are susceptible to dehydration (Adams and Huskins, 1992). Dehydration of the meniscus during exposure to air has been visually observed in the form of surface undulations (Moshurchak and Ghadially, 1978; Ghadially et al., 1983). Also, as meniscal tissue and articular cartilage are exposed to air, water loss and concomitant shrinkage may occur. Material and structural properties also could be affected by water loss particularly those that are influenced by the viscoelastic behavior which is governed in part by the water content (Chimich et al., 1992; Haut and Haut, 1997; Race et al., 2000; Hoffman et al., 2005).

Meniscal tissue hydrated with water is exposed to air during harvesting and dissection procedures, and during allograft sizing. Previous studies have shown that meniscal size and shape can strongly affect the tibiofemoral contact pressure distribution and the stresses within the meniscus. A 10% change in meniscal dimensions can result in as much as a 10% change in contact pressure variables

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(Donahue et al., 2004). Also meniscal size and shape have a greater effect on stresses in the meniscus than material properties (Meakin et al., 2003). Currently, there is no information available on the effects of dehydration on meniscal tissue size and shape. Therefore, one objective of this study was to determine the percent change in meniscal dimensions over time due to dehydration.

Articular cartilage is also exposed to air during harvesting, dissection and experimentation. With a large surface area-to-volume ratio, the effects of dehydration on the size and shape of articular cartilage may be even more pronounced than those of the meniscus. Thus, another objective of this study was to determine the percent change in articular cartilage thickness over time due to dehydration.

To study the effect of dehydration on meniscal tissue and articular cartilage, a fast and highly accurate method of measuring the size and shape of the tissues is needed. Laser scanning is an accurate method of measuring the size and shape of soft tissues (Bhat and Smith, 1994; Ibbett et al., 1994; Patete et al., 1996; Kusnoto and Evans, 2002; Tognola et al., 2003; Avis et al., 2004; Harrison et al., 2004). However, to scan structures within the knee joint, the joint must be disarticulated to expose the soft tissue or bone surface to the laser beam. Common methods of soft tissue specimen hydration, such as saline baths, cannot be used because they interfere with laser measurements. Thus, the tissues must remain exposed to air.

Although a previous non-contacting, laser-based, three-dimensional coordinate digitizing system (3-DCDS) has been used to record the three-dimensional size and shape of the menisci and articular cartilage in human knee joints (Haut et al., 1998), one possible limitation of this system is the time in which a full scan can be completed. The five scans needed to develop a complete three-dimensional model of the meniscus require a total time of about 60 min. Exposing the meniscus to air for this amount of time may cause dehydration of the tissue and resultant error in size and shape. Therefore, the final objective of this study was to develop a new laser scanning system that can perform a complete scan of either the meniscus or articular surface within a time such that there is less than a 5% change in measurements of meniscal size and shape, as well as cartilage thickness due to dehydration.

2. methods

2.1. Design of 3-DCDS II

The high-speed 3-DCDS II diagrammed in Fig. 1 was based on the 3-DCDS developed by Haut et al. (1998). X- and Y-coordinates are controlled by X and Y positioning stages while Z-coordinates of surface points are measured with a semiconducting laser-based displacement sensor. An X–Y table (102004P, Parker Automation, Daedal Division, Irwin, PA), composed of two stepper-motor-driven, linear positioning stages, was disassembled into individual stages. The motor drivers are controlled by a 2-axis controller (6K2, Parker Automation, Daedal Division, Irwin, PA) that communicates to the PC through an ethernet connection.

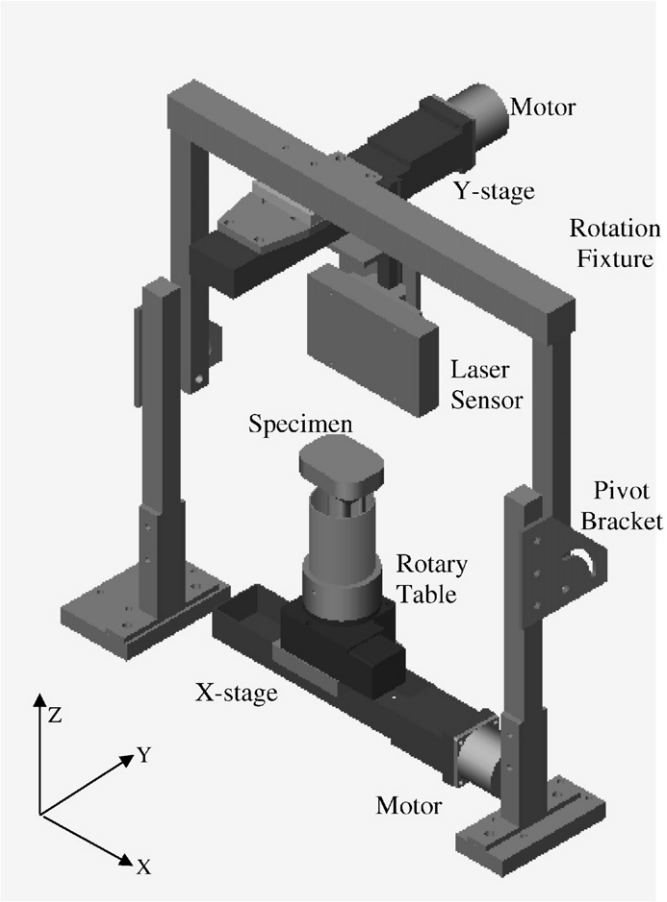

Fig. 1. Diagram of the 3-DCDS II in the configuration to scan the superior surface of the meniscus. The X stage, mounted to a solid marble base, controls translation of the specimen in the X-direction, while the Y stage, mounted above the specimen, controls translation of the laser displacement sensor in the Y-direction. The laser displacement sensor is mounted above the tissue on the Y stage by a guide block that can be easily translated in the Z-direction to achieve the working distance of the laser displacement sensor.

Table 1
3-DCDS II component specifications

X–Y positioning table (102004P, Parker Automation, Daedal Division, Irwin, PA)
Resolution: 101,600 steps/rev
Travel: 100 mm
Laser (AR600-1, Schmitt Measurement Systems, Portland, OR)
Wavelength: 650 nm
Beam size: 60 μm
Working distance: 76.2 mm
Measurement range: ±12.7 mm
Measurement precision: 2.54 μm
Sampling rate: 1250 Hz maximum

The laser displacement sensor is an inexpensive, commercially available device (AR600-1, Schmitt Measurement Systems, Portland, OR) that uses laser triangulation to measure distance in the Z-direction with a precision of 2.54 μm. The device sends measurements to the PC through serial RS-232 communication at a baud rate of up to 56 kBd/s. Refer to Table 1 for specifications for both the X–Y positioning table and laser displacement sensor.

A Visual Basic program, running on a PC, sends positioning commands to the 2-axis controller to move the specimen beneath the laser in rows until the entire surface of the specimen is scanned. As the specimen moves beneath the laser, the computer continuously records X- and Y-coordinates from the controller, as well as Z-coordinates from the laser displacement sensor. The result is a point cloud of coordinates taken from the tissue surface with row spacing of 500 μm and point spacing within each row of 200−500 μm.

When scanning a meniscus, the side of the tissue can be scanned by manually rotating the laser and Y stage by 90° about the X-axis (Fig. 2). To scan the remaining sides of the meniscus, the specimen is rotated about the Z-axis by a computer-controlled, stepper-motor-driven rotary table (RT-3, Newmark Systems, Mission Viejo, CA). The rotation fixture consists of an aluminum pivot bracket that rotates 90° ± 1° (80/20 Inc., Columbia City, IN). By keeping the specimen horizontal in the gravity field, this instrument design eliminates measurement errors that might occur due to shifts in tissue position caused by changing the orientation of the tissue in the gravity field.

The coordinate data captured by the 3-DCDS II can be built into a three-dimensional computer model using either a solid modeling program, such as SolidWorks (SolidWorks Corporation, Concord, MA), or a finite element package such as PATRAN (MSC Software Corp., Santa Ana, CA) or ABAQUS (Hibbitt, Karlsson, Sorensen Inc., Pawtucket, RI). Coordinate data from the sides of the meniscus can be transformed back into the original coordinate system of the tibial plateau and the surfaces combined to create a complete surface or solid model (Haut et al., 1998).

2.2. Accuracy testing

To evaluate the bias (i.e. systematic error) and precision (i.e. random error) of the motion control along the X and Y stages, movement along either the X or Y stage was commanded by the computer and the change in position for 15 random movements along each stage was measured using a coordinate measuring machine (CMM) (BRT 504, Mitutoyo Corp., Kawasaki, Japan) with a ruby-tipped probe and a precision of 9 μm. For each stage, the errors were calculated as the differences between the commanded changes in position and the measured changes in position. Bias and precision were calculated as the average and standard deviation, respectively, of the errors.

The fixture was also examined to determine whether it introduced any error. With the 3-DCDS II in the position to scan the superior surface of the meniscus (Fig. 1), the angles of the vertical support bars were measured with a digital protractor-level-inclinometer with a precision of 0.1° (SmartTool, M-D Building Products, Oklahoma City, OK). The fixture was then rotated into the position to scan the sides of the meniscus (Fig. 2) and the angle of the support bars was measured again. The measured change in angle was compared against the expected 90.0° and the measurements were repeated 10 times to determine repeatability (i.e. precision). Using previously described methods (Haut et al., 1998), overall system bias and precision for various surface slopes were calculated by combining the bias and precision of the X and Y stages, and the rotating fixture.

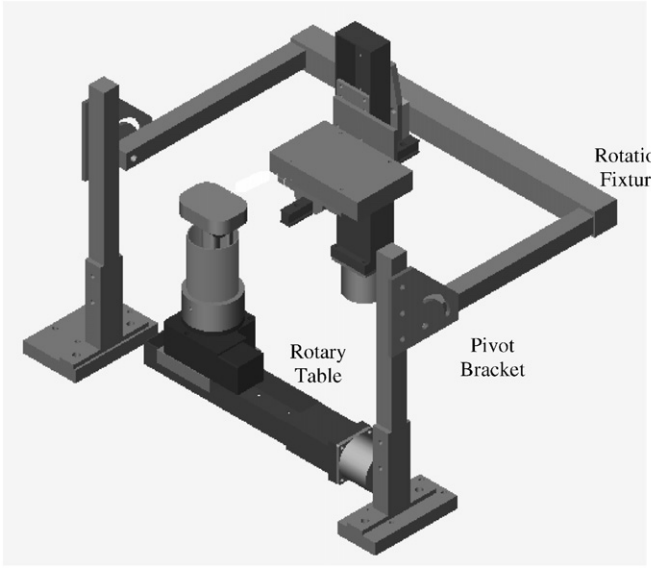

Fig. 2. The 3-DCDS II in the configuration to scan the side of the meniscus. The rotation fixture has been rotated 90° using the pivot bracket so that the laser beam is directed towards the side of the meniscus.

2.3. Determination of dehydration rates

The dehydration rates of meniscus and articular cartilage were determined by scanning six human cadaveric knee specimens (average age 69 years, range 48–79 years) over time. All specimens were free from degenerative arthritis and meniscal tears. The meniscal data from one of the specimens were corrupted, however, and could not be included in the meniscal study. Stored at −20 °C until use in sealed plastic bags, each knee was thawed at room temperature and disarticulated. The soft tissue was removed leaving the medial and lateral menisci attached to the tibial plateau. The distal end of the tibia was potted using PMMA into a fixture for mounting into the 3-DCDS II. The tibial plateau was submersed in saline for 12 h to ensure full hydration of the meniscus based on a pilot study which showed that 4 h were needed for full hydration (Pham, 2007). Approximately every 5 min for 120 min, nine lines (three lines across each region: anterior, middle and posterior) were scanned over the medial meniscus (Fig. 3). Data point spacing was between 200 and 500 μm and each set of scans took approximately 10 s. After 2 h, the menisci were removed and the same nine lines were scanned on the articular cartilage surface.

After the menisci were removed and the articular cartilage was scanned, the cartilage was hydrated for a minimum of 12 h in saline. Articular cartilage thickness was measured by scanning a grid of points uniformly separated by 2 mm on the cartilage surface every 10 min for 130 min. Each scan took between 2 and 4 min and resulted in 190–430 data points, depending on the size of the tibial plateau. The cartilage was then removed by submersing the tibia in 5.25% sodium hypochlorite solution for at least 12 h (Donahue et al., 2002) and the same grid of points was scanned on the tibial plateau. Cartilage thickness was calculated as the difference between Z-coordinate measurements of the intact cartilage and the tibial plateau at identical X–Y-coordinate pairs.

2.4. Data analysis

To evaluate whether the biases of the motion control and fixture rotation were significantly different from zero, t-tests (significance level $\alpha = 0.05$) were performed.

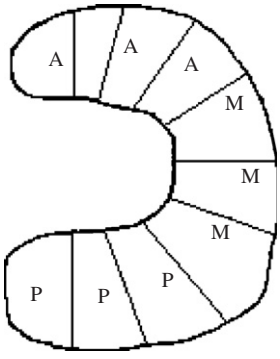

Fig. 3. Lines scanned over the surface of the medial meniscus of a left knee. A = anterior, M = middle, P = posterior.

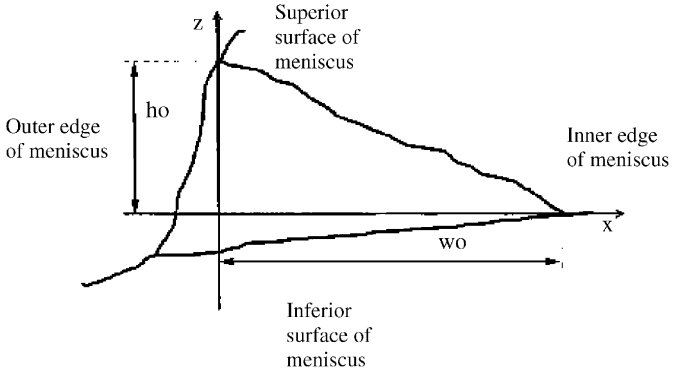

Fig. 4. Cross-sectional standard parameter measurements. A transverse standardized plane was determined for each specimen by a least squares regression of the articular cartilage data points scanned immediately after the meniscus was removed. For cross-sectional scans, a local x-axis was drawn through the inner edge of the meniscus and parallel to the transverse standardized plane and a local z-axis was drawn through the highest point of the meniscus and perpendicular to the x-axis. Meniscal height ho was measured as the distance from the highest point of the meniscus to the x-axis, while meniscal width wo was measured as the distance from the inner edge of the meniscus to the z-axis.

Because the tibiofemoral contact pressure computed via finite element models was most sensitive to meniscal height (ho) and cross-sectional width (wo) (Donahue et al., 2004), these standard parameters were determined using methods described previously (Fig. 4) (Haut et al., 1998) and compared over time. For each specimen, the percent changes in ho and wo at each time point were calculated for each of the nine line scans relative to their original, time zero, heights and widths. For each time point that the meniscus was scanned, the average percent changes in ho and wo were calculated for each region (anterior, middle and posterior) by averaging the three values of percent changes in ho and wo measurements, respectively, of each region of each specimen.

To evaluate whether changes in dimensions were statistically different within the different regions of the meniscus, a one-way analysis of variance (ANOVA) was performed. The independent variable was region with 3 levels (anterior, middle and posterior) and the dependent variable was the percent change in ho or wo after 120 min of exposure to air.

The percent changes in articular cartilage thickness (th) over time were calculated relative to their original, time zero, thickness. Data from all specimens were averaged to calculate an average percent change in thickness each time the articular cartilage was scanned.

The rates of dehydration of the meniscus (percent change in ho over time and percent change in wo over time) and articular cartilage (percent change in thickness over time) were determined by performing a quadratic regression analysis which was forced through zero. Because the ANOVA showed that the region did not significantly affect the percent change in either dimension of the meniscus, the data from all 3 regions were used to determine an overall average percent change in ho and wo at each of 24 time points. The resulting regression coefficients were tested for significance against zero.

3. Results

The high-speed 3-DCDS II was able to scan a 60×80 mm area (the typical depth and width of a human tibial plateau) in 8 min and all five sides needed for a full scan of a meniscus (superior, anterior, posterior, medial and lateral) in 24 min. This was 2.5 times faster than the 20 and 60 min, respectively, achieved with the previous instrument (Haut et al., 1998). The resolution between

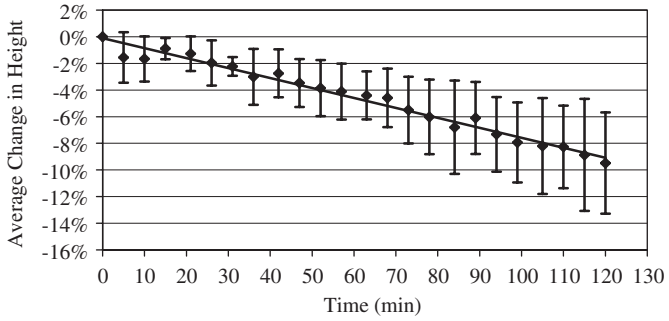

Fig. 5. Average percent change in medial meniscal height ho (±1 standard deviation) over all regions during exposure to air. The height decreased linearly with time. The line is the simple linear regression fit (average change in ho (%) $= -0.0749 * t$ where t is in minutes).

coordinate points was between 200 and 500 μm while the previous instrument resolution was 500 μm.

Over the full range of motion, the X and Y stages had biases of −1.7 and −2.2 μm, respectively, and precisions of 42.8 and 31.0 μm, respectively. The biases were not statistically different from zero ($p = 0.884$ for the X stage and $p = 0.799$ for the Y stage). The bias and precision of the rotation of the fixture were -0.1° and 0.1°, respectively. The bias was significantly different from zero ($p = 0.001$). The overall system bias and precision were bounded by 31.1 and 22.6 μm, respectively, for a slope of 45°, which is the highest slope necessary using the rotating fixture of the 3-DCDS II. The minimum bias and precision for a 0° slope were 0.0 and 2.6 μm, respectively.

The initial average heights of the meniscus for the five specimens were 6.4, 7.7 and 7.2 mm in the anterior, middle and posterior regions, respectively, while the initial average widths were 7.0, 10.8 and 12.1 mm in the anterior, middle, and posterior regions, respectively. The initial average cartilage thickness over the medial tibial plateau for the six specimens was 1.9 mm.

There was no significant difference between the dehydration rates of the anterior, middle and posterior regions of the meniscus ($p = 0.613$ for change in height ho, $p = 0.783$ for change in width wo). While meniscal height ho decreased linearly at the rate of 4.5% per hour (Fig. 5), there was no significant change in meniscal width wo during exposure to air (Fig. 6).

Articular cartilage thickness also decreased over time when exposed to air but the decrease was nonlinear (Fig. 7). Cartilage thickness decreased nonlinearly at a rate of 6% per hour after 10 min, and the rate of thickness decrease increased to 16% per hour after 130 min.

4. Discussion

To measure the dehydration rate of meniscal tissue and articular cartilage, a fast and accurate method of measuring soft tissue size and shape was needed. Thus, one objective of our study was to develop a highly accurate 3-DCDS that was fast enough to capture the size and shape

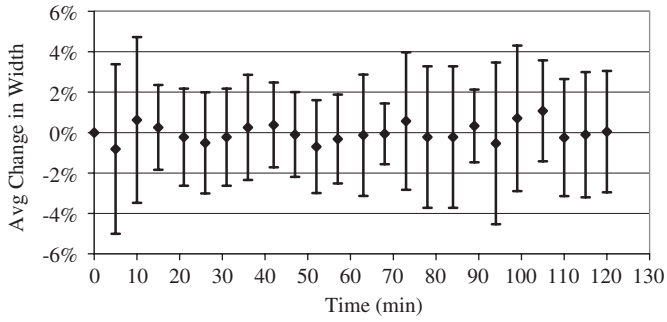

Fig. 6. Average percent change in medial meniscal width wo (±1 standard deviation) over all regions during exposure to air. The width did not change over time.

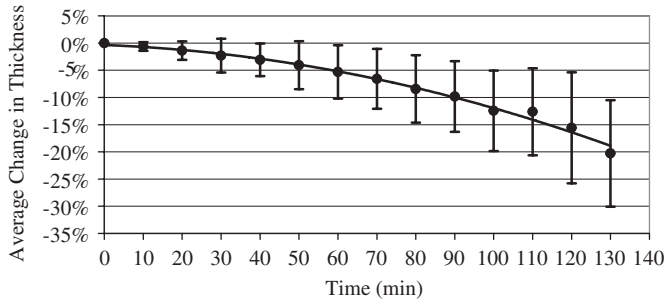

Fig. 7. Average percent change in medial articular cartilage thickness th (±1 standard deviation) during exposure to air. The thickness decreased nonlinearly with time. The line is the quadratic linear regression fit (average change in th (%) $= -0.0382 * t - 0.000818 * t^2$ where t is in minutes).

of musculoskeletal tissues with less than a 5% change in meniscal dimensions due to dehydration. The 3-DCDS II developed to make these measurements was shown to be a fast and highly accurate instrument for capturing the geometry of musculoskeletal tissues. With a total scan time of 24 min, this instrument can scan an entire meniscus with less than 2% change in size and shape. An articular cartilage surface can be scanned in 8 min, with less than 0.4% change in thickness. The speed of this instrument was better than that of the previously built instrument (Haut et al., 1998) by a factor of 2.5. Also, by implementing new software that collects data along a continuous scanning path, the resolution of the 3-DCDS II reached a range of approximately 200−500 μm while the discrete grid of scanned points limited the original 3-DCDS to a resolution of 500 μm. Finally, by maintaining the orientation of the tissue in the gravity field, any changes in shape particularly for the menisci that might occur due to change in the orientation were avoided.

Laser scanning is more accurate than other methods which have been used to quantify the size and shape of musculoskeletal tissues. For example, cartilage thickness has been measured using MRI (McGibbon et al., 2003), CT (Wyler et al., 2007) and ultrasound (Yao and Seedhom, 1999). While these imaging modalities are attractive because disarticulation of the joint is not required, all three modalities are at a disadvantage because the worst case accuracy of laser scanning, which occurs when the surface slope is 45°, is at least 10 times better than MRI and CT both of which are much better than ultrasound. Although stereophotogrammetry offers better accuracy than either MRI and CT (Ateshian et al., 1991; Ronsky et al., 1999), as with laser scanning the joint must be disarticulated and the setup is complex for highest accuracy. Even with multiple cameras (required for highest accuracy), the accuracy is still only comparable to the worst case accuracy for laser scanning.

The improved 3-DCDS II would be useful in a wide variety of applications particularly those that demand high accuracy. In orthopedics, one such application is the development of finite element models of joints. Strains in both hard and soft tissues resulting from contact are of particular interest to characterize the mechanical environment of the cells for accurate study of mechanotransduction (Upton et al., 2006). In the case of the human knee, computations from these models have implications for the design and development of meniscal repair devices and replacements (Meakin et al., 2003; Donahue et al., 2004), provide insight into the etiology of osteoarthritis that frequently is a sequel to partial and/or complete meniscectomy (Pena et al., 2005) and serve to assess functional roles of various tissues (Yao et al., 2006). Another example application is the study of implant wear where high accuracy is needed to quantify the amount of wear.

Applications for our laser scanner abound outside of orthopedics. For example, a highly accurate laser scanner is advantageous in the study of wound healing (Bhat and Smith, 1994; Patete et al., 1996), in determining the size and volume of skin ulcers (Ibbett et al., 1994), in varied orthodontic applications including treatment changes, growth and surgical simulations (Kusnoto and Evans, 2002) and in developing solid models of internal organs (Tognola et al., 2003; Avis et al., 2004).

Because meniscal size and shape are a major determinant of the contact pressure distribution in the tibiofemoral joint within the knee and the stresses developed within the meniscal tissue (Donahue et al., 2004) and because there is currently no information available on the effects of dehydration on meniscal tissue, another objective of our study was to determine the effects of exposure to air on meniscal size and shape. When exposed to air, meniscal height decreased while meniscal width did not change. Based on our regression model, meniscal tissue can be exposed to air for approximately 88 min before heights shrink by 5% and significant changes occur in contact pressure variables in the knee and stresses within the meniscus. One explanation for why the height changed but the width did not is that the menisci were attached to the tissues around its periphery. This likely restricted movement in the direction of the width while leaving the height free to change.

Using the point cluster provided by the 3-DCDS II to create a solid model of the meniscus makes the assumption

that the superior surface of the articular cartilage is in direct contact with the inferior surface of the meniscus during the scanning process. This is a reasonable assumption because the knee is positioned in the 3-DCDS II in an anatomical position. The meniscus is always resting upon the tibial plateau and thus the articular cartilage surface.

One aspect of the methods that merits mention is that the articular cartilage was not re-hydrated between removal of the meniscus and scanning of the articular cartilage for use in meniscal dehydration measurements. However, during scanning of the meniscus, the articular cartilage beneath the meniscus was not exposed to air. Therefore, full hydration of the articular cartilage was maintained.

Because there is no information available on the dehydration rate of articular cartilage and because of the possible susceptibility of articular cartilage to dehydration, another objective of our study was to determine the change in articular cartilage thickness due to dehydration. Based on our regression model, articular cartilage can be exposed to air for approximately 60 min before a 5% change in thickness occurs. The dehydration rate of articular cartilage was higher than the dehydration rate of the meniscus, most likely due to the larger exposed surface-to-volume ratio of articular cartilage compared to meniscal tissue. For an area of the medial meniscus equal to 731 mm^2 covering the medial tibial plateau (Ahmed and Burke, 1983) and a volume equal to 3000 mm^3 (Bowers et al., 2007), the surface area-to-volume ratio becomes about 0.29/mm when corrected for the slope. For an average thickness of 1.9 mm measured in this study for the articular cartilage covering the medial tibial plateau, the surface-to-volume ratio becomes 0.53/mm, which is about a factor of 2 greater than that of the medial meniscus. The somewhat higher water content of articular cartilage relative to the meniscus also might explain the higher dehydration rate for articular cartilage.

Because the age of the specimens tested was elderly, it is of interest to consider whether our findings regarding dehydration rates would apply to younger specimens as well. Presumably, dehydration rate would be influenced by the water content so that an answer to the above question can be obtained by examining the effect of age on water content. One previous study, which tested articular cartilage samples from a single location on the human patella, showed no difference in mechanical properties which depended on water content with age (Armstrong and Mow, 1982). However, another previous study, which tested cartilage samples from two different locations on the femoral head, showed a significant decrease in water content with age at one location but not the other (Venn, 1978). Hence, it is difficult to determine whether age affects the dehydration rate of articular cartilage on the tibial plateau.

Cartilage thickness was measured as the distance between the top surface of the articular cartilage and the surface of the tibia after the cartilage had been removed in the Z-direction. Defining articular cartilage thickness in the Z-direction instead of along a line perpendicular to the surface of the tibia after cartilage removal may overestimate the true thickness of the articular cartilage. However, the articular cartilage thickness of the medial tibial plateau of 1.9 ± 0.9 mm measured in our study was not significantly different from that in previous studies (1.6 ± 0.2 mm, $p = 0.382$ for Eckstein et al., 2001; 1.2 ± 0.2 mm, $p = 0.086$ for females and 1.4 ± 0.2 mm, $p = 0.152$ for males of Faber et al., 2001). Also, because we measured changes in thickness and because it is reasonable to assume that cartilage thickness would change at the same relative rate, our method of measuring cartilage thickness should not affect our dehydration rate results.

In summary, a new high-speed laser scanning system was developed in this study and was used to quantify the effect of dehydration on the size and shape of the meniscus and the thickness of the articular cartilage. Meniscal tissue dehydrated at the same rate across all regions (anterior, middle or posterior). Meniscal height decreased linearly at a rate of 4.5% per hour exposed to air while meniscal width was not affected. Articular cartilage thickness decreased nonlinearly and at a much faster rate of up to 16% per hour after 130 min. Thus, when dissecting and experimenting on meniscal tissue and articular cartilage, they can be exposed to air for 67 and 60 min, respectively, before a 5% change in dimensions occurs due to dehydration.

Conflict of interest

No conflicts.

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