Bony Structures at the Attachment Areas of the Cruciate Ligaments in Human Knee Joints and Their Clinical Significance-Studied Using the P45 Plastination Technique

Estructuras Óseas en las Áreas de Inserción de los Ligamentos Cruzados en las Articulaciones de la Rodilla Humana y su Importancia Clínica, Estudiadas Mediante la Técnica de Plastinación P45

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SUMMARY: For treating cruciate ligament injuries, especially for characterizing the mechanics of the tunnel in cruciate ligament reconstruction, correctly understanding the bony information of the attachment area of the cruciate ligaments is significant. We studied 31 knee joints of middle-aged Chinese adults using the P45 sheet plastination technique, focusing on the attachment areas of the cruciate ligaments, especially the bony structures. The trabeculae at the attachment area were distributed radially and extended deep into the medial wall of the lateral condyle of the femur. However, in the anterior part of the intercondylar eminence, the trabeculae of the anterior group were parallelly arranged along the tendinous fibers of the anterior cruciate ligament fibers. Similarly, at the attachment area of the lateral wall of the medial condyle of the posterior cruciate ligament, the trabeculae extended radially toward the deep medial condyle. Deep in the posterior part of the intercondylar eminence, the trabeculae were parallelly arranged along the cruciate ligament, the trabeculae were arranged longitudinally. In the anterior part of the intercondylar eminence, the trabeculae were parallelly arranged along the perpendicular directions of ligament fibers. The distribution patterns of the trabeculae were arranged longitudinally. In the anterior part of the intercondylar eminence, the trabeculae were arranged longitudinally. The distribution patterns of the trabeculae were art the attachment areas of the cruciate ligaments at the ends of the femur and tibia were different. This difference should be considered when orthopedic surgeons reconstruct anterior cruciate ligaments.

KEY WORDS: Knee joint; Cruciate ligament; Trabecula bone; P45 plastination technique.

INTRODUCTION

The knee joint is the largest and most complex joint in the human body. The knee joint injury is an essential and difficult point in clinical work, especially in treating the ligament injury of the knee joint (Ayre *et al.*, 2017). The cruciate ligament is one of the important factors in stabilizing the knee joint during movement. If a cruciate ligament injury is not cured for a long time, knee osteoarthritis (KOA) is very likely to be progressed, causing great pain to the patient (Schelin *et al.*, 2017). Therefore,

scholars have researched a lot in anatomy (Anderson *et al.*, 2012), mechanics (Pache *et al.*, 2018), treatment modalities (Chiang *et al.*, 2020), etc., aiming to further understand cruciate ligament injuries. In anatomy, a ligament-bone junction is called an enthesis. Since the tension applied to a ligament can be transmitted to the bone of the enthesis, the characteristic direction of the trabecula can indicate the distribution and direction of the force to the ligament insertion area (Suzuki *et al.*, 2020). Hence,

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properly understanding the structural characteristics of the trabecula bone (TB) and precise anatomical features of the tendon-bone junction of the cruciate ligament is significant for studying the cruciate ligament injury, especially for characterizing the mechanics of the tunnel in cruciate ligament reconstruction (Zhang et al., 2018; Luo et al., 2021). However, there is a lack of research literature on using existing techniques (for example, computerized tomography) to exposit information on TBs and soft tissue (Rachmat et al., 2014; Zhang et al., 2018), especially on the extraction of anatomical information on ligaments, such as their boundaries and their relationship with the surrounding tissue (Galbusera et al., 2014). In this project, we aimed to reveal the anatomical characteristics of the TBs at the attachment area of the cruciate ligaments using the P45 plastination technique. This research contributes to understanding the mechanical characteristics of the tunnel and provides a morphological basis to further develop better biocompatible materials in cruciate ligament reconstruction.

MATERIAL AND METHOD

A total of 31 formalin-fixed specimens of adult knee joints were studied. The imaging examination showed no signs of tumors, congenital malformations, fractures, severe osteoarthritis or other related diseases in all specimens. Then, continuous plastinated sections were prepared by Dalian Hoffen Biotechnology Co., Ltd. using the P45 sheet plastination technique. Finally, we selected sagittal plastinated sections 20 cases and coronal plastinated sections 11 cases.

P45 sheet plastination technique

The experimental procedure is as follows (Sui & Henry, 2007):

Slicing: The specimens were frozen at -70 °C for 2 weeks, embedded in polyurethane in an embedding box, frozen again at -70 °C for 2 days, and sliced using a high-speed band saw with a thickness of 3 mm.

Bleaching: The slices were rinsed in cold water overnight and soaked in 5 % hydrogen peroxide overnight.

Dehydration: After bleaching, the slices were pre-cooled. Then, we dehydrated them in 85 % acetone at -25 °C for 5 days and in 93 % acetone at -15 °C for 5 days. Next, degreasing was performed on them at room temperature. Finally, they were dehydrated in 100 % acetone. **Vacuum impregnation:** We removed the slices from the acetone bath and clamped them with a double glass plate. The slice infiltration mold was made and filled with Hoffen polyester P45 (Dalian Hoffen Biotechnology Co., Ltd., Dalian, China). The mold filled with the infiltration and embedding materials was placed vertically in a vacuum cabinet for impregnation at room temperature. We slowly reduced the pressure to 20, 10, 5 and 0 mmHg according to the size and release rate of the bubbles. The pressure of 0 mmHg was maintained until the bubbles stopped. The impregnation time was more than 8 hours.

Curing: After releasing the vacuum, the bubbles on the plate were checked and removed. The mold was clamped with a clip on the top and placed vertically in a 40 °C water bath to cure for 3 days. After curing, we removed the slices from the water bath and cooled them on a shelf to room temperature. The slices were removed from the glass plate and properly covered with bonded plastic film for protection.

Observation and photography: We placed the slices on an X-ray reading lamp for observation and took photographs with a Canon 7D camera (Canon Inc. Tokyo, Japan). The attachment sites of the cruciate ligaments and the morphology and distribution of the TBs beneath the attachment sites were observed. The slice observation was conducted by three independent researchers (W-B J, T-W S, and S-Z S), the disagreements were resolved through discussion or consultation, and the final decision was made by the fourth author (S-B Y).

RESULTS

The structures of the knee joints can be clearly shown in the P45 plastinated sections. The attachment ranges of the tendons and ligaments around the joints on the cortical bones (CBs) were clear, and the boundaries between the CBs, cancellous bones and TBs can be observed.

Sagittal P45 sections of the knee joints

Through the lateral part of intercondylar eminence (IE) (Fig. 1): The posterior upper part of the anterior cruciate ligament (ACL) and the posterior lower part of the posterior cruciate ligament (PCL) can be observed clearly. The CB at the posterior attachment area of the ACL significantly thickens on the lateral wall of the femoral intercondylar fossa, and its thickness is similar to that of the cortex of the femur metaphysis. Beneath the CB at the attachment area of the ligament, the dense thickening TBs are radially arranged and extend deep into the lateral condyle of the femur (Fig. 1a). The PCL is attached to the posterior part of the tibial IE, and the CB at the attachment area is slightly thicker than the surrounding area (Fig. 1b). Deep in the anterior part of the attachment area of the ligament, the enlarged TBs are arranged parallelly and perpendicular to the ligament. Beneath the posterior part of the attachment area of the ligament, the thickening TBs are arranged longitudinal and parallelly, deeply pass through the epiphyseal line and terminate at the CB of the tibial metaphysis. A weak area of the tibial cortex exists in the dorsal of the ligament attachment point, and there are still TBs arranged horizontally anterior to the area (Fig. 1b). **Through the medial part of IE** (Fig. 2): The sections show the anterior lower part of the ACL and the anterior upper part of the PCL. The ACL is attached to the anterior part of the tibial IE. A significantly thick CB at the attachment area of the ACL, deep within which TBs thicken and concentrate. The thickening TBs extend in two directions. The TBs of the anterior group are parallelly arranged along the extension line of the ligament, while those of the posterior group are perpendicular to the ACL (Fig. 2b). At the anterior attachment area of the PCL, the CB on the medial wall of the femoral intercondylar fossa is obviously thicker than the surrounding area, where the thickening TBs are radially arranged into the deep intercondylar fossa (Fig. 2a).



Fig. 1. Sagittal P45 sections of the knee joint (through the lateral part of the IE). The CB at the attachment of the ACL, the trabeculae arranged densely and radially (black triangles). The trabeculae at the attachment site of the PCL slightly thicken, and beneath the attachment site of the PCL, the trabeculae are thick and arranged in two groups. The trabeculae of the posterior group are arranged parallelly and extended vertically through the epiphyseal line to terminate at the bony cortex of the tibial metaphysis (black arrowheads). The trabeculae of the anterior group are concentrated horizontally (white arrowheads). The CB posteroinferior to the attachment area of PCL is thin. There are still TBs arranged horizontally anterior to the weak area of the CB (white triangles). F: Femur; T: Tibia; P: Patella; TT: Tibial tuberosity; ACL: Anterior cruciate ligament; PCL: Posterior cruciate ligament; PM: Popliteus muscle; Gas: Gastrocnemius; IE: Intercondylar eminence; EL: Epiphyseal line. Black arrow: The vertical arrangement of the trabeculae of the posterior group; White arrow: The horizontal arrangement of the trabeculae of the cB; Stars: The thickening CB at the attachment area of the cruciate ligament; a, b: The enlargement of Fig.s a and b (black box).



Fig. 2. Sagittal P45 sections of the knee joint (through the medial part of IE). The CBs at the attachment area of the PCL significantly thickens, and the TBs are distributed at its depth in a radially distributed form (black triangles). The CB at the attachment site of the ACL thickens. Deep in the thickening CB, the trabeculae are thick and dense and arranged in two groups. The trabeculae of the anterior group are concentrated parallelly in the direction of the ligament extension line to the epiphyseal line (black arrowheads). The trabeculae of the posterior group are arranged parallelly in the direction perpendicular to the long axis of the ligament (white arrowheads). F: Femur; T: Tibia; P: Patella; TT: Tibial tuberosity; ACL: Anterior cruciate ligament; PCL: Posterior cruciate ligament; PM: Popliteus muscle; Gas: Gastrocnemius; IE: Intercondylar eminence; EL: Epiphyseal line; Stars: The thickening CB at the attachment area of the cruciate ligament; a, b: The enlargement of Fig. a and b (black box).

Coronal P45 sections of the knee joints

Through the posterior part of IE (Fig. 3): In this sections, the posterior attachment areas of the cruciate ligaments are shown. At the attachment area of the medial wall of the lateral femoral condyle of the ACL, the CB is thicker than the surrounding area. Beneath the attachment area of the ACL, the TBs thicken and are distributed radially deep in the lateral femoral condyle, with the upper part thicker and more concentrated than the lower part (Fig. 3a). At the posterior attachment area of the tibial IE of the PCL, the CB thickens, under which thickening trabeculae are arranged transversely and parallelly (Fig. 3b).

Through the anterior part of IE (Fig. 4): In this sections, the anterior attachment areas of the cruciate ligaments are shown. At the tibial IE and on the lateral wall of the medial

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condyle, the CB at the attachment area of the cruciate ligament thickens. Deep in the attachment site of the ACL, the TBs thickens and are arranged in a grid pattern (Fig. 4b). On the lateral wall of the medial condyle, the CB at the attachment area of the PCL significantly thickens, and the TBs are distributed at its depth in a plaque-like form (Fig. 4a).

DISCUSSION

Clinical observation has shown a high incidence of knee joint injuries, especially the injuries of cruciate ligaments (Shirazi-Adl & Moglo, 2005; Lee *et al.*, 2013). However, the optimal treatment for cruciate ligament ruptures is still controversial (Weber *et al.*, 2019; Chiang *et al.*, 2020). Satisfactory cruciate ligament reconstruction



Fig. 3. Coronal P45 sections of the knee joint (through the posterior part of IE). The CB at the femoral attachment area of the ACL thickens, and the trabeculae at depth are distributed radially and laterally (black arrowheads), in which the upper part is thicker than the lower one. The CB at the tibial attachment area of the PCL thickens, and the trabeculae at depth thicken and are arranged horizontally (white arrowheads). MCF: Medial condyle of the femur; LFC: Lateral condyle of the femur; T: Tibia; Fi: Fibula; ACL: Anterior cruciate ligament; PCL: Posterior cruciate ligament; Black arrow: The trabeculae distributed radially; Stars: The thickening CB at the attachment area of the cruciate ligament; a, b: The enlargement of Fig.s a and b (black box).



Fig. 4. Coronal P45 sections of the knee joint (through the anterior part of IE). The CB at the tibial attachment end of the ACL thickens. Deep in the CB, the trabeculae thicken and are arranged in a grid pattern (black arrowheads). The CB significantly thickens at the femoral attachment of the PCL. Deep in it, the thickening trabeculae are distributed in a plaque-like form (black triangles).. MCF: Medial condyle of the femur; LFC: Lateral condyle of the femur; T: Tibia; ACL: Anterior cruciate ligament; PCL: Posterior cruciate ligament; Stars: The thickening CB at the attachment area of the cruciate ligament; a, b: The enlargement of Fig. a and b (black box).

depends on various factors, such as graft materials, graft fixation and graft strength. For cruciate ligament reconstruction, the most important is to characterize the mechanics of the tibial and femoral tunnel. However, determining proper tunnel characteristics remains to be a difficult task in cruciate ligament reconstruction (Sheps et al., 2005; Tsukada et al., 2008). Some scholars observed that the cruciate ligament rupture and ligament repair were related to the decrease in bone mineral density around the knee joint (Nyland et al., 2010). Several studies have shown that in ligament reconstruction, adequately fixing the graft is crucial to ensure the stability of the knee joint, and the bone mineral density and structures of the TBs significantly affect ligament reconstruction (Klein et al., 2005; Lee et al., 2012). Hereby, an accurate bone distribution of the cruciate ligament fixation area is the key to ligament reconstruction (Balci et al., 2016). The identified morphology mechanisms leading to an effective and directional versatile stress reduction at the tendon-bone interfaces may provide guidance for biomimetic strategies aiming to design such hard-soft interfaces (Rossetti et al., 2017).

Our study showed that at the attachment areas of the ACL and PCL to the femoral condylar fossa and the tibial IE, the CBs thickened, while the bony cortex at the surrounding area was weak. The TBs arranged deep in the attachment areas of these two ligaments were thick and dense, indicating that the junctions between the ligaments and bones normally undertake strong tension force, which can be transferred deep in bones. Furthermore, the arranging pattens of the TBs were different at the femoral and tibial attachment areas of the ligaments. At the femoral attachment areas of the ACL and PCL, the thickening TBs extended radially deep in the femoral condyles. The most characteristic structure of TBs emerged at their tibial attachment areas. Beneath the attachment area of the ligament of the tibial IE, the TBs became thicker and more concentrated into two groups in the sagittal section. One group of TBs was parallelly arranged along the extension line of the ligament and finally reached the epiphyseal line, while the other group of TBs aggregated parallelly in the direction perpendicular to the ligament fibers. This phenomenon indicated that the stress or loading modes of the cruciate ligaments at the attachment sites of the tibial and femur were different. According to the TB distribution patterns, it can be inferred that the stress in the femur may be radially transferred at the femoral attachment area of the cruciate ligament. In addition, the stress loaded by the ACL in the lateral condyle of the femur may be more complex because the TBs were strengthened in both sagittal and coronal directions. The TBs are arranged according to the compressive direction; it has been experimentally shown that the trabeculae develop in the compressive direction (Lanyon, 1974). However, based on the distribution pattern of TBs deep in the IE, the stress loaded by the cruciate ligaments at their attachment areas in the tibia may be mainly composed of two types and transferred deeply in two directions. One is tensile stress, being conducted profoundly along the long axis of the ligaments, and the other is compressive stress in the direction perpendicular to the long axis of the ligaments. These results also enriched the available data of Suzuki *et al.* (2020). It is noteworthy that the tibial attachment area of the PCL was close to the posterior margin of the tibial plateau; thus, the thickened trabeculae were gathered horizontally and vertically rather than in the direction perpendicular to the ligament fibers or the direction of the extension line of the ligament.

In ligament reconstruction, due to the lack of TB information at the attachment areas of cruciate ligaments in the past, the selection of grafts, the location of the attachment area of the tibia and femur, and the depth of the reconstruction into the bone all depended on the experience of clinicians (Mariani *et al.*, 2005), increasing the risk of surgical failure. Our study revealed the two biomechanical phenomena of the cruciate ligaments at the attachment areas of the tibiae. The compressive stress of TBs of the cruciate ligaments factually extends to the epiphyses of the tibiae. This anatomical information is an important reference for clinical surgery. Poor fixation during ligament reconstruction or reconstruction penetrating the epiphyseal line in the tibial tunnel may violate the anatomical and biomechanical properties of the ACL and cause instability of the knee joint.

In summary, our research showed the TB structures at the attachment areas of cruciate ligaments in detail, providing anatomical evidence for the functional analysis of cruciate ligaments, especially for clinical repair strategies and operational improvement of cruciate ligaments.

Limitation and prospect. Some limitations need to be noted regarding the present study. First, in this study, knee joint specimens were not analyzed by sex and age. Second, morphometric information is lacking. In future research, quantifying the information, such as porosity, anisotropy degree, and the number of trabeculae, of TBs with soft tissue boundaries at the attachment areas of cruciate ligaments will be important for understanding the mechanical information of the tunnels in cruciate ligament reconstruction.

CONCLUSION

The CBs of the cruciate ligaments thicken at the attachment areas of the femur and tibia, deep within which TBs were enhanced. The deep TBs thickened and were radially arranged at the attachment area of the femoral cruciate ligament. There were two directions of TB enhancement at the attachment area of the tibia. One was parallel along the extension line of the ligament, and the other was parallelly in the direction perpendicular to the long axis of the ligament. The arrangement patterns of TBs in the attachment areas of the cruciate ligaments were different at the femoral and the tibial ends, reflecting the difference in stress distribution at the attachment areas.

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Ethics approval and consent to participate. This study was approved by the Ethics Committee of the body and Organ donation Center of Dalian Medical University. The subjects were 31 middle-aged knee joints from China Human and Organ donation Center. According to the regulations of the Ethics Committee, the written informed consent of the donors involved in this study was obtained before death.

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RESUMEN: Para el tratamiento de lesiones de los ligamentos cruzados, especialmente para caracterizar la mecánica del túnel en su reconstrucción, es importante comprender correctamente la información ósea del área de inserción de estos ligamentos. Estudiamos 31 articulaciones de rodilla de individuos chinos, adultos, de mediana edad, utilizando la técnica de plastinación de láminas P45, centrándonos en las áreas de unión de los ligamentos cruzados, especialmente en las estructuras óseas. Las trabéculas en el área de inserción se distribuyeron radialmente y se extendieron profundamente en la pared medial del cóndilo lateral del fémur. Sin embargo, en la parte anterior de la eminencia intercondílea, las trabéculas del grupo anterior estaban dispuestas paralelamente a lo largo de las fibras tendinosas del ligamento cruzado anterior, mientras que las trabéculas del grupo posterior estaban dispuestas paralelamente a lo largo de la dirección perpendicular de las fibras del ligamento cruzado anterior. De manera similar, en el área de inserción en la cara lateral del cóndilo medial del ligamento cruzado posterior, las trabéculas se extendían radialmente y profundas hacia el cóndilo medial. Profundamente en la parte posterior de la eminencia intercondílea, las trabéculas estaban dispuestas longitudinalmente. En la parte anterior de la eminencia intercondílea, las trabéculas estaban dispuestas paralelamente a lo largo de las direcciones perpendiculares de las fibras del ligamento. Los patrones de distribución del tejido óseo trabecular en las áreas de unión de los ligamentos cruzados en los extremos del fémur y la tibia eran diferentes. Estas diferencias deben tenerse en consideración cuando los cirujanos ortopédicos reconstruyen los ligamentos cruzados anteriores.

PALABRAS CLAVE: Articulación de la rodilla; Ligamento cruzado; Hueso trabecular; Técnica de plastinación P45.

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