Quasi-Steady-State Displacement Response of Whole Human Cadaveric Knees in a MRI Scanner

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It is important to determine the three-dimensional nonuniform deformation of articular cartilage in its native environment. A new magnetic resonance imaging (MRI)-based technique (cartilage deformation by tag registration (CDTR)) has been developed, which can determine such deformations provided that the compressive load-displacement response of the knee reaches a quasi-steady state during cyclic loading. The objectives of this study were (1) to design and construct an apparatus to cyclically compress human cadaveric knees to physiological loads in a MRI scanner, (2) to determine the number of load cycles required to reach a quasi-steady-state load-displacement response for cyclic loading of human cadaveric knees, and (3) to collect sample MRI images of undeformed and deformed states of tibiofemoral cartilage free of artifact while using the apparatus within a MRI scanner. An electropneumatic MRI-compatible apparatus was constructed to fit in a clinical MRI scanner, and a slope criterion was defined to indicate the point at which a quasi-steady-state load-displacement response, which would allow the use of CDTR, occurred during cyclic loading of a human knee. The average number of cycles required to reach a quasi-steady-state load-displacement response according to the slope criterion defined herein for three cadaveric knee joints was 356 ± 69. This indicates that human knee joint specimens can be cyclically loaded such that deformation is repeatable according to MRI requirements of CDTR. Sample images of tibiofemoral cartilage were obtained for a single knee joint. These images demonstrate the usefulness of the apparatus in a MRI scanner. Thus the results of this study are a crucial step toward developing a MRI-based method to determine the deformations of articular cartilage in whole human cadaveric knees. [DOI: 10.1115/1.2978986]

1 Introduction

It is important to determine the in vitro deformation of articular cartilage in whole human knees for many reasons. First, chondrocytes have been shown to respond to many factors, including their mechanical environment [1,2], by altering rates of synthesis and degradation of cartilage constituents [3]. Though direct mechanisms leading to osteoarthritis (OA) are not well known, it is likely that chondrocyte activity is involved in cartilage degradation [4]. Determining complex cartilage deformation may help explain how OA occurs in the knee. Extensive knowledge of in vivo deformation of articular cartilage in whole human knees additionally will allow validation of constitutive models [5] and biomechanical evaluation of cartilage replacements [6] and procedures for cartilage repair.

MRI has been demonstrated as a noninvasive tool to investigate deformation of articular cartilage. Surface deformation of patellofemoral cartilage has been investigated in vivo [7,8], and thickness and volume deformations of cartilage have been determined in vivo [9,10] using MRI. New MRI-based techniques have been developed to determine three-dimensional (3D) nonuniform deformations throughout the volume of articular cartilage explants. During steady-state cyclic loading of cartilage explants, these techniques allow for noninvasive deformation measurements through the use of either the cartilage deformation by tag registration (CDTR) technique that employs tag line registration [11] or displacement encoding based on phase contrast methods [12].

To use CDTR to obtain cartilage deformation for a knee loaded in its native environment, an apparatus meeting several design criteria is needed. First, cartilage must be cyclically loaded and must reach a quasi-steady-state load-displacement response prior to image acquisition. This is to ensure that image data collected over many cycles are not biased by motion artifacts due to tissue creep. Second, the apparatus must be constructed using MRI-compatible materials. Lastly, the apparatus must induce physiological knee loading. While many devices described in literature are capable of loading cartilage in its native environment in the knee to physiological load levels, these devices are not MRI compatible [13–20]. MRI-compatible knee-loading devices are limited to static loading of the knee in an anterior direction [21] or the patella directly [8], or static loading of small animal models [22]. Some studies investigate cartilage deformation in vivo; however, results may be subject to motion artifact because the knee is not at steady state during imaging, which takes several minutes [9,10]. To the authors’ knowledge, the only MRI-compatible cyclic loading device is limited to the use of cartilage explants [23].

Given the need for a knee-loading apparatus meeting several design criteria to investigate cartilage deformations using CDTR, this study had three objectives. The first objective was (1) to design and construct an apparatus to cyclically compress human cadaveric knees to physiological loads in a MRI scanner. While quasi-steady-state load-displacement has been demonstrated for articular cartilage explants [24], it is unknown whether quasi-steady-state load-displacement can be achieved for whole human cadaveric knees loaded to physiological levels. Therefore, the second objective was (2) to determine the number of load cycles
required to reach a quasi-steady-state load-displacement response for cyclic loading of human cadaveric knees. To indicate the potential for conducting future MRI-based studies of cartilage deformation, a final objective was (3) to collect sample artifact-free images of undeformed and deformed states of tibiofemoral cartilage while using the apparatus within a clinical MRI scanner.

2 Methods

2.1 Design Description. An electropneumatic apparatus was designed and built to cyclically load a whole human cadaveric knee in a clinical MRI scanner (Fig. 1). Restricted to plywood, plastics, aluminum, and 300-series stainless steels, key components of the apparatus included an actuating pneumatic cylinder, femur holding clamp, four degree-of-freedom (4DOF) adjustable attachment device, and laser displacement sensor fixed to a frame designed to fit in a clinical 3.0 T MRI scanner (Siemens Medical Systems, Inc., Erlangen, Germany) having a 58-cm-diameter bore. The apparatus located the knee joint at the isocenter of the scanner bore where a receiving coil could be placed around the knee within the frame. No metallic materials were located within the receiving coil, and metallic loops on the apparatus were located as far away from the imaging site as was practical to minimize image distortion due to local magnetic fields caused by induced current from changing magnetic fields.

Cyclic pressure-controlled compressive loading was applied by a double-acting pneumatic cylinder (3000DVS3.00-2, American Cylinder Co., Inc., Peotone, IL) powered by a computer-controlled system previously used in our laboratory [23]. A maximum load of 1500 N was used to approximate a typical physiological load experienced by the knee during walking of two times body weight [25] for an average male [26] and was expected to result in detectable cartilage deformations. While fixed during loading, the location of the cylinder was adjustable in four degrees of freedom for alignment purposes (details in Sec. 2.2).

To minimize deflection of the apparatus during loading such that it would not affect displacement measurements, a box-shaped plywood frame was used. Deflection due to bending was estimated to be less than 15 μm based on the calculation of bending displacement for an unsupported beam model with a 1500 N load parallel to but offset 0.1 m from the long axis of the frame (the approximate location of the actuating cylinder).

Displacement of the piston rod, and consequently the compensatory displacement of the tibia relative to the femur, was measured with a laser displacement sensor (LB-041/1001(W), Keyence Corporation, Woodcliff Lake, NJ) having 8 μm resolution. The sensor was mounted above the pneumatic cylinder and measured displacement as the change in length of the piston rod extending from the cylinder by sensing an aluminum plate fixed to the piston rod. The sensor could be translated toward or away from the aluminum plate for optimal sensing or removed entirely for apparatus use in a MRI scanner.

2.2 Specimen Alignment. A tibial coordinate system was first established to define the mechanical axis of the tibia. The origin of the coordinate system was the point located midway between the tips of tibial eminences, and the $Z_t$-axis, or the mechanical axis of the tibia [27], was defined as a line passing through a point midway between the lateral and medial maleoli and the origin of the tibial coordinate system ($+Z_t$ proximal). The $X_t$-axis was defined as the cross-product of $Z_t$ and a line connecting the tips of the tibial eminences ($+X_t$ anterior). The $Y_t$-axis was then defined as the cross-product of $Z_t$ and $X_t$ ($+Y_t$ medial for a right knee).

Once the tibial coordinate system was defined, specimens were prepared and attached to a 4DOF adjustable attachment device (Fig. 2), which was subsequently used to locate the tibial mechanical axis for each specimen in 3D relative to the loading axis of the device and thus the loading axis of the apparatus. Whole human cadaveric legs were first prepared by dissecting soft tissues from the tibia and femur at a distance of approximately 13 cm in either direction from the joint line. The foot was removed while maintaining relative alignment of the lateral and medial maleoli by preserving connective tissue between distal ends of the tibia and fibula, which would be important for later imaging of the specimen. The tibia was then potted in an acrylic tube using polymethylmethacrylate (PMMA) and was expected to result in detectable cartilage deformations. While fixed during loading, the location of the cylinder was adjustable in four degrees of freedom for alignment purposes (details in Sec. 2.2).

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Images were obtained using a 3D gradient recalled echo sequence $=1.48$ mm$^2$, and field of view = 380 $\times$ 380 cm$^2$, and knee images were obtained using a 3D gradient recalled echo (GRE) sequence $=20.6$ ms, $=9.625$ ms, flip angle = 20 deg, slice thickness = 1.5 mm, spacing between slices = 0 mm, number of averages = 1, in-plane resolution = 0.47 $\times$ 0.47 mm$^2$, and field of view = 240 $\times$ 240 cm$^2$.

The locations of landmarks defining the mechanical axis of the tibia and the loading axis of the 4DOF device were determined and used to find adjustment values for the 4DOF device. First, the group of three Delrin spheres on the 4DOF device (Fig. 2) was used to establish a local coordinate system located at the center of rotation of the device. Then, using transformation matrices, the tibial landmarks and center of the single sphere on the end of the 4DOF device were found relative to this local coordinate system [28]. These markers were used to compute anterior-posterior (A-P), medial-lateral (M-L), flexion-extension (F-E), and varus-valgus (V-V) adjustments required to align the mechanical axis of the tibia with the loading axis of the device.

Once adjustment values were determined, the plastic 4DOF device was replaced with an aluminum version of the device, aligning the loading axis with $Z_t$. After removing the plastic 4DOF device, the acrylic tube was potted in an aluminum tube using PMMA to increase the rigidity of the tibial end of the specimen. Using computed adjustment values, the aluminum 4DOF device was attached to the distal end of the tibia, achieving rotation adjustments to the closest 0.5 deg increment and translation adjustments to the nearest millimeter. A pin with an orthogonal thru hole projected from the most distal hinge part (in lieu of the cylindrical compartment located on the plastic version of the device), which could be slipped into a mating part on the end of the piston rod of the cylinder to fix the alignment of the loading axis of the apparatus with the mechanical axis of the tibia.

A reference position was then determined for each specimen. The knee was flexed and extended repeatedly outside of the apparatus with the tibia fixed horizontally to locate the flexion-extension axis (or the cylindrical axis [29]) through the centers of circles estimated from the posterior portions of the femoral condyles [30]. This axis was marked on the knee. The femoral head and greater trochanter were next removed from the femur, and the remaining femoral bone was potted in an aluminum tube using PMMA. The femoral bone tube was inserted into the femur clamp on the loading apparatus, and the 4DOF device was fixed to the piston rod of the cylinder (Fig. 3). The apparatus allowed adjustment of the knee specimen in six degrees of freedom, and the specimen was moved such that the F-E axis marked on the specimen was aligned with the F-E axis denoted on the apparatus. A-P, M-L, and compression-distraction (C-D) translations in addition to V-V and internal-external (I-E) rotations were achieved by moving the slotted plate on which the pneumatic cylinder was attached relative to the frame of the apparatus, while F-E rotation, additional I-E rotation, and additional C-D translation were achieved with the femur clamp. After locking the tibial side of the specimen in place and locking I-E rotation and C-D translation in place on the femoral side, the tibial mechanical axis was aligned with the loading axis of the apparatus and the F-E axis was located in a horizontal plane. The third V-V axis was allowed to exist as the cross-product of these two axes [31]. The specimen was then manually extended on the femoral side until resistance was felt and defined to be in a 0 deg flexion reference position from which either hyperflexion or extension angles could be achieved.

2.3 Determination of Cycles to Quasi-Steady State. The number of load cycles required to reach a quasi-steady-state load-displacement response was determined for three human cadaveric
knees. Each of three cadaveric knees (all male, age range of 36–52 years) free of OA or knee surgery as determined by physical examination and viewing A-P and lateral radiographs was prepared as described in Sec. 2.2, flexed 10 deg from its 0 deg reference position, and loaded to 1500 N for 900 cycles in which each cycle had a total cycle duration of 10 s with a load duration of 2 s. Loading occurred within a few hundred milliseconds and was of sufficient duration to allow for MRI data acquisition [23]. Nine hundred cycles were applied to ensure that once quasi-steady state was met, it was maintained over the amount of time required to acquire images (320 cycles or approximately 53 min). Displacement was recorded in the direction of compression during loading using the laser displacement sensor, and the average number of cycles required to reach a quasi-steady-state load-displacement response according to a slope criterion defined below was determined for the three specimens. Average variation of the displacement after quasi-steady-state was achieved was also ascertained.

The number of cycles required to reach quasi-steady state was determined by comparing the slope of recorded displacement during load application versus time data with a slope criterion defined by linear regression analysis to avoid motion artifacts in MR images [23]. The slope criterion was defined as being equal to 150 μm/3200 s in which 150 μm corresponded to half the desired MRI spatial resolution of 300 μm, and 3200 equals (10 s/cycle)×(320 cycles). A spatial resolution of 300 μm was chosen because cartilage in the knee was estimated to be 3–5 mm thick, which would allow for up to five to eight tag lines in CDTR [11] or 10–15 pixels to characterize deformation through the thickness using phase contrast methods [12]. Data were recorded every three load cycles for each experiment to minimize data processing time and storage requirements, and the slope of groups of ten data cycles (derived from a total of 28 load cycles) was iteratively compared with the slope criterion. Specifically, the slope was first computed for cycles 3–30 and compared with the slope criterion. If quasi-steady state was not achieved, then the slope for cycles 6–33 was computed and compared with the slope criterion. The number of cycles required to achieve quasi-steady state was reported as the first cycle of the first group of ten data cycles, which was less than or equal to the slope criterion. To express variability of displacement data once quasi-steady state according to the slope criterion was achieved, the range for which all quasi-steady-state displacement data were within 95% of the mean quasi-steady-state displacement was calculated as ± two standard deviations about the mean of quasi-steady-state displacement data recorded over 320 cycles starting with the first cycle at which quasi-steady state occurred. Variability of displacement once quasi-steady state was achieved was computed to determine whether these displacements were within CDTR resolution requirements.

2.4 Demonstration of Use. MR images of tibiofemoral cartilage in the knee of one specimen were collected using the apparatus in a 3.0 T clinical MRI scanner (Siemens Medical Systems, Inc., Erlangen, Germany). A single knee specimen (male, 36 years), free of either OA or previous knee surgery as determined by physical examination and A-P and lateral radiographs, was prepared as described above and flexed 10 deg in the apparatus. After application of over 500 cycles to ensure a quasi-steady-state load-displacement response of the specimen, load was removed while an image depicting the uncompressed state of cartilage of the medial femoral condyle and tibial plateau in the sagittal plane was obtained using GRE (TR=40 ms, TE=7.45 ms, flip angle =10 deg, number of averages=12, slice thickness=8 mm, resolution=446×446 μm², and field of view=200×200 mm²). A constant load of 1500 N was then applied, during which an image depicting the compressed state of cartilage in the same plane was obtained using the same imaging parameters. The undeformed and deformed images were each acquired in approximately 5 min.

3 Results

A quasi-steady-state load-displacement response according to the slope criterion described herein was reached during cyclic loading of whole human cadaveric knee specimens (Fig. 4). The average number of cycles required to reach quasi-steady state defined by the slope criterion was 356 (range 276–399) and the average variability over 320 cycles once quasi-steady-state was reached was ±79 μm.

Sample MR images were collected, illustrating the usefulness of the apparatus to load cadaveric knees in a MRI scanner (Fig. 5). Two sagittal views of the same image plane (446×446 μm² in-plane resolution) depict unloaded and loaded states of cartilage on the tibial and femoral surfaces of the medial condyle after having been cyclically loaded to quasi-steady state. Deformation can be observed between the undeformed and deformed images in the same area indicated by arrows in Figs. 5(b) and 5(c).

4 Discussion

There is a need to better characterize the 3D deformation of articular cartilage in its natural environment and to establish the feasibility of using MRI-based methods, which require that cartilage be cyclically loaded. Accordingly, the objectives of this study were (1) to design and construct an apparatus capable of applying cyclic compressive physiological loads to human cadaveric knees while operating in the bore of a MRI scanner, (2) to determine the number of load cycles required to reach a quasi-steady-state load-deformation response for cyclic loading of knees, and (3) to collect sample MR images of undeformed and deformed states of tibiofemoral cartilage free of artifact while using the apparatus within a MRI scanner. The key findings were that (1) the system consisting of the apparatus with a cadaveric knee reached a quasi-steady-state load-displacement response according to the slope criterion described herein within 400 cycles for a load cycle having a 10 s total cycle duration and 2 s of load application during which 1500 N of compression were applied, and (2) the apparatus was used within the bore of a MRI scanner to load a whole cadaveric knee while images free of artifact were collected of articular cartilage in undeformed and deformed states. Through these
key findings, this study significantly advances MRI-based methods for determining cartilage deformation. Aspects of the methods used to provide these findings should be reviewed to appreciate the conditions of applicability of the method.

Despite use of a physiological load level in compression, the knee was likely subjected to constraint loads. Sophisticated knee-loading machines allow coupled motion to occur during loading because restricting this motion causes unnatural constraint loads [20]. Under compression loading at fixed angles of flexion as occurred in this study, coupled motions occur in all four remaining degrees of freedom [32]. Consequently, the pattern of articular contact would likely be affected by coupled motions as would the corresponding 3D deformations throughout the volume of the tissue. Although incorporating mechanisms to allow coupled motion was considered, it was deemed impractical because the inertia (of the specimen and associated apparatus mechanisms) estimated in allowing coupled motions indicated that the time limits required for deformation measurements by MRI were insufficient for motion to be completed.

To meet imaging and load level requirements, the timing of the load cycle was necessarily different than what might be approximated with a normal walking cadence [33, 34]. A 2 s load application time compared with less than 1 s in walking was used primarily because lengths of tubing to supply the air cylinder resulted in a relatively long time to full load application (approximately 0.9 s). Additional time would then be required to image deformed cartilage. A relatively long total cycle duration of 10 s was used because this total cycle duration was predicted to result in a smaller number of cycles required to reach quasi-steady-state, which was observed to occur for cartilage explants [23]. To reduce the amount of time required to collect image data and hence achieve a load cycle more representative of normal walking, the lengths of tubing could be shortened, the rigidity of the apparatus could be increased, and/or faster imaging protocols could be used [35].

Because viscoelastic materials were used to construct the apparatus to meet material constraints, a performance evaluation was conducted to determine the number of cycles required to minimize these effects [28]. Results obtained during cyclic loading of a steel rod, preconditioned apparatus and steel rod, 4DOF device, and specimens without knees suggest minimal advantages to preconditioning the apparatus. Because the average number of cycles required to reach quasi-steady state according to the slope criterion defined herein for whole knee specimens was greater than an order of magnitude from the other tests, the apparatus should reach quasi-steady state during loading of a whole knee specimen without preconditioning the apparatus.

The average number of cycles to reach quasi-steady state in cyclic loading of cadaveric knees according to the slope criterion defined herein is consistent with expectations. While a quasi-steady-state load-displacement response was observed to occur after 35–50 cycles for cartilage explants [23], a greater number of cycles as observed in this study was expected for whole knee joints because there are more biological tissues (quantity and type) being loaded. The value of the quasi-steady-state slope criterion used for whole knee specimens as compared with cartilage explants should be considered because adjusting this value will change the number of cycles required to reach quasi-steady state. A greater quasi-steady-state slope criterion (defined in Sec. 2.3) was used for whole knee specimens as compared with cartilage explants [23] because a clinical scanner cannot achieve the same high resolution that a small-bore 7.05 T scanner can achieve for cartilage explants. That is, the numerator of the slope criterion was 150 μm (half the desired image resolution possible with a 3.0 T clinical scanner) instead of 25 μm for a 50 μm in-plane image resolution possible with a 7.05 T research scanner. A greater number of cycles to reach quasi-steady state would be expected if the slope criterion used to evaluate cartilage explants for a 10 s total cycle duration (25 μm/(10 × 160 cycles)) was used for this study. In cartilage deformation studies, image acquisition with 300 μm resolution results in a strain bias of zero and a strain precision less than 5.3% [36]. Improved strain precision can be achieved by either increasing the tag line spacing (in thick cartilage) or increasing the image spatial resolution.

Although the approximate 53 min required to image the tissue after reaching quasi-steady state is reasonable, this time might be reduced. Faster imaging techniques such as steady-state free-precession (SSFP) [35] as compared with fast spin echo (FSE) sequences used with CDTR [11] would reduce the time to image cartilage by allowing more data to be obtained during each load cycle. Alternatively, faster imaging techniques could allow acquisition of additional image slices during the 53 min time interval. This is because the denominator of the slope criterion is based on the total cycle duration and the number of cycles required to acquire a specified amount of data. Acquisition of additional image slices could be used to increase the resolution of data in the direction orthogonal to image planes (smaller spacing between im-

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**Fig. 5** Example images in the sagittal plane of a specimen depicting undeformed (b) and deformed (c) states of cartilage of the knee taken from the region of interest shown in the original unloaded cartilage image (a). Solid arrows denote an area of observable deformation. Deformation analysis cannot be performed between these two images because 3D registration of the cartilage volume is required to account for any out-of-plane cartilage displacement. A 3D registration was not performed because CDTR has not yet been adapted for use with a clinical scanner and therefore could not be applied to establish and track tag lines over a 3D volume.
age slices over a given distance) or could be used to capture data over a larger volume of tissue (more slices with the same spacing between images).

Sample MR images, depicting cartilage in undeformed and deformed states (Fig. 5), indicate the potential for using the apparatus to obtain MR images free from artifact required to determine accurate 3D nonuniform cartilage deformations in whole cadaveric knees. Nonuniform strain fields are known to occur in cartilage explants [24] and will occur in the whole knee. Cartilage deformation (i.e., change in tissue thickness) is difficult to observe between MR images of Fig. 5 and may be due in part to out-of-plane motion and relatively large slice thickness. However, coupled motion of the knee was fixed during loading, and negligible overall motion of the knee was observed aside from that in the C-D direction. Given that there was an apparent shift in the location of the knee and cartilage in the C-D direction in the image (as evidenced by performing an image subtraction), we estimated that any out-of-plane displacement was negligible in comparison to any in-plane displacement visible in the image. These images also may be subject to small amounts of rebound and creep given that images took several minutes to obtain. Future imaging protocols will require gating of MRI and loading apparatus actions. Gating of MRI and load apparatus actions is critical given that the displacement-sensitive spin state decays on the order of $T_1$ of the tissue [11]. Nevertheless, the apparatus described herein allowed highly repeatable displacement of tibiofemoral cartilage in a whole cadaveric knee in a MR scanner. This is an important step in adapting CDTR from use with a 7.05 T research scanner, in which cartilage explants can fit, to use with a 3.0 T clinical scanner, which can accommodate whole knee specimens.

Development of the MRI techniques will aid in more detailed measurements of cartilage deformations. To effectively use the CDTR technique, in-plane image resolution should be improved to at least $300 \times 300 \ \mu m^2$, and gating of the pulse sequence for cyclic loading should be coded into the MR sequences to obtain images over many loading cycles. Alternatively techniques other than CDTR might be developed, which enable pixel-level deformation measurements. For example, either navigator echoes [37] or displacement encoding with stimulated echoes (DENSEs) followed by a fast imaging sequence [12] would enable high resolution of tissue deformation at each pixel without motion artifacts.

With further advancement of the MR imaging techniques, a method to obtain 3D nonuniform deformations in articular cartilage in the knee will have valuable applications. Three-dimensional strain tensors obtained using CDTR may be used to validate and enhance current constitutive models of cartilage, which currently account for anisotropic material properties and heterogeneous strain distributions [38]. Such a method might also be extended to validate and enhance finite element tibiofemoral contact models involving additional anisotropic and viscoelastic tissues such as the meniscus [39]. Importantly, recently emerging cartilage tissue engineering techniques driven by the prevalence of OA and other degenerative processes will require gross biomechanical evaluation in a living subject. While engineered cartilage replacements depend on a great number of factors [40,41], determination of 3D strain patterns may be one way in which replacements can be functionally evaluated.

In summary, this study advances MRI-based methods for determining 3D cartilage deformation. The apparatus applies cyclic compressive loads at physiological levels to human cadaveric knees in the bore of a MRI scanner. Using this apparatus, the ability of whole human cadaveric knees to reach quasi-steady-state load-displacement was demonstrated, and the number of cycles required to reach a quasi-steady state was determined. Finally, sample MR images of undeformed and deformed states of tibiofemoral cartilage were collected to indicate that high-quality MR images can be obtained while using the apparatus in a MR scanner. Hence the contributions of our study are an important and essential step toward developing a MRI-based method to determine 3D cartilage deformations in whole human cadaveric knees.

References
