


RESEARCH ARTICLE

Intra-Articular Biomechanical Changes of the Meniscus and Ligaments During Stance Phase of Gait Circle after Different Anterior Cruciate Ligament Reconstruction Surgical Procedures: A Finite Element Analysis

Zi-mu Mao, MD^{1,2†} , Zhen-wei Wang, MD^{1†}, Chao Xu, MM^{3,4†}, Chen-he Liu, MM^{5†}, Zhi-yu Zhang, MM⁶, Xiao-li Ren, MM⁷, An-qi Xue, ME^{8,9}, Ze-nan Li, MM¹⁰, Feng Zhao, PhD⁸, Qi Yao, MD¹, Jia-kuo Yu, MD, PhD²

¹Department of Joint Surgery, Beijing Shijitan Hospital, Capital Medical University, ²Institute of Sports Medicine, Peking University, ⁸Key Laboratory for Biomechanics and Mechanobiology of Ministry of Education, Beijing Advanced Innovation Center for Biomedical Engineering, School of Biological Science and Medical Engineering, Beihang University, ⁹Beijing Institute of Medical Device Testing and ¹⁰Fengtai Fourth Outpatient Department, Beijing Garrison, Beijing, ³Xinjiang Key Laboratory Neurological Disorder Research, Key Laboratory of Autonomous Region and ⁴The Department of Orthopaedics, The Second Affiliated Hospital of Xinjiang Medical University, Urumchi, ⁵Department of Orthopaedics, First Hospital of Shanxi Medical University and ⁷Shanxi Institute of Sports Science, Taiyuan, Taiyuan and ⁶Department of Sports Medicine, Yan'an Traditional Chinese Medicine Hospital, Yan'an, China

Objective: The debate on the superiority of single- or double-bundle for anterior cruciate ligament reconstruction has not ceased. The comparative studies on intra-articular biomechanics after different surgical reconstructions are rare. This study is to evaluate the biomechanical stress distribution intra-knee after single- and double-bundle anterior cruciate ligament reconstruction by three-dimensional finite element analysis, and to observe the change of stress concentration under the condition of vertical gradient loads.

Methods: In this study, magnetic resonance imaging data were extracted from patients and healthy controls for biomechanical analysis. Patients included in the three models were matched in age and sex. The strength and distribution of induced stresses were analyzed in two frequently used procedures, anatomical single-bundle anterior cruciate ligament reconstruction and anatomical double-bundle anterior cruciate ligament reconstruction, using femoral-graft-tibial system under different loads, to mimic a post-operation mechanical motion. The three-dimensional finite-element models for normal ligament and two surgical methods were applied. A vertical force simulating daily walking was performed on the models to assess the interfacial stresses and displacements of intra-articular tissues and ligaments. The evaluation results mainly included the stress of each part of ligament and meniscus. The stress values of different parts of three models were extracted and compared.

Results: The stress of ligament/graft at femoral side of three finite-element models was significantly higher than at tibial side, while the highest level was observed in single-bundle reconstruction finite-element model. With the increase of force, the maximum stress in the medial (7.1–7.1 MPa) and lateral (4.9–7.4 MPa) meniscus of single-bundle reconstruction finite-element model shifted from the anterior horn to the central area ($p = 0.0161, 0.0479$, respectively). The stress was shown to be at a lower level at femoral side and posterior cruciate ligament of intra-knee

Address for correspondence Zi-mu Mao, Department of Joint Surgery, Beijing Shijitan Hospital, Capital Medical University, No. 10 Tieyi Road, Yangfangdian, Haidian District, Beijing, 100038, China. Email: mzm@pku.edu.cn; Jia-kuo Yu, MD, PhD, Institute of Sports Medicine, Peking University, No. 49, North Garden Road, Haidian District, Beijing 100191, China. Email: yujiaquo@126.com; Feng Zhao, PhD, Key Laboratory for Biomechanics and Mechanobiology of Ministry of Education, Beijing Advanced Innovation Center for Biomedical Engineering, School of Biological Science and Medical Engineering, Beihang University, No. 7 Xueyuan Road, Haidian District, Beijing 100083, China. Email: fzhao@buaa.edu.cn

[†]These authors contributed equally.

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in two reconstruction finite-element models than that in normal finite-element models, while presented higher level at the tibial side than normal knee ($p = 0.3528$). The displacement of the femoral side and intra-knee areas in reconstruction finite-element models was greater than that in normal finite-element model ($p = 0.0855$).

Conclusion: Compared with the single-bundle technique, the graft of double-bundle anterior cruciate ligament reconstruction has better stress dissipation effect and can prevent postoperative meniscus tear more effectively.

Key words: Anterior cruciate ligament; Displacement; Double bundle; Finite-element model; Intra-knee; Single bundle; Stress

Introduction

Anterior cruciate ligament (ACL) rupture is a serious knee joint disease, which can lead to osteoarthritis with absence of effective and timely treatment.^{1,2} Currently, arthroscopic autologous tendon transplantation to reconstruct broken ACL is the most common and effective approach to treat patients with ACL rupture. With the rapid development of the anatomic study, accumulated evidence have proved that the ACL is mainly composed of two bundles, the anteromedial bundle (AMB) and the posterolateral bundle (PLB).^{3,4} This has led to the concept and technique of anterior cruciate ligament reconstruction (ACLR) closer to the natural anatomical location of femoral and tibial footprint. The double-bundle reconstruction technique by using two grafts to reconstruct the AMB and PLB has also been developed.⁵⁻⁷

Up to now, there are variety of methods to evaluate the clinical effect of different surgical procedures of ACLR in clinical practice.⁸⁻¹⁰ However, the main limitation of these methods is the inability to directly measure the mechanical response to relative positional differences between graft and tunnel that may result from different surgical procedures. Meanwhile, it is still challenging to continuously track the status of daily knee joint use and the degeneration of cartilage for a long-term follow-up period. Further, the clinical effect could not be deduced passively until articular cartilage degeneration worsens 10 years postoperatively. There is an urgent need to develop an effective method for predicting meniscal tears due to changes in the biomechanical environment. Studies have shown that biomechanical analysis of three-dimensional finite-element model (3D-FEM) can help solve these clinical problems.^{11,12} FEM analysis is a computational procedure that can be used to calculate the stress in an element by reproduce a complex structure. This technology can perform a model solution to simulate the irregular geometries and mimic the postoperative

condition of ACLR, and thus provide abundant information about the biomechanical effects on the reconstructed ligament and adjacent organizational structure. Following this analysis, the stress and displacement in each area of intra-knee under the postoperative loads can be quantitatively measured, and the possible clinical outcomes related to these factors, such as the maturity of grafts, the influence of the physical factor on the widening of bone tunnel caused by the grafts, wear and tear caused by compression of the medial and lateral meniscus, and the risk of osteoarthritis, can also be predicted.

The purpose of study are as follows: (i) to evaluate the stress response in femoral-graft-tibial structures under daily walking weight load; (ii) to evaluate stress concentration between the proximal and distal parts in the posterior cruciate ligament (PCL) and in the medial and lateral meniscus from the anterior horn to the posterior horn of three obtained FEMs; (iii) to evaluate the displacement of the graft and PCL under different loads.

Materials and Methods

ACL Model Selection

The geometry of the intact knee was extracted from images performed by a magnetic resonance imaging (MRI) scanner (3.0T GE Discovery MR750w, General Electric Company, USA). Before the start of this study, the clinical follow-ups of surgical patients were carried out and clinical cohorts were established to obtained the demographic baseline indicators required for modeling to calibrate the model parameters (Table 1). Knee models was obtained from two healthy 33-year-old male volunteers. After initial modeling, the original models were adjusted according to the average knee size of patients based on previous surgical cohorts (Table 1). All participates involved in this study gave informed consent. The

TABLE 1 Demographic and Baseline Characteristics of Patients in Previous Follow-up Cohorts

	Age at last follow-up, y	Sex, male/female, n (%)	Height, cm	Weight, kg	Body mass index (BMI), kg/cm ²
SB-ACLR (N = 38)	33 (30–49)	25/13	172 (160–181)	73 (61–85)	24 (21–27)
DB-ACLR (N = 34)	33 (30–48)	25/9	173 (161–185)	74 (60–88)	24 (20–28)
p value	N.S.	N.S.	N.S.	N.S.	N.S.

^aValues are presented as the median (range) unless noted otherwise.

TABLE 2 Parameters of MRI sequence

Index	Setting value
TR	1280
TE	11,000
Echo train length	54
Bandwidth	50
FOV	16.0 × 16.0
Slice thickness	1.0
Phase	288 × 288

physical indicators of the two volunteers were matched with the median values of the baseline data of the two cohort of single bundle anterior cruciate ligament reconstruction (SB-ACLR) and double bundle anterior cruciate ligament reconstruction (DB-ACLR) (Table 1). Before the MRI scan, the mechanical axis of the lower limbs had been measured in order to maintain the consistency of the basic conditions of the legs. The specific indexes of MRI were shown in Table 2. Based on the principle of least cost, the knee joint data of one of the volunteers were used to reconstruct the normal model and the SB-ACLR model. The SB-ACLR and DB-ACLR with autologous hamstring autografts procedures were performed by one single surgeon. The graft preparation, tunnel techniques, femoral and tibia fixation methods and devices, and the postoperative rehabilitation were conducted as previously described.¹³⁻¹⁵

Model Designs

The obtained MRI images were used to generate 3D surface creation of the bony parts and soft tissues by using MIMICS 17.0 image processing software (Materialize, Leuven, Belgium). The generated FEMs of femoral-normal/grafted ACL-Tibial structures consisted of two bony parts (femur and tibia), meniscus and cartilage layers, and ligaments (ACL and PCL). Then Geomagic Studio 2014 software (Geomagic, North Carolina, USA) was performed to smooth the knee model and remove redundant features on 3D

surface models. After the polygon module was processed, the 3D models with smooth fitting surface were transferred to SolidWorks 2016 3D software (Dassault Systèmes, Vélizy-Villacoublay, France), where solid 3D models could be assembled. The stress and displacement of different parts of normal ACL, grafted ACL, and PCL under different vertical forces, and the stress of meniscus under the vertical pressure of femoral condyle were further analyzed.

Finite-Element Mesh

The 3D mesh models generated from three different MRI images of knees were shown in Figure 1. The models were imported to ANSYS 17.0 (Ansys, Pennsylvania, USA) for reconstruction and meshing. These elements were modified by ANSYS Engineering Data Sources and the meshes were refined in regions of high gradients for accurate representation. A mesh sensitivity study was conducted to measure the quality of the outcome results. To avoid volumetric locking and to maximize model accuracy, advanced hexahedral meshes were preferred.

Interface Condition and Vertical Load

The femoral condyle cartilage, tibial plateau cartilage, medial and lateral meniscus from the intra-articular knee were selected as the interaction modules, while the bound and contacted surfaces of them were also analyzed. Generally, the load on the knee joint mainly comes from the body mass above the knee joint and the normal position related to tibiofemoral joint, which requires the muscle contraction force in the knee joint. Therefore, the mass of the body above the knee joint and the muscle contraction force were regarded as the entire load on the knee. In this study, those vertical loads applied on the FEMs were to simulate the stance phase of gait circle, when one leg stands upright, the other leg swings off the ground, and thus the center of gravity just falls on the longitudinal femoral-tibial axis. The flexion and extension angle of the knee joint under this condition was between -5° and 5° . According to the

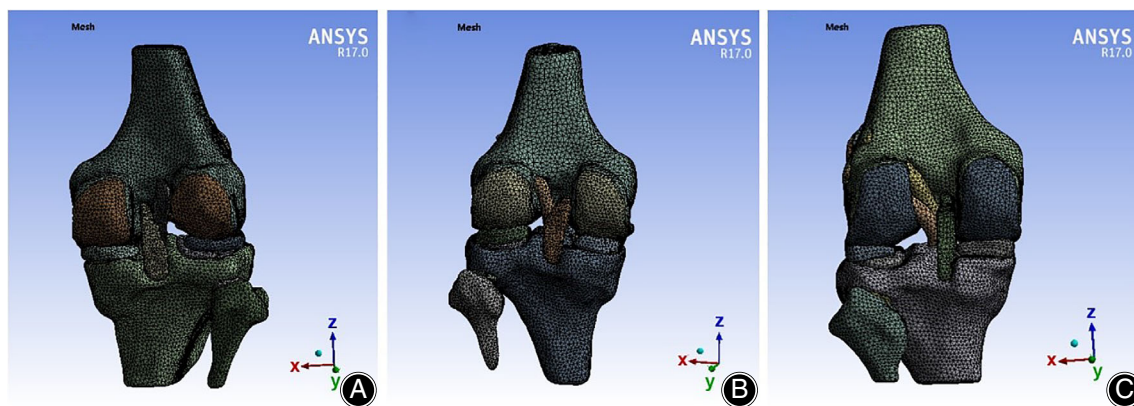


Fig. 1 3D mesh models of three different knees by using ANSYS software. (A) Normal knee. (B) SB-ACLR. (C) DB-ACLR. Node/element number: (A) 515506/351745, (B) 406374/272436, (C) 579075/394332

previous cohorts, the median body weight of surgical patients and healthy controls were close to 70 kg, based on the previous follow-up data, the body weight parameters of the patients were simplified to 70 kg during modeling. International standard of ISO 14243-3 and ATSM Committee were used for reference to determine the relative position of the diameters and opposite lines of the femur and tibia, as well as the temporal correspondence between tibiofemoral stresses and gait phases. The calculation of stress is mainly based on the lever principle of tibiofemoral joint angulation and stress load. In order to reduce the amount of calculation and better observe the changes of measurement indexes, the stress interval of knee joint (500–800 N) was calculated under the condition of 70 kg body weight, and further simplified into four stress gradients, 500 N, 600 N, 700 N, and 800 N.

Boundary Conditions

In the FEM, the femur was completely free, the knee joint was straightened at 0°, and the distal tibia and fibula were completely restrained and fixed without any constraint. No restrictions were present on the degrees of freedom nor rotational degrees of freedom in other directions. In order to evaluate the stress change in the three models of knee joint, every load of 5S was continuously applied at the femur end, and stress in the femoral condyle cartilage and meniscus was analyzed.

Material Properties

The skeletal structure is characterized with rigid bodies in the majority of the numerical studies of heterogeneity and anisotropy. But bone itself has the characteristics of heterogeneity and anisotropy. At the same time, the purpose of this study is to evaluate the stress effect of the graft intra-knee. Therefore, a better method should be to define skeletal structure as homogeneous and isotropic. With this approximation method, the elastic modulus property equivalent to the related bone could be obtained without affecting the accuracy of the results. The ligaments are usually assumed to be non-linear, hyper-elastic, and transversely isotropic fibered materials, and generally modeled by an incompressible Neo-Hookean behavior with the energy density function.^{16–20} Based on previously reported studies,^{21–24} the material properties of all components were evaluated and shown in Table 3.

Outcome Measures

All mechanical data are automatically generated in ANSYS software (ANSYS, Inc. Pittsburgh, Pennsylvania). The stress values of the specific parts were extracted to be observed under gradient loads, so as to obtain the trend of stress variation. The statistical unit of all mechanical data is MPa.

Statistical Analysis

Statistical analysis was performed using the SPSS 23.0 software package (SPSS Inc., IBM, USA). The unpaired, two-

TABLE 3 Material properties of all components on the finite-element

Components	Young modulus E (MPa)	Poisson's ratio ν
Femur	11,000	0.30
Tibia	11,000	0.30
Cartilage	5	0.45
Meniscus	59	0.49
Normal ACL	Nonlinear (215.3 for reference)	Nonlinear (0.40 for reference)
Autograft	Nonlinear	Nonlinear
PCL	215.3	0.40

Abbreviations: ACL, Anterior cruciate ligament; PCL, posterior cruciate ligament.

tailed Student's T test and Mann-Whitney U nonparametric two-tailed test were used to compare the pre- and postoperative scores. Demographic data and subjective scores were compared between SB-ACLR group and DB-ACLR group by unpaired, two-tailed Student's T test or the chi-square test. The MRI indicators were compared between the two groups by Mann-Whitney U nonparametric two-tailed test or the chi-square test. *p* value of 0.05 was considered statistically significant.

Results

Generation of Normal and Grafted ACL Models

Three models, including normal knee joint, SB-ACLR, and DB-ACLR, were successfully established (Figure 1). The Nodes/Elements for three models were calculated (normal knee: 515506/351745, SB-ACLR: 406374/272436, DB-ACLR: 579075/394332). The constraints and parameters defined previously were analyzed, and the mechanical stress distribution on each component of three FEM systems under different loads was detected. In the analysis of ANSYS software, the system will get the stress or displacement value of each part and automatically generate the hot zone, and was visible to the readers. The color gradient from blue to red was corresponding to the value from low to high. This displayed method can observe how the mechanical stress and displacement in each part of FEM are distributed. FEMs was used to simulate the walking pressure of the knee joint under the condition of single-leg stance phase. We extracted the specific values of the effective region and summarized these detailed values into Figures 2–4.

Stress and Displacement in Normal ACL and Grafts

The stress effects in normal ACL and grafts after operation was shown in Figure 2. Generally, for normal ACL, the stress level is consistent at the femoral side (8.7–13.9 MPa), middle part intra-knee (9.4–15.0 MPa), and tibial side (7.9–12.6 MPa), at the same time, no change in this trend was observed when the preset load was changed. Importantly,

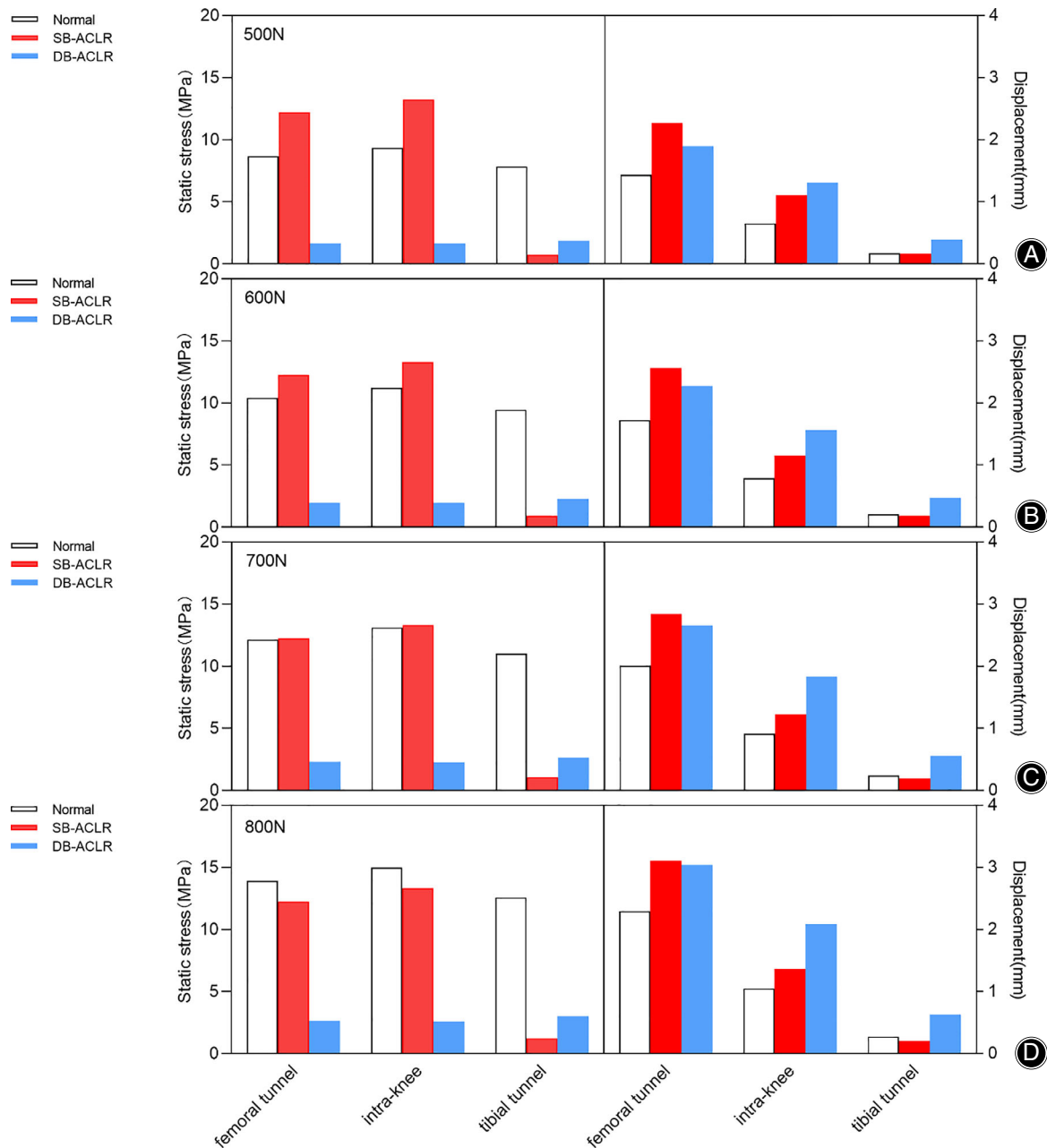


Fig. 2 Stress and displacement of ACL in three FEMs under gradient vertical loads from 500 N to 800 N (A–D). ACL, anterior cruciate ligament. Under different load conditions, the reading value of each part is the maximum stress value

overall stress of DB-ACLR graft in those areas showed a much smaller level than that in normal ACL when the load was applied (1.6–2.6 MPa, 1.6–2.6 MPa, 3.9–3.0 MPa, respectively), while no force-dependent manner of stress was found in DB-ACLR graft as well. For SB-ACLR, with the increase of load, the stress level of the graft did not change significantly, but remained at a relatively consistent level (12.2–12.2 MPa, 13.2–13.3 MPa, 0.7–1.2 MPa, respectively). At the same time, the stress level at femoral side and middle part was significantly higher than that of tibia (normal).

Basically, the displacements of ligament/graft in the three models were gradually increased as the force was increased from 500 to 800 N (Figure 2A–D). All models showed highest level of displacement at the femoral side, followed by the middle part, while the smallest displacement was observed at the tibial side.

Stress and Displacement at Meniscus

In order to explore the differences of stress in different parts of the meniscus, the medial and lateral meniscus were

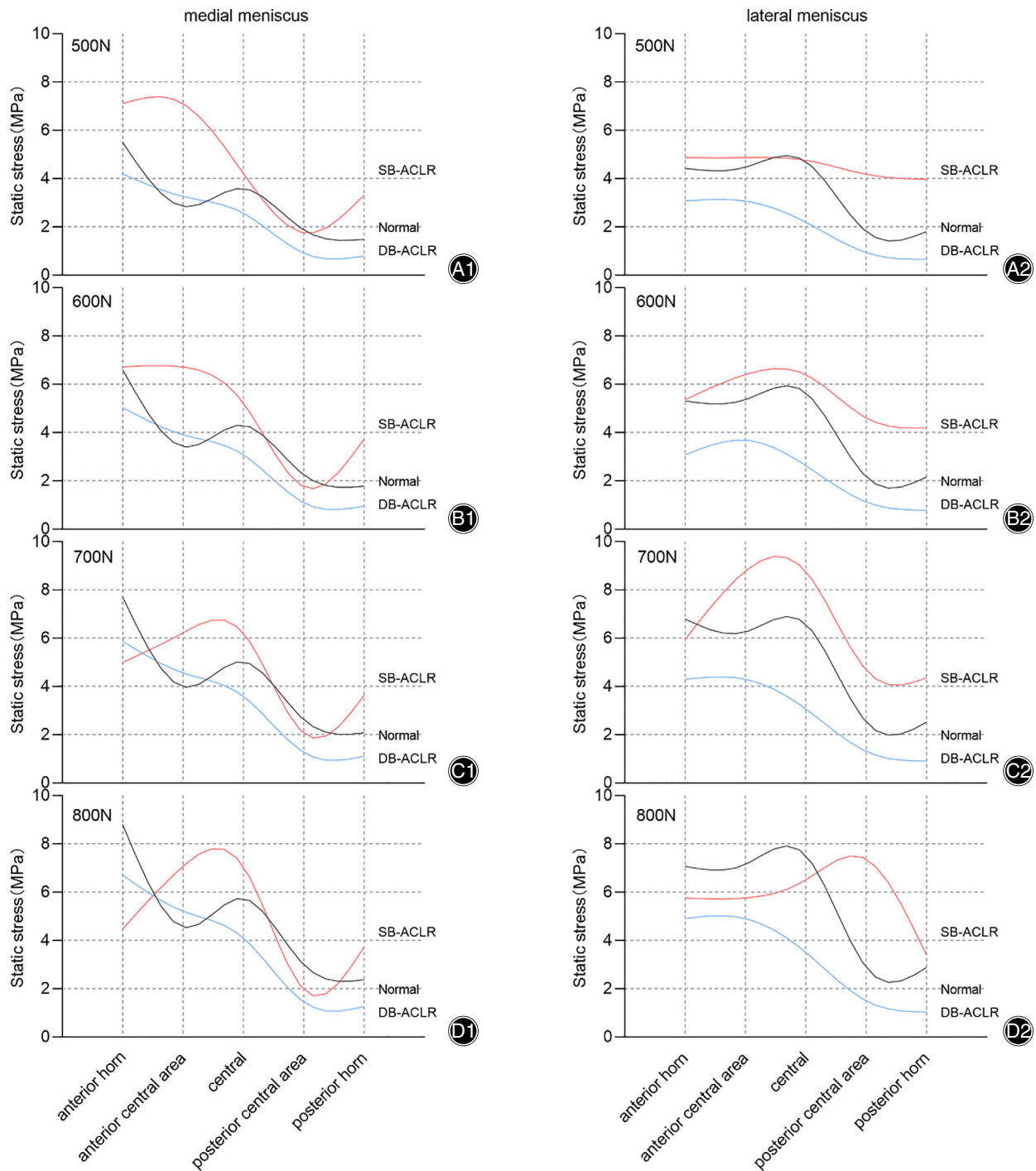


Fig. 3 Stress of medial and lateral meniscus in three FEMs under gradient vertical loads from 500 N to 800 N. The medial (A1–D1) and lateral (A2–D2) meniscus were divided into five regions: anterior horn, anterior central area, central area, posterior central area, and posterior horn

divided into five regions, including anterior horn, anterior central area, central area, posterior central area, and posterior horn, respectively, as shown in Figure 3. For the medial meniscus, the maximum stress at the anterior horn of the three models was higher than that at the posterior horn (Figure 3A1–D1). Under the gradient load from 500 to 800 N, the stress in the normal and DB-ACLR models at five

regions was gradually increased. However, the stress in the SB-ACLR model was higher in the anterior horn and anterior central area at a load of 500 N and 600 N, while the maximum stress position shifted to the central area when the load changed to 700 N and 800 N (Figure 3A1, B1 vs C1, D1). Similarly, the stress at five regions of lateral meniscus in the three models was increased with the enhancement of the

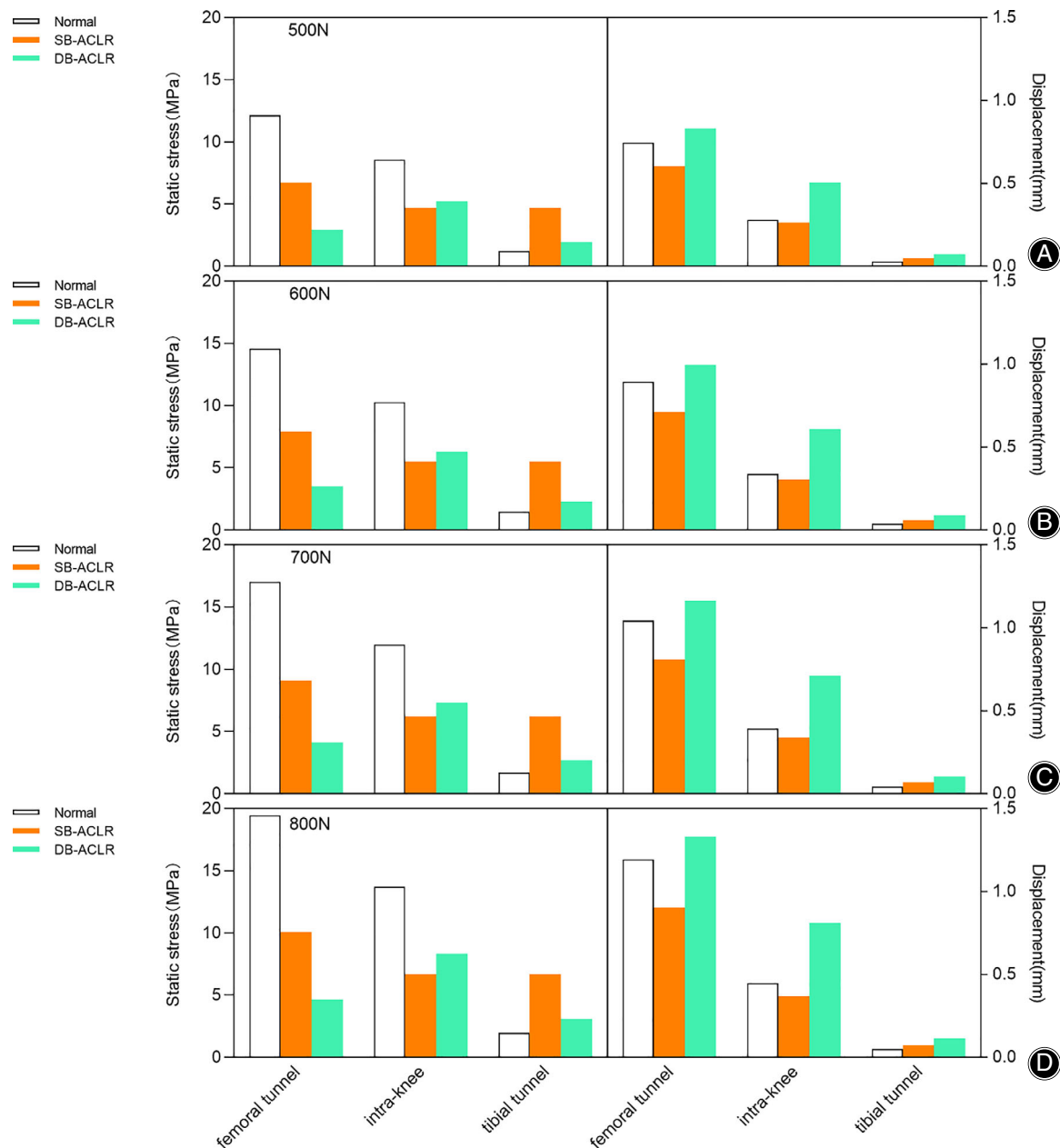


Fig. 4 Stress and displacement of PCL in three FEMs under gradient vertical loads from 500 N to 800 N (A–D). PCL, posterior cruciate ligament

force, while the stress in each region of the DB-ACLR model was significantly lower than that of the other two models (Figure 3A2–D2). In addition, as the increased load was applied on the SB-ACLR, the stress was gradually transferred from the middle part to the middle rear part of the lateral meniscus (Figure 3A2, B2 vs C2, D2).

Stress and Displacement at PCL

Given that the ACL is quite close to PCL in the knee joint, changes in the mechanical stress and displacement at PCL may help understand the long-term function of the knee

after ACL grafted surgery. Therefore, we further evaluated the performance of PCL in three FEMs under different gradient loads. Generally, the PCL stress was increased in three models when the load was changed from 500 to 800 N, as shown in Figure 4. Under different loads, the maximum stress at PCL in the normal model was present at the femoral side (12.1–19.4 MPa), followed by the middle part intra-knee (8.6–13.8 MPa), and the lowest stress was shown at the tibial side (1.2–2.0 MPa). In the SB-ACLR model, the stress at the femoral footprints was found largest, while there was not much difference of stress between the middle part and the

tibial tunnel. However, in the DB-ACLR model, the maximum stress was located in the middle part. For the displacement of the PCL, the maximum values of the three models was all shown at the femoral side, which demonstrated that more tension was obtained at this position. Meanwhile, the displacement of the PCL at the middle part was found highest in the DB-ACLR model when compared with that in other two models.

Discussion

Main Finding of the Study

The most important finding of the study is that FEM tests showed that in SB-ACLR, the maximum stress of graft was concentrated in the proximal side of femoral tunnel, the middle part of middle segment, and the distal part of tibial tunnel, which indicated the possible locations of loosened fixation and intra-articular fracture in the clinical practice. At the same time, the graft of SB-ACLR in the whole tibial tunnel did not play the role of relieving the internal stress under vertical forces. Meanwhile, the anatomical arrangement (along the long axis of the ACL) of the femoral-ACL-tibial complex showed higher stiffness, ultimate load and energy absorption values than the arrangement of complex along the longitudinal axis of the tibia.²⁵

The ACL is normally composed of many small collagen fiber bundles, while according to the tibial insertion footprints, the ACL can be divided into two big bundles on the macro level, including anteromedial bundle (AMB) and posterolateral bundle (PLB).^{3,26,27} Generally, the length and tension of these two ligaments constantly change in the whole range of motion of the knee joint, which can eliminate their own load and external force, and assist ligament reconstruction evolved from the past way of reconstruction of a single tendon to the way of double tendon reconstruction.⁴ When the load exceeds the limit, the ACL ruptures are typically present at the middle part and footprint of the ligament,²⁸ this is consistent with this study. Moreover, after the operation of different ACL reconstruction, the biomechanical changes at the bundles of ligament may result in different long-term clinical outcomes. Specifically, the increased and uneven contact stress in the joint is considered to be highly correlated with meniscus tear and cartilage degeneration.²⁹ At present, though short-term and mid-term follow-up studies have compared the SB-ACLR and DB-ACLR in patients in terms of the clinical outcomes, a difference of the failure rate between the two procedures still remains unclear.^{5,14,30,31} Meanwhile, it has been reported that surgical fixation method is associated with failure rates of procedures.^{32,33}

This study explored and compared the contact stress in the femoral tunnel by using different ACL models (normal ACL, SB-ACLR, and DB-ACLR). Although multiple studies have compared the biomechanical behaviors of normal ACL and ACLR grafts,^{26,34,35} the operation introduced here is a relatively new approach for autograft transplantation.¹⁵ In

this study, when the tension in different areas of the knee joint becomes greater, the impact energy of the graft on the bone tunnel will increase. This may lead to tunnel widening, slowed tendon bone healing progress, poor maturity of the grafts, and increased risk of surgical failure. In addition, the stress in different areas of grafts of DB-ACLR model was much lower than that in the normal ACL. This superior behavior of DB-ACLR may be caused by the fact that the two bundles of grafts with same thickness can bear the mechanical effects at the same time, so that the average force on each part is much smaller to keep the biological activity of the grafts from fatigue failure. As the inserted location for the femoral and tibial tunnels in SB-ACLR procedure is closer to the end of AMB, the entrance of the femoral tunnel may rotate when the stress is applied, which made the graft act like a “wiper,” pounding and scrubbing the bone tunnel, widening it, and affecting healing.

Based on these findings, we proposed that more care should be taken in clinical practice when the grafts of SB-ACLR are applied in the femoral tunnel, and whether this graft would have a higher rate of re-fracture, a lower maturity, and a higher occurrence of tunnel widening after the procedure are required to be fully considered.

Mechanical Effects of Intra-Articular Structural Changes Caused by Different Surgical Methods on Meniscus

The meniscus is the most important part of the knee joint to digest and absorb the collision energy between the femur and tibia, while improper force may cause various types of meniscus tear. Similarly, the cartilage surface of the medial and lateral femoral condyles would inevitably wear out under the excessive load, and eventually result in osteoarthritis. Previous studies have compared the clinical efficacies of SB-ACLR and DB-ACLR, however, the results were presented controversially.^{6,7,36} Some studies have demonstrated that DB-ACLR technology has greater effects on control of knee stability by protecting meniscus and cartilage than SB-ACLR after surgery,^{14,36} while some other studies proved that there is no significant difference between these two procedures.^{5,7} However, most of these studies proved that ACLR procedure could not effectively avoid the occurrence of postoperative osteoarthritis. In this study, we found the maximum stress at the medial meniscus of SB-ACLR model gradually shifted from the anterior horn and anterior central area to the central part as the load was increased, which suggested that the SB-ACLR could not fix the stress concentration in the anterior horn and central part of meniscus. This result implied that the different surgical procedures may exhibit different control effects on the antero-posterior position of femur and tibia when loads are applied.

Mechanical Effects of Grafts with Different Surgical Methods on PCL

In this study, the stress at the medial and lateral meniscus of the DB-ACLR model was lower than that of normal and SB-ACLR FEMs. This may be caused by stress absorption

effect which was simultaneously exerted by the reconstructed AMB and PLB. It should be noted that this result cannot be directly obtained from the clinical follow-up measurements. Therefore, 3D model applied here to simulate and predict the mechanical response of different parts of knee models under various load parameters is a main advantage over other studies. This approach may be used as an important supplement during clinical follow-up to evaluate the long-term clinical efficacy after ACL reconstruction and to reveal the possible reasons for postoperative meniscus injury and osteoarthritis occurrence. Additionally, it was shown that the displacement at PCL of the DB-ACLR model was higher than that of the other two FEMs, which may be due to the fact that the PCL is objectively squeezed by the two same-thickness bundles. When the stress is applied, the middle segment of the PCL arch is raised posterior and up towards, which may result in tightened PCL and increased displacement. Mesfar *et al.*³⁷ found the mechanical contribution of ACL was strongly dependent on the force of PCL under flexion applied by quadriceps load. A larger force in the PCL or earlier initiation to resist force can result in greater ACL forces. The alterations in ligament stiffness or pretension used during reconstruction surgery would influence the mechanical role of both treated and untreated cruciate ligaments. Tension or loss of function of the PCL can also lead to increased contact stress within the joint.^{38,39} Therefore, the effect of PCL on controlling the relative position of the tibia and femur should not be ignored.⁴⁰ As joint contact forces and contact areas are highly sensitive to ACL and PCL pre-strains,⁴⁰ long-term increased load may bring negative effects on knees. However, in this study, it is interesting that the increase of the displacement of PCL at the middle part in DB-ACLR model does not lead to its stress exceeding that of the normal model under the same condition and position, which provides a new idea for the selection of clinical surgical methods in the future; that is, maybe the value of the mechanical advantages of the DB-ACLR is far greater than sense of constriction caused by the space occupied by two bundles.

Limitations of this Study

There are some limitations in the current study. Firstly, only two commonly used types of postoperative biomechanical response were simulated to reflect the general clinical utility of the typical ACLR in this study, while the different ACLR procedures, the fixation methods, and the bone tunnel positions have also been proved to affect the failure rate of ACL reconstruction in other studies.⁴¹⁻⁴³ Secondly, only the geometry of ACL, PCL, and meniscus was considered in the FEMs, the other knee joint structures, such as the medial/lateral collateral ligament and patella, were not systematically evaluated here. Meanwhile, the gradient vertical loads on knee joint under the straightened phase condition were performed to evaluate the different stress on these areas, other movements (such as knee extension and flexion) were not extensively studied in this study. In this study, the

mechanical properties of ACL and PCL are isotropic; however, the cartilage covered by the surface of the internal and external condyles of the femur, and the overlap between the lower surface of the meniscus and the tibial plateau were considered to be integrated on the whole contact surface. Further studies are required to refine and improve the reported models, and eventually to provide a more detailed framework as to reduce the failure rates of ACLR for patients.

Conclusion

This study indicates that the vertical loads in the knee result in a difference between normal knee and the operational knees. The DB-ACLR procedure can help absorb stress in the knee joint and reduce the stress heterogeneity in different parts of the joint, but SB-ACLR has less impact on the PCL displacement. Therefore, the operation scheme should be chosen according to the specific situation of the patients.

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Conflict of Interest

The authors declare that they have no conflicts of interest for this paper.

Authors Contributions:

Zi-mu Mao, Zhen-wei Wang, Chao Xu, Chen-he Liu: substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work.

Zhi-yu Zhang, Xiao-li Ren, An-qi Xue, Feng Zhao, Ze-nan Li: acquisition, analysis, or interpretation of data for the work, drafting the work or revising it critically for important intellectual content.

Qi Yao, Jia-kuo Yu: acquisition, analysis, or interpretation of data for the work, drafting the work or revising it critically for important intellectual content. Final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethical Approval

The study was approved by Peking University Third Hospital Medical Science Research Ethics Committee (No. IRB00006761-2011097), all partners are bound by or agree to this ethics document.

Consent to Participate

All participants involved in this study provided informed consent, the study did not involve examination of the patients, so there was no direct contact with the patients.

Consent to Publish

All co-authors involved in this study gave consent to publish.

Competing Interests

No conflict of interests.

Availability of Data and Materials

This manuscript has no associated data, or the data will not be deposited.

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