Quantitative comparison of soft tissue–bone interface at chondral ligament insertions in the rabbit knee joint

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ABSTRACT

At chondral ligament insertions the calcified fibrocartilage interdigitates deeply with the lamellar bone. The shape of this interface is formed under physiological loading conditions. For the purpose of morphological comparison between different ligament entheses in the rabbit knee, the number and frequency of interdigitations and thickness of calcified fibrocartilage were quantitated at the femoral insertion of the medial collateral ligament, both insertions of the cruciate ligaments, and the tibial insertion of the patellar ligament. Among the insertions, the femoral insertion of the medial collateral ligament showed the lowest frequency and depth of interdigitations at the soft tissue–bone interface, but had the thickest zone of calcified fibrocartilage. An inverse relationship was found at the insertion interface of the cruciate and patellar ligaments. The frequency and depth of interdigations at the bone–soft tissue interface at different chondral entheses seem to be related to the mechanical strength of the respective ligament; meanwhile it may be hypothesised that the thickness of the calcified fibrocartilage might be more related to the amount of motion which takes place at an insertion.

Key words: Enthesis; fibrocartilage; image analysis.

INTRODUCTION

Knee ligament anatomy and physiology have for long been the focus of research because of the frequent need for reconstructive surgery in this area. Restoration of a normal ligament–bone junction (enthesis) after reconstruction is regarded as prerequisite for satisfactory results (Rodeo et al. 1993). Most of the knee ligament entheses are of epiphyseal or apophyseal origin; they contain different fibrocartilages and have therefore also been called chondral entheses (Knese & Biermann, 1958). The fine morphology of chondral knee ligament, tendon, and meniscus entheses have been extensively described (Petersen, 1930; Schneider, 1956; Knese & Biermann, 1958; Hurov, 1986; Woo et al. 1990; Ralphs et al. 1992; Rufai et al. 1992; Wei & Messner, 1995), but few quantitative data are available from this anatomical region (Cooper & Misol, 1970; Benjamin et al. 1991; Gao et al. 1994). Recently, the differences in the amounts and distribution of calcified fibrocartilage and cortical lamellar bone in the region immediately deep to the patellar ligament and quadriceps tendon attachments have been analysed quantitatively (Evans et al. 1991) and from the data it was concluded that differences in maximum force alone can produce a greater density of calcified tissue at ligament or tendon attachments. It also has been postulated that the fibrocartilaginous zones within chondral insertions may prevent fatigue failure by providing a more gradual transition from soft tissue to the hard bone (Schneider, 1956). The interface between lamellar bone and calcified fibrocartilage at a chondral enthesis forms an irregular border where the cartilaginous zone deeply interdigitates with the bone tissue (Cooper & Misol, 1970; Hurov, 1986; Gao et al. 1994). At this interface the ligament collagen fibres attach to bone after having passed through the different fibrocartilaginous zones. Schneider (1956) postulated that the shape of this interface is related to

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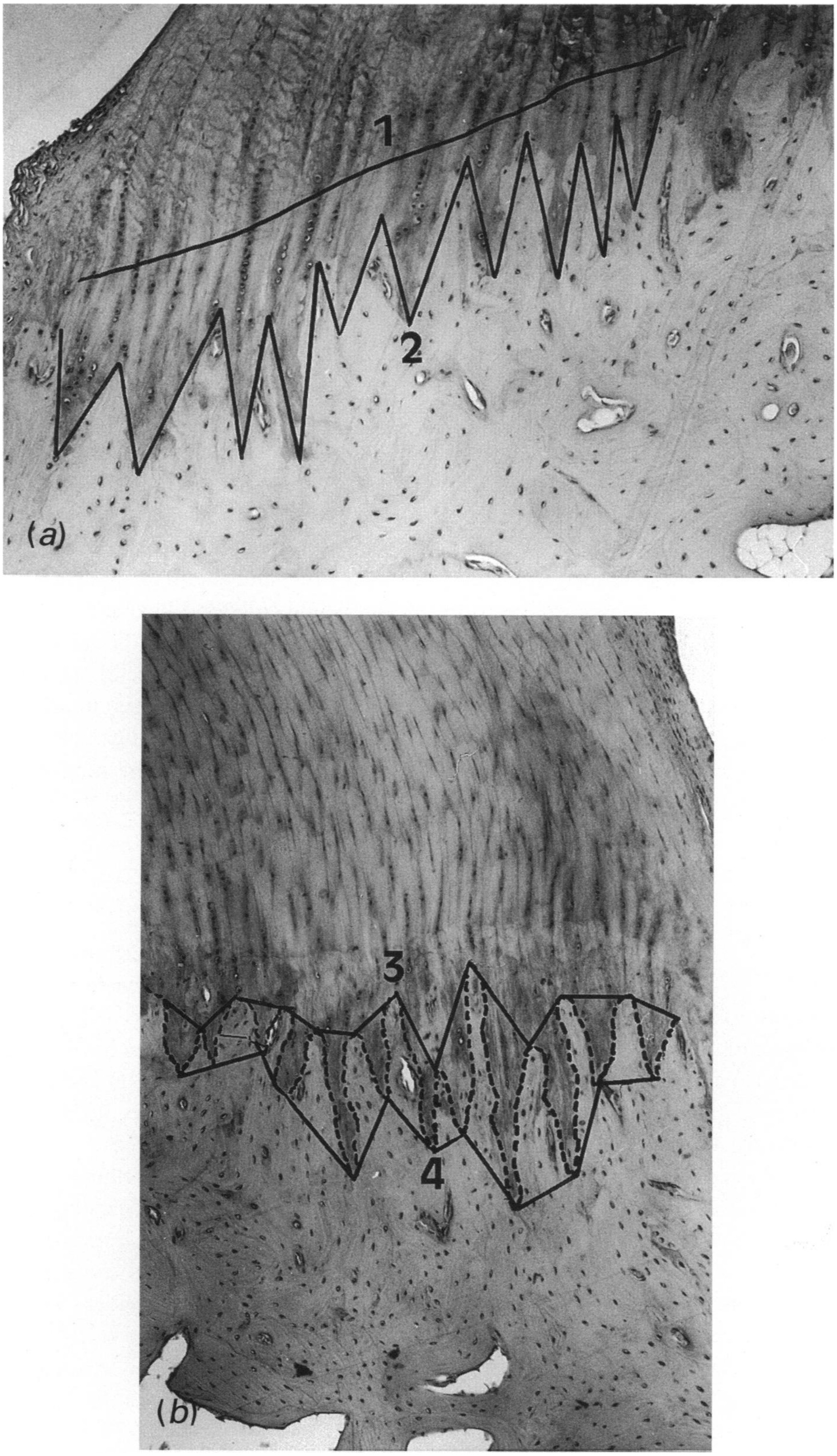

Fig. 1. (*a*) Calculation of the thickness of calcified fibrocartilage (tibial insertion of the anterior cruciate ligament). The tidemark is marked (line 1) and another line (line 2) is drawn following the interdigitations between calcified fibrocartilage and bone (cement line), and the mean distance between these lines calculated (AB/PAS, × 12.5). (*b*) Calculation of interdigitation depth at the soft tissue–bone interface (femoral insertion of the posterior cruciate ligament). The top (line 3) and bottom (line 4) of the interdigitations were connected separately and the mean distance between these 2 lines calculated. The frequency of interdigitation/mm was also calculated (AB/PAS, × 12.5).

local loading conditions. He further compared this anatomical region with the interface between epidermis and dermis and hypothesised that similar load adaptation mechanisms as are known for the skin might take place at this site. However, there are at present no quantitative morphological data on this specific region. The purpose of the present approved animal study was to compare the morphology of chondral insertions around the knee joint quantitatively with special emphasis on the shape of the bone–soft tissue interface and thickness of calcified fibrocartilage.

MATERIALS AND METHODS

The right knee joints of 10 healthy adolescent New Zealand white rabbits (age range 6–8 months; weight range 3.4–4.2 kg) were dissected immediately after death. The joint cavity was opened by cutting the quadriceps tendon and parapatellar tissues. After excision of the inferior patellar fat pad and synovial tissue, the joint surfaces were examined grossly. All joints appeared free from osteoarthrosis or other abnormalities. The soft tissue around the knee joint including the menisci was removed, leaving the ligaments intact. The femur and tibia were cut 2 cm from the joint line. The whole joint was then fixed in formalin for 2 wk. After decalcification in 10% EDTA for about 4–6 wk, the medial collateral ligament, the anterior and posterior cruciate ligaments, and the patellar ligament were transected at their midpoints; the femoral and tibial specimens of each ligament including a piece of underlying bone at the insertion were removed separately. For the present study, we evaluated the femoral insertion of the medial collateral ligament, the femoral and tibial insertions of both cruciate ligaments, and the tibial insertion of the patellar ligament. Each specimen was divided into 2 halves by cutting it parallel to the ligament fibres and perpendicular to the bony insertion surface. Thus we were able to start with the histological preparation of the central part of the ligament insertion. Sections of 5 μm thickness at 100 μm intervals were cut parallel to the collagen fibres, perpendicular to the bony insertion surface throughout the attachments, stained with haematoxylin–eosin (H & E) and Alcian blue/periodic acid-Schiff (AB/PAS). The array of collagen fibres was observed with polarised light. The thickness of the calcified fibrocartilaginous zone and the depth and number of the interdigitations between the calcified fibrocartilage and subjacent bone were measured using computerised image analysis (Image-Pro-Plus Program, Media Cybernetics, USA). For the purpose of measurement, the tidemark was marked, and a second line was drawn following carefully the interdigitations (cement line) between the calcified fibrocartilage and underlying bone. The mean distance between these 2 lines, representing the thickness of calcified fibrocartilage, was calculated (Fig. 1*a*). Interdigitation depth at the bone-calcified fibrocartilage interface was determined by calculating the mean distance between 2 further lines which connected the tops and the bottoms of the interdigitations separately (Fig. 1*b*). Also the frequency of interdigitations per measurement unit (mm) was calculated. These 3 measurements were repeated throughout each sample by consecutively covering the entire length of the insertion site. For each specimen, a minimum of 2 separate samples from the central area of the insertion was evaluated and average values were calculated. All measurements were performed with a 12.5 magnification.

Statistics

Paired *t* tests with adjustment for multiple comparisons were chosen to calculate differences among the measurements within individual knees.

RESULTS

The femoral and tibial insertions of both cruciate ligaments showed chondral insertion characteristics throughout with a distinct cement line and different fibrocartilaginous layers; accordingly, the entire length of these specimens was subjected to the abovementioned measurements and calculations (Fig. 1*a*, *b*). At the 2 purely extra-articular insertions—the tibial insertion of the patellar ligament (Fig. 2) and the femoral insertion of the medial collateral ligament (Fig. 3)—the superficial portion of collagen fibres attached via periosteum to bone and lacked the cartilaginous zones. In these specimens only the deep portion of the insertion was subjected to the measurements. In addition, the proportion between the 2 types of tissue patterns within a single insertion differed between the patellar and the medial collateral ligament. The chondral type of insertion dominated at the medial collateral ligament but, at the insertion of the patellar ligament, the size of the periosteal part almost equalled the size of the chondral part. At the chondral part of the insertion the ligament collagen fibres passed the cartilaginous zones and attached at the irregular cement line without being cemented into the

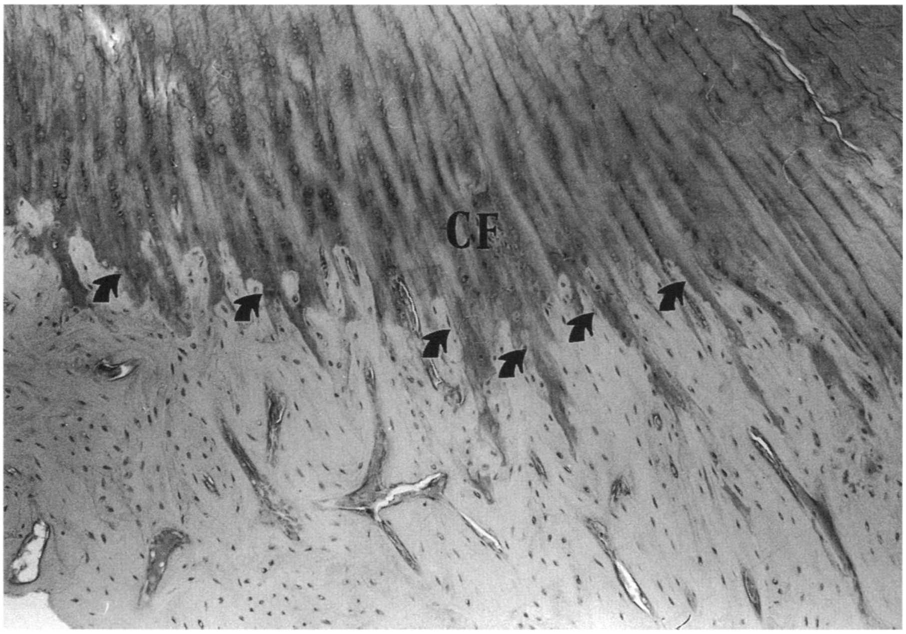

Fig. 2. The tibial insertion of the patellar ligament (deep part). The deep part of the patellar ligament has the characteristics of a chondral insertion. CF, calcified fibrocartilaginous zone; arrows, cement line (AB/PAS, × 12.5).

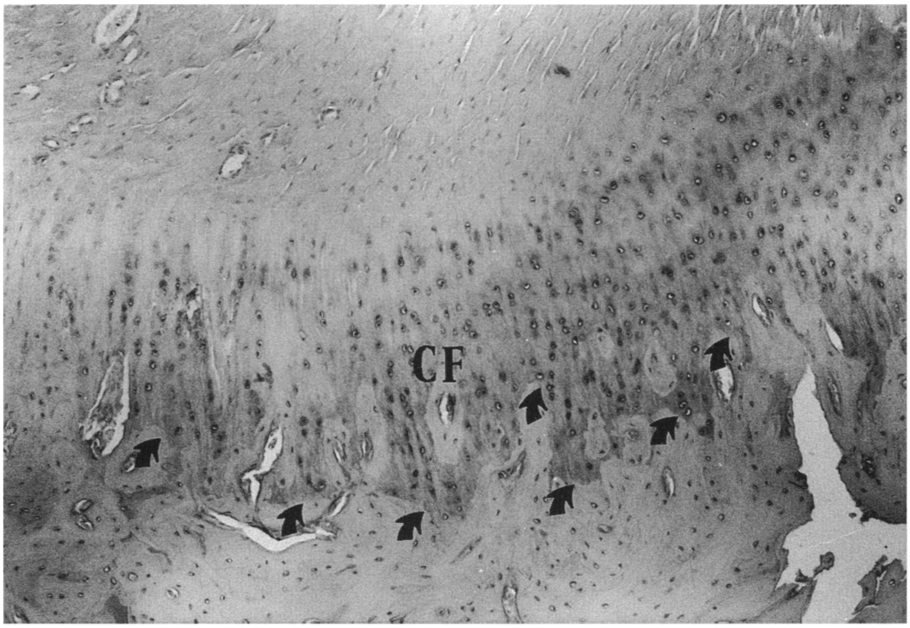

Fig. 3. The femoral insertion of the medial collateral ligament (deep part). The insertion has a lower frequency and depth of interdigitations at the calcified fibrocartilage–bone interface (arrows) and a thicker calcified fibrocartilaginous zone (CF) than other insertions (AB/PAS, × 12.5).

cortical lamellar bone in the region immediately deep to the insertion (Fig. 4).

The femoral insertion of the medial collateral ligament had a lower frequency of interdigitations per measurement unit at the cement line than all other insertions ($P < 0.01$), and a lower interdigitation depth than the femoral insertions of the anterior and posterior cruciate ligaments ($P < 0.01$) (see Table). On the other hand, the medial collateral ligament insertion had the thickest zone of calcified fibrocartilage among the investigated insertions ($P < 0.01$) (Table). There were no statistically significant differences of interdigitation frequency and depth and thickness of calcified fibrocartilage between other insertions, nor were there any significant correlations between the different measurements.

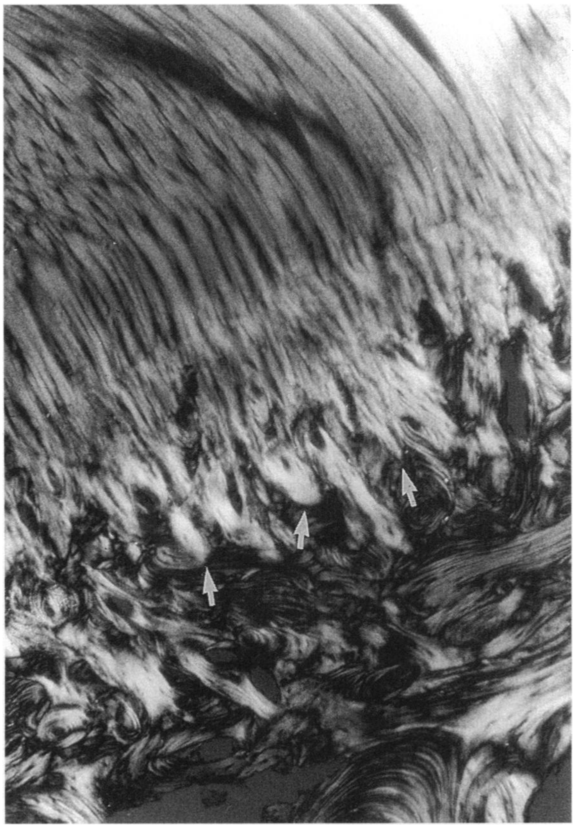

Fig. 4. Tibial insertion of the anterior cruciate ligament. The ligament collagen fibres attach to bone without merging with it (arrows) (AB/PAS, × 12.5, polarised light).

DISCUSSION

The interface between calcified fibrocartilage and subchondral bone showed the lowest frequency and depth at the insertion of the medial collateral ligament. According to Schneider (1956) this ligament insertion should therefore be exposed to lower tensile forces than other ligament insertions. This also agrees with a number of biomechanical studies, which show that the ultimate strength of the medial collateral ligament is lower than that of the anterior cruciate or patellar ligaments (Woo et al. 1992; Danto & Woo, 1993). The close similarity in shape of interface between the femoral and tibial insertions of the anterior or posterior cruciate ligaments might be explained by the identical force at either end of these structures. The calcified fibrocartilage at chondral insertions differentiates around puberty in the rabbit or rat knee joint (Woo et al. 1990; Wei & Messner, 1995) and from human cadaver studies it is known that in adulthood only slight remodelling takes place at the interface between calcified fibrocartilage and bone (Schneider, 1956). Accordingly, the specific shape of this interface—number and frequency of interdigitations between the calcified fibrocartilage and bone and interdigitation depth—forms postnatally during a short period under specific loading conditions. In contrast, increase of calcified fibrocartilage and lamellar bone was observed beyond puberty in the rat or rabbit (Gao et al. 1994; Wei & Messner, 1995), and was interpreted as a strengthening of the unit during the maturation process. In addition, greater amounts of lamellar bone and calcified fibrocartilage were found in man related to larger forces that the attached structure transmits (Evans et al. 1991). Besides the importance for tissue strengthening, it was postulated by Schneider (1956) that the different fibrocartilaginous layers within a chondral insertion may diminish the risk for fatigue failure during motion by making this transition between soft tissue and bone more gradual, analogous to the protection of electric wires by interposing a piece of stiffer material between the relatively soft wire and the hard wall. In a study of the human menisci Benjamin et al. (1991) found a thicker layer of the calcified tissue in the lateral meniscal attachments which exhibit larger displacements during knee flexion than the medial meniscal attachments (Thompson et al. 1991; Bylski-Austrow et al. 1994). From these reflections, it may be hypothesised that the shape of the soft tissue–bone interface and the thickness of calcified fibrocartilage at a ligament insertion may be capable of 2 different kinds of physiological adaptation: the former may form and adapt according to the tensile loads around puberty, the latter may respond to loads beyond that period and possibly also adapt to the

Table. *Thickness of calcified fibrocartilage and frequency and depth of interdigitations at the soft tissue–bone interface*†

	MCL [F]	ACL [F]	ACL [T]	PCL [F]	PCL [T]	PL [T]
Thickness (mm)	0.28* (0.07)	0.22 (0.07)	0.21 (0.03)	0.24 (0.02)	0.22 (0.04)	0.24 (0.04)
Depth (mm)	0.10** (0.04)	0.13 (0.04)	0.11 (0.03)	0.12 (0.03)	0.12 (0.02)	0.13 (0.02)
Frequency/mm	9.9* (1.6)	12.2 (2.2)	11.4 (1.5)	10.8 (2.0)	11.8 (1.0)	12.8 (2.4)

† Mean values (S.D.).
MCL, medial collateral ligament; ACL, anterior cruciate ligament; PCL, posterior cruciate ligament; PL, patellar ligament; [F], femoral insertion; [T], tibial insertion. * Significant difference compared with all other insertions ($P < 0.01$). ** Significant difference compared with the femoral insertions of both cruciate ligaments ($P < 0.01$).

amount of motion within this vulnerable site of transition between soft tissue and bone. This would also explain the lack of a correlation between interface characteristics and size of calcified fibrocartilage as found in the present study. Following the above-mentioned considerations, it may be suggested that a larger amount of motion takes place at the peripherally located, acute medial collateral ligament insertion than at the more perpendicular insertions of the centrally located cruciate ligaments (Müller, 1982; Kapandji, 1987), thus explaining the thicker calcified cartilaginous zone in the insertion of the former. Unfortunately, the few studies dealing with morphological change of ligament insertions after experimental immobilisation of the knee (Laros et al. 1971; Newton et al. 1995) do not include quantitative measurements and can, therefore, not answer whether loss of motion might cause a decrease of the calcified fibrocartilaginous zone as hypothesised here. They only report qualitatively unchanged insertion morphology after immobilisation. Calcification and interdigitation characteristics need also to be interpreted by taking the entire insertion area into account, which was not measured in the present investigation. Area and tissue homogeneity within an insertion may play important roles in the load-adaptation mechanism. In this context, the femoral insertion of the medial collateral ligament and the tibial insertion of the patellar ligament which are partly chondral, partly periosteal attachments may not be comparable to the purely chondral attachments of the cruciate ligaments. Also the available mechanical data may not be representative for the amount of physiological load within an insertion: first because the ligament seldom failed within the attachment zone, but rather by a subchondral bone fracture or ligament intrasubstance rupture (Crowninshield & Pope, 1976; Woo et al. 1990); and secondly, because of the unphysiological in vitro test arrangement. The failure strength during these tests has been found to be influenced by load direction, knee joint flexion angle, and strain rate (Noyes et al. 1974; Woo et al. 1987; Danto & Woo, 1993; Mommersteeg et al. 1995). However, the fact that frequency and depth of interdigitations at the bone–soft tissue interface were consistently higher at stronger structures such as the cruciate and patellar ligaments in contrast to the medial collateral ligament insertion may indicate some positive relation between the size of interface and strength of the unit and therefore support the proposals by Schneider (1956). The fact that no mechanical failure occurred at the irregular cement line, but rather through the zone of calcified cartilage or subchondral bone (Crowninshield & Pope, 1976) may underline the effectiveness of interdigitations between 2 different types of tissue to resist mechanical forces.

From the presented data it is impossible to draw definite conclusions about physiological loading and motion conditions at different insertions and possible morphological adaptations to them because of the lack of data on insertion size and mechanics. However, the specific pattern of the soft tissue–bone interface and calcified fibrocartilage at each individual insertion points to possible relations between structural characteristics and the ultimate strength of the attached ligament.

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