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Research Paper

An update on the constitutive relation of ligament tissues with the effects of collagen types

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ABSTRACT

The musculoskeletal ligament is a kind of multiscale composite material with collagen fibers embedded in a ground matrix. As the major constituent in ligaments to bear external loads, collagens are composed mainly of two collagen contents with different mechanical properties, *i.e.*, types I and III collagen. The constitutive relation of ligaments plays a critical role in the stability and normal function of human joints. However, collagen types have not been distinguished in the previous constitutive relations. In this paper a constitutive relation for ligament tissues was modified based on the previous constitutive relation by considering the effects of collagen types. Both the collagen contents and the mechanical properties of sixteen ligament specimens from four cadaveric human knee joints were measured for determining their material coefficients in the constitutive relation. The mechanical behaviors of ligaments were obtained from both the uniaxial tensile and simple shear tests. A linear regression between joint kinematic results from *in vitro* and *in silico* experiments was made to validate the accuracy of this constitutive relation. The high correlation coefficient ($R^2=0.93$) and significance ($P<0.0001$) of the regression equation revealed that this modified constitutive relation of ligaments was accurate to be used in studying joint biomechanics. Another finite element analysis with collagen contents changing demonstrated that the effect of variations in collagen ratios on both joint kinematics and ligament biomechanics could be simulated by this constitutive relation.

1. Introduction

As a key contributor to the stability and normal function of musculoskeletal joints, the ligament is a kind of composite biological material consisting of collagen fibers embedded in a ground matrix. Collagens are the major constituent in ligaments to bear external loads (Ushiki, 2002). It is known that the mechanical properties of composite materials, such as musculoskeletal ligaments, depend on their microstructures and compositions.

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Fibril morphology is one of the main microstructure features of ligaments. It is found that both the mean value and distribution of fibril diameters in ligaments/tendons change during joint immobilization, graft ligamentation, ligament/tendon maturing, healing and ageing (Binkley and Peat, 1986; Frank et al., 1992; Goh et al., 2008; Moore and Beaux 1987; Shino et al., 1995). Many studies have been investigated for determining whether fibril morphology correlates with mechanical properties of ligaments or not, but no consensus has been achieved. Some researchers reported that the variations in collagen fibril diameters did not affect the mechanical properties of ligaments, (Derwin and Soslowsky, 1999; Frank et al., 1992; Kongsgaard et al., 2010; Lavagnino et al., 2005) whereas a significant correlation between fibril morphology and mechanical properties of ligaments was found by some others (An et al., 2004; Rigozzi et al., 2010).

Collagens of types I and III are the two major contents of the collagen composition in ligaments (Ushiki, 2002). It is demonstrated that both the two collagen contents greatly change during ligament healing, maturing and ageing as well as graft ligamentization (Amiel et al., 1986; Frank et al., 1988; Hoffmann and Gross. 2007; Liu et al., 1995; Sakai et al., 2001). The proportion of type III collagen is about 10% of total collagen mass in normal rabbit medial collateral ligaments while reaches up to 40% of the total collagen mass 14 weeks after injury (Amiel et al., 1987). Besides, the contents of the different collagen types also become abnormal in patients suffering some diseases like Ehlers–Danlos syndrome, osteogenesis imperfecta, and hypermobility syndrome (Ogilvie-Harris and Khazim, 1994; de Paepe and Malfait, 2012; Russek, 1999).

The type III collagen molecule is more flexible than the type I collagen molecule, which results in that fibers rich in type I collagen are greatly stiffer than those rich in type III collagen (Majewski et al., 2012; Silver et al., 2002). An experimental comparison of rabbit medial collateral ligaments with different type I and III collagen contents has come to a similar conclusion that ligaments with more type I collagen contents are stiffer than those with more type III collagen contents (Wan et al., 2014b). The changes of type I and III collagen contents alter the mechanical behaviors of ligaments and further induce some clinical manifestations in joint stability, mobility, and ligamentous ruptures (Herbort and Raschke, 2011; Ogilvie-Harris and Khazim, 1994; de Paepe and Malfait, 2012; Russek, 1999). However, to our knowledge, there is no published constitutive relation with the effects of collagen types for ligament tissues. In the previous studies some other variables have been considered in constitutive relations of ligament so far, such as total collagen volume, fiber orientation, fibril crimp, fibril diameter and density (Ault and Hoffman, 1992; Freed and Doehring, 2005; Gardiner and Weiss, 2003; Goh et al., 2008; Grytz and Meschke, 2009; Kahn et al., 2013; Peña et al., 2006; Weiss et al., 1996). The lack of collagen types in constitutive relations makes it difficult to quantitatively analyze the effect of variations in collagen types of ligaments on the ligament biomechanics and joint stability during human lives (including different normal metabolic processes and abnormal diseases).

In this study, we modified a constitutive relation for ligaments by considering collagen types. Both mechanical tests and measurements of different collagen types were performed to determine the material coefficients of the constitutive relation for human knee joint ligaments. In order to validate the modification of the constitutive relation, *in silico* joint kinematic results were simulated by finite element (FE) joint models under four loading conditions and compared with joint kinematics of the corresponding *in vitro* experiments. It was demonstrated that the modified constitutive relation was appropriate for determining the mechanical behaviors of ligaments. The collagen-type-involved constitutive relation may contribute to predicting the effect of changes in collagen types on ligament biomechanics as well as joint kinematics.

2. Material and methods

2.1. Specimen preparation

Four normal human knee joints (3 male and 1 female, 30–59 yr, 156–168 cm, 67–75 kg) were obtained from patients in the Department of Orthopaedic Oncology Surgery of Peking University People's Hospital following research ethics committee approval. The patients suffered from pelvic tumor, and needed lower limb amputations. To preserve the water content and the mechanical properties of these joint tissues, skin and muscle around the joints were reserved. The joints were double-wrapped using gauze soaked with phosphate buffered solution (PBS), and sealed in airtight plastic bags at $-20\,^{\circ}$C. The cadaveric joints were thawed at room temperature for 24 h before testing. Briefly, the main ligaments from all the four knee joints were used for developing the constitutive relation with collagen types, and two of the four joints were randomly selected to validate the constitutive relation by comparing the *in silico* joint kinematic results with the *in vitro* results.

In the development of the collagen-type-involved constitutive relation, four main ligaments with bone attachments were resected from each joint, corresponding to anterior cruciate ligament (ACL), posterior cruciate ligament (PCL), medial collateral ligament (MCL), and lateral collateral ligament (LCL), respectively. To measure the strain of ligament specimens accurately and properly, the epiligament tissues covering the ligaments were striped carefully by surgeons using scalpel because of their weakly adherence to the ligament surface. Two different tests were included for obtaining the mechanical properties of the ligaments: uniaxial tensile tests and simple shear tests. First, there were 16 ligament specimens used in the uniaxial tensile tests for acquiring the longitudinal mechanical behaviors of the ligaments. These specimens were defined as the mid-substance of each ligament between two sections that were 2 mm far from the mid-position of ligaments; the mid-position was defined as the middle location between the tibial and femoral insertions of the ligaments. The contents of collagn types were also measured from these specimens ($n=16$). Whereas, specimens for the simple shear tests ($n=4$) were extracted from the MCLs of each joint because of their flat ribbonlike dimensions. It was demonstrated that simple shear properties of ligaments were dependent on ground substance rather than their fiber components (Wan et al., 2014a). Hence the transverse mechanical behaviors of other three ligament specimens (ACL, PCL and LCL) could be determined by the MCL specimens from the same joint with the assumption of the same mechanical properties of ground substance for the four ligaments of the same joint.