available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jmbbm

Research paper

Microstructure of the ligament-to-bone attachment complex in the human knee joint

Damien Subit[a,b,*], *Catherine Masson*[a,1], *Christian Brunet*[a,2], *Patrick Chabrand*[c,3]

[a] *Laboratoire de Biomécanique Appliquée, Faculté de Médecine Secteur Nord, Boulevard Pierre DRAMARD, 13916 Marseille Cedex 20, France*
[b] *Laboratoire de Mécanique et d'Acoustique, 31 chemin Joseph-Aiguier, 13402 Marseille Cedex 20, France*
[c] *Laboratoire d'Aérodynamique et de Biomécanique du Mouvement, Parc Scientifique et Technologique de Luminy, Université de la Méditerranée et CNRS, Case Postale 918, 13288 Marseille, France*

ARTICLE INFO

Article history:
Received 24 July 2007
Received in revised form
21 November 2007
Accepted 6 February 2008
Published online 13 February 2008

Keywords:
Ligament-to-bone attachment
Fibrocartilage
Microstructure
Knee
Collagen fibres
Mechanical function

ABSTRACT

Clinical and experimental studies have shown that injuries in the human knee ligaments occur in the ligament midsubstance, at the transition between bone and ligament, and in the bone in the vicinity of the ligament-to-bone attachment site. Whereas ligament and bone have been thoroughly described, the way they connect to each other remains unclear. The goal of this study is to provide a description of the microstructure of the ligament-to-bone insertion, with the view of providing a mechanical model capable of predicting the injuries that occur at this insertion. The preparatory literature review showed that there was no description of the insertion microstructure for the human ligaments. The results found for human tendons and animal tendons/ligaments were used to lead the histological and electron — scanning and transmission — microscopy analysis. The posterior cruciate ligament (PCL), and the lateral collateral ligament (LCL) were sampled from one post mortem human subject. Slices were cut along the longitudinal direction of the ligaments, following the fibers direction. The histology analysis showed that the insertion has the same structure as reported in the literature: it is made of a mineralization front between calcified and uncalcified fibrocartilage, which is not crossed by the ligament fibers. The transmission electron microscopy analysis of the calcified fibrocartilage revealed a collagenous structure which has a direction drastically different from the direction of the ligament fibers. The mechanical function of the insertion was discussed and combined with the histological findings to hypothesize the microstructure of the insertion.

* *Corresponding author at:* University of Virginia, Center for Applied Biomechanics, 1011 Linden avenue, Charlottesville, VA, 22902, USA. Tel: +1 434 296 7288, fax: +1 434 296 3453.

E-mail addresses: subit@virginia.edu (D. Subit), masson@inrets.fr (C. Masson), brunet@inrets.fr (C. Brunet), chabrand@univmed.fr (P. Chabrand).

URLs: http://www.lma.cnrs-mrs.fr, http://www.centerforappliedbiomechanics.org (D. Subit), http://www.inrets.fr (C. Masson), http://www.inrets.fr (C. Brunet), http://www.labm.univ-mrs.fr (P. Chabrand).

[1] Tel.: +33 4 91 65 80 15; fax: +33 4 91 65 80 19.
[2] Tel.: +33 4 91 65 80 00; fax: +33 4 91 65 80 19.
[3] Tel.: +33 4 91 26 62 38; fax: +33 4 91 41 16 91.

1751-6161/$ - see front matter
doi:10.1016/j.jmbbm.2008.02.002

Nomenclature

PCL	Posterior cruciate ligament
LCL	Lateral collateral ligament
FOV	Field of view
OM	Optical microscopy
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
HES	Haemotoxylin-eosin-saffron
MB	Methylene blue
CFC	Calcified fibrocartilage
UFC	Uncalcified fibrocartilage
ECM	Extra cellular matrix
PMHS	Post mortem human subject

1. Introduction

The structural and material properties of human knee ligaments have been studied for decades, with a focus on injuries caused by impacts, such as sport and road accident related injuries. Experimental and clinical studies of the knee ligament have shown that injuries occur in the ligament itself (ligament midsubstance), in the bone or at the ligament-to-bone insertion site (Danto and Woo, 1993; Lee and Hyman, 2002; Noyes et al., 1974). While the ligament and bone structures are well known, the way the ligament inserts into the bone remains obscure. The location of the failure is likely to be explained by the specific structural and material properties of each component (ligament, bone, transition) and how they interact. The dependency of the failure location to the strain rate has been observed experimentally (Arnoux et al., 2005; Lee and Hyman, 2002; Noyes et al., 1974; Subit, 2004), but has not been linked yet to the material properties of the components of the bone-ligament-bone complex. The failure mechanisms in the ligament midsubstance and in the bone have been reported and used to develop the computational models currently utilized to predict injuries (Arnoux et al., 2002; Cardot et al., 2006; Pioletti et al., 1998; Pithioux et al., 2004; Weiss and Gardiner, 2001). However, these models do not account for the mechanical response of the insertion site, which is generally represented as a sudden transition from ligament material to bone material (Puso and Weiss, 1998; Song et al., 2004; Thomopoulos et al., 2006): the mechanical response of the insertion site was described by looking at the stress concentration due to the sudden change in material stiffness (Matyas et al., 1995; Thomopoulos et al., 2006). Indeed, the insertion is the transition between a soft tissue – the ligament – which can stretch of about 10% before failure, and a hard tissue – the bone – which can bear only about 1% of stretch before failure. The insertion is then an astonishing structure from the mechanical point of view. The literature review set about showed that there is no description of the microstructure of the human ligament-to-bone insertion. Therefore this review was extended to human tendon,[4] because of the similarities between ligament and tendon anatomy and function. As a matter of fact, tendon and ligament are often considered identical in the literature (Benjamin et al., 2002; Cooper and Misol, 1970; Evans et al., 1990; Schneider, 1956). Furthermore, animal ligaments studies were as well included. Schneider (1956) and Cooper and Misol (1970) gave the first description of the insertion site (dog patellar tendon and lateral collateral ligament), later confirmed by Benjamin et al. (1986) for human tendon insertions. It is divided in 4 layers: the ligament or tendon, the uncalcified fibrocartilage (UFC), the calcified fibrocartilage (CFC), and the bone. The transition line between uncalcified and calcified fibrocartilage is named *tidemark* or *blue line*, this is the mineralization front. The transition line between the calcified fibrocartilage and the bone is named *cement line* (Fig. 1). The tidemark is a moving mineralization front (human Achilles tendon (Milz et al., 2002), rat Achilles tendon (Rufai et al., 1996)), there is no change in the constituting materials. Besides the mineralization, the difference between UFC and CFC is that the collagen fibres in the latter are not crimped (quadriceps tendon fibers insertion into the patellae of adult rabbits, humans, dogs and sheep (Cooper and Misol, 1970)). The actual frontier between bone and tendon/ligament is the cement line (rabbit knee joint (Gao and Messner, 1996)). The collagen fibres do not cross the cement line (Gao et al., 1996; Milz et al., 2002). Clark and Stechschulte (1998) observed that the tendinous fibres interdigitate with the bone lamellar structure, without merging with them however. A molecular glue could be responsible for binding them together. Some authors attempted to relate the mechanical role of the insertion to its structure. For Schneider (1956), the fibrocartilage prevents the tendon from narrowing when loaded, so that it does not damage the bony part of the insertion. It was suggested similarly that the fibrocartilage would ensure a gradual transition (Knese and Biermann, 1958) from the ligament, which is very soft (E = 20–70 MPa (Arnoux, 2000; Pioletti, 1997)), to the bone (E = 10 000–20 000 MPa (Yamada, 1970)), and even that the insertion has the ability to adapt itself to the load by modifying the amount of calcified tissue (Gao and Messner, 1996) and the layout of the fibres (Rufai et al., 1996). The tendon or ligament fibres can penetrate the UFC at various angles relative to the surface of the UFC (Benjamin et al., 1986): the UFC ensures that the tendon does not bend when it meets the bone, and that the collagen fibres reach the tidemark at an angle approaching a right angle. Therefore, the mechanical function of the UFC would be to offer some protection from wear and tear to the tendon/ligament fibres, by preventing them from being bent, splayed out or compressed during the motion of the joint to which the tendon is attached. Thus the ligament-to-bone attachment would be a way to reduce the mechanical loading transmitted by the ligament to bone.

This paper presents the results of the study performed on the knee ligament-to-bone attachment in human to determine its microstructure. The goal was to choose a mechanical model capable of describing its behaviour and its failure (Arnoux et al., 2005; Subit, 2004). Optical microscopy, scanning and transmission electron microscopy devices were used to look at the knee ligament insertion sites in the knee joint of a Post Mortem Human Subject (PMHS). The preparation of the samples is first described, followed by

[4] The term *enthesis* is widely used to refer to the ligament-to-bone junction, even though the word is devoted to the tendon-to-bone attachment in anatomy.