Arch Orthop Trauma Surg (2003) 123 : 283–288
DOI 10.1007/s00402-003-0527-7

ORIGINAL ARTICLE

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Structure and vascularization of the acetabular labrum with regard to the pathogenesis and healing of labral lesions

Received: 15 August 2002 / Published online: 7 June 2003

Abstract *Introduction:* The aim of this study is to examine the structure and vascularization of the acetabular labrum with regard to the pathogenesis and healing of labral tears. *Materials and methods:* The labral tissue was characterized immunohistochemically and under light microscopy; the collagen fibril texture was demonstrated by scanning electron microscopy after temporary staggered maceration of the tissue; and the vascularization of the acetabular labrum was studied immunohistochemically using antibodies against laminin. *Results:* The peripheral aspect of the acetabular labrum consists of dense connective tissue. The internal layer consists of type II collagen-positive fibrocartilage. Scanning electron microscopy revealed three distinct layers in the acetabular labrum: (1) the articular surface was covered by a meshwork of thin fibrils; (2) beneath the superficial network, there is a layer of lamella-like collagen fibrils; (3) the majority of the collagen fibrils are oriented in a circular manner. Blood vessels enter the labrum from the adjacent joint capsule. The distribution of vessels within the labrum is not homogenous. Blood vessels can be detected only in the peripheral one-third of the labrum. The internal part is avascular. *Conclusion:* The result of this study demonstrates that the structure of the acetabular labrum is highly significant for the direction of traumatic and dysplastic labral lesions. The biomechanical analysis of the structure suggests that the labrum is stressed by compressive load. Therefore, excision or removal of the labrum may alter physiological functions such as enhancing joint stability and load distribution. The vascular pattern identified should encourage surgeons to develop repair strategies of peripheral labral tears to maintain its functions in the hip.

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Keywords Fibrocartilage · Circular tensile stress · Labral lesions · Healing potential

Introduction

The acetabular labrum is a joint lip that increases the depth of the acetabulum and enhances joint stability. Labral tears are a rare condition, but they may cause recurrent and severe hip pain in younger patients [7, 10, 13]. Tears of the acetabular joint lip may be caused by trauma or by degenerative changes due to hip dysplasia [25].

When looking at the development and direction of tears and lesions of other joint lips or the menisci, the collagen fibril texture is highly significant [19, 20]. Collagen fibrils are always directed along the greatest tensile stress, and the tissue is most fragile perpendicular to the main direction of the collagen fibrils.

Today, most authors suggest excision of the torn acetabular labrum as the treatment of choice. Although patients experience promt pain relief [8], excision of the labrum might compromise its physiological functions [4, 5, 6]. The healing potential of intraarticular structures such as menisci or joint lips is closely associated to the spatial distribution of blood vessels. In the human knee joint meniscus, lesions of the vascularized external third have the ability to heal, while lesions of the avascular inner aspect mostly fail to heal [18].

The aim of this study was to describe the structure and blood supply of the acetabular labrum with regard to the pathogenesis and treatment of acetabular labral tears.

Materials and methods

Light microscopy

Acetabular labra were removed from 25 subjects of different ages (13 women, 12 men, aged 23–77 years) during autopsy. After exarticulation of the femoral head, the labra were removed with the adjacent bone using a chisel. Then the labra were divided into anterior, superior and posterior segments and fixed in 4% formalin (Fig. 1). After decalcification in 10% EDTA at 40°, each segment

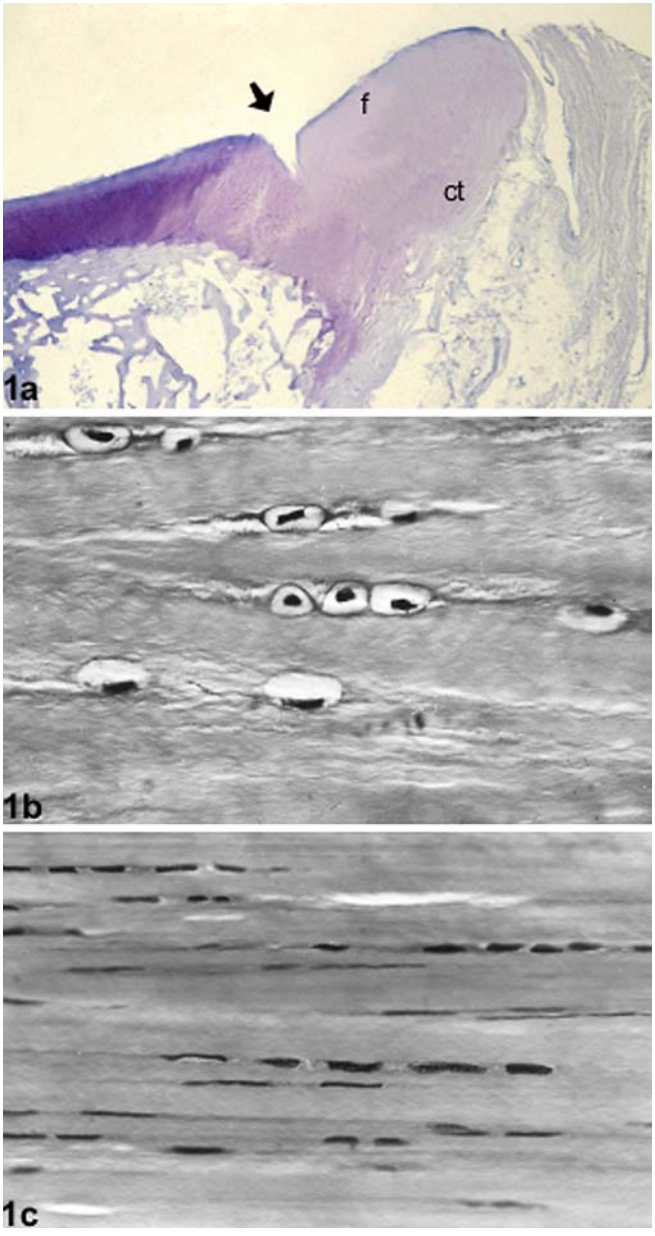

Fig. 1a–c Light microscopy. **a** Cross-section of the acetabular labrum at the superior portion. The labrum is separated from the hyaline cartilage by a physiological cleft (*arrow*). It is composed of two different tissues. The internal part which is directed towards the femoral head is composed of fibrocartilage (*f*), the external part consists of dense connective tissue (*ct*). Between the fibrocartilage and dense connective tissue, there is a large transition zone; x25, 62 years, female. **b** Chondrocytes of the internal fibrocartilage, x540. **c** Longitudinal fibroblasts in the dense connective tissue of the external circumference, x540

was embedded in paraffin. Transverse sections were performed and mounted on gelatin-coated slides. The following stains were used: toluidine blue (pH 5.8), Goldner and Gomori. The slides were examined under a Zeiss-Axiophot microscope.

Scanning electron microscopy

Twenty acetabular labra (10 men and 10 women aged 45–76 years) were studied under the scanning electron microscope in order to analyse the texture of the collagen fibrils. The labra were fixed in 4% formalin and subdivided into anterior, superior and posterior segments to permit a topographical classification. The tissue was removed layer by layer chemically to evaluate the texture of the collagen fibrils throughout the entire labrum cross-section. To expose the deeper layers, specimens were macerated in hypochlorous acid (HClO) in a graduated procedure. Ohtani's method was used to demonstrate the collagen fibrils in the exposed layers and the superficial layer [15]. To do so, the different layers of the labrum segments were kept in 10% NaCl solution at room temperature over a period of 4–5 days. Subsequently, the specimens were rinsed in distilled water for 1–2 days and then saturated in tannic acid for 4–5 h. After rinsing the specimen in distilled water for 24 h, they were counter-fixed in OsO_4, dehydrated in an ascending alcohol series and dried using the critical point method. The specimens were sputtered with gold chloride and studied under a Phillips XL 20 scanning electron microscope.

Immunohistochemistry

For the immunohistochemical investigations 20 acetabular labra were obtained from autopsies performed within 48 h after death (age of subjects 39–80 years). The acetabular labra were divided into three different segments as for light microscopy. All samples were shock-frozen in liquid nitrogen. Transverse and longitudinal sections of each segment were cut with a cryostat at –30°C and mounted on gelatin-coated slides. For immunohistochemistry, frozen sections were pretreated with testicular hyaluronidase (Boehringer, Ingelheim, Germany) in Tris-buffered saline (TBS) in a moist chamber at 37°C for 30 min. The sections were washed three times with TBS and incubated with goat serum for 45 min at room temperature. Incubation with the primary antibody was carried out for 60 min at room temperature. The antibodies used were polyclonal laminin antibodies (Medac, Hamburg, Germany), polyclonal type I collagen antibodies (Prof. Müller, Lübeck), monoclonal type II collagen antibodies (Developmental Hybridoma Bank, USA) and type III collagen antibodies (Prof. Müller, Lübeck). Sections were labelled with the respective secondary antibody, fluorescein thiocyanate-conjugated (FITC) goat anti-rabbit IgG (Medac) for 45 min. Control sections were incubated only with the FITC-conjugated antibody. Positive controls including a tissue with defined antigen sites (laminin: skeletal muscle, type I collagen: biceps tendon, type II collagen: articular cartilage, type III collagen: spleen) were used. For the exact localisation of the immunoreactions, each section was analysed under polarized light.

Results

Light microscopy

Under light microscopy the acetabular labrum can be divided into two different zones (Fig. 1): The part which is directed towards the joint capsule consists of dense connective tissue (Fig. 1). The collagen fibrils were divided into bundles by reticular fibres. Between the joint capsule and labrum there was a small recessus in the majority of specimens (21 of 25). In this zone the labrum is covered by a layer (approx. 200 µm) of loose, well vascularized connective tissue.

In the region facing the femoral head, chondrocytes are embedded between the collagen fibrils. This fibrocartilaginous layer measures between 200 and 300 µm in thickness. There is a continuous transition from the dense connective tissue of the periphery to the fibrocartilage of the internal part.

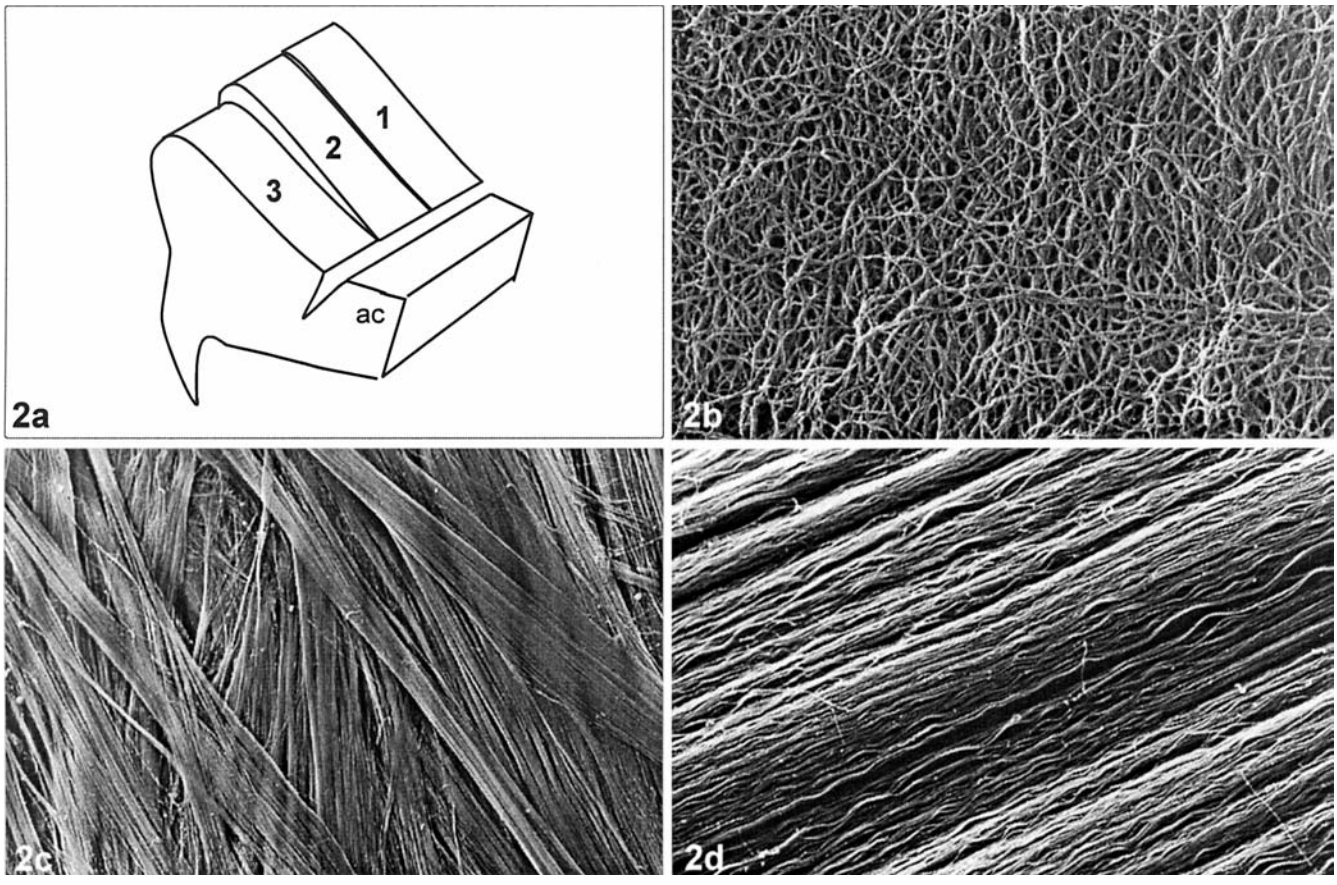

Fig. 2a–d Scanning electron microscopic results. **a** Schematic drawing of the different layers (**b–d**), detected under the scanning electron microscope. **b** Surface of the labrum acetabulare (layer 1), consisting of a network of randomly arranged delicate fibrils, x15,000, 55 years, male. **c** Below the superficial layer there are lamellae of collagen fibrils which intersect at various angles (layer 2), x1900, 55 years, male. **d** The majority of the collagen fibrils have a circumferential orientation (layer 3), x7000, 55 years, male

The transition between the labrum and hyaline cartilage of the acetabulum corresponds also to the structure of fibrocartilage with isogenic groups of chondrocytes. In 19 specimens there was a cleft dividing the labrum and cartilage partially (n=13) or completely (n=6).

Scanning electron microscopic results

Scanning electron microscopy of the acetabular labrum reveals three distinct layers (Fig. 2a):

1) a fibril network covering the surface of the labrum (Fig. 2b),
2) a lamellar layer beneath the superficial network (Fig. 2c),
3) an external main portion (Fig. 2d).

The surface of the acetabular labrum is covered with a 10-µm-wide network of delicate fibrils with a diameter of approx. 30 nm (layer 1). The fibrils do not show a preferred orientation (Fig. 2b). Collagen fibrils measuring approx. 100–130 nm in diameter are found below the superficial network (layer 2). These fibrils lie together in tight, approx. 40-µm-wide lamellar bundles, forming a fibril layer corresponding to the superficial fibrocartilage (Fig. 2c). The lamella-like fibril bundles of this zone intersect at various angles (Fig. 2c). In the cranial part of the labrum, the average thickness is between 200 and 300 µm, but the thickness of layer 2 decreases continuously towards the anterior and posterior ends of the labrum.

Collagen fibrils with a circular orientation form the main part of the acetabular labrum. In the entire labrum the prime orientation of the collagen fibrils within the main portion (layer 3) is circular in the external as well as in the internal circumference (Fig. 2d). Fibrils with a diameter of approx. 120 nm form bundles of varying thickness, which are covered by thin collagen fibrils with a diameter measuring approx. 30 nm. In the anterior and posterior portions of the labrum, the circular fibres are continuous with the transverse acetabular ligament.

Immunohistochemistry

In every age group the majority of collagen fibrils stained positive for type I collagen (Fig. 3a). The type I-positive fibrils were divided into bundles by thin fibrils stained by antibodies against type III collagen (Fig. 3b). In the fibrocartilage facing the joint cavity, apart from types I and III

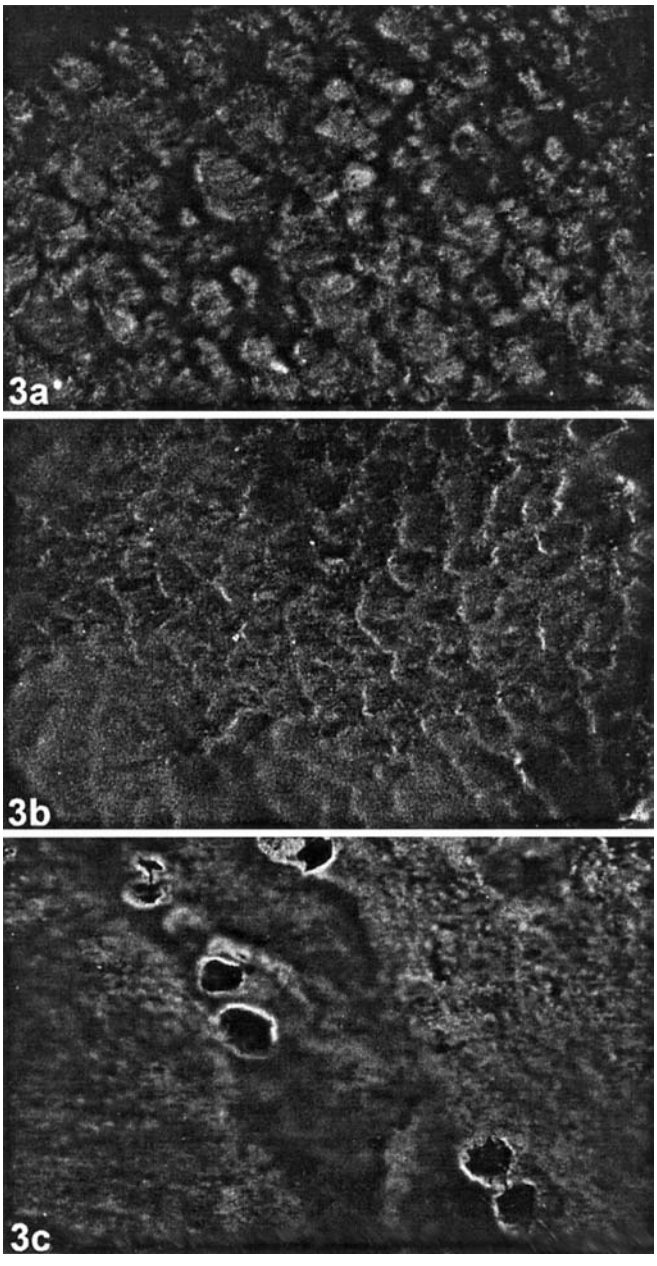

Fig. 3a–c Immunohistochemical analysis of the extracellular matrix. **a** Immunostaining for type I collagen. The majority of the collagen fibrils could be immunostained with antibodies against type I collagen, FITC-conjugated secondary antibody, ×260, 67 years, female. **b** Immunostaining for type III collagen. The bundles of type I collagen are separated by fibrils which were immunostained with the antibody against type III collagen, FITC-conjugated secondary antibody, x260, 67 years, female. **c** Immunostaining for type II collagen. It was positive in the pericellular matrix of the fibrochondrocytes, FITC-conjugated secondary antibody, x854, 67 years, female

collagen, immunostaining for type II collagen was positive (Fig. 3c). Type II collagen was restricted to the pericellular matrix of chondrocytes. The collagen fibrils of the superficial network were stained by antibodies against type III collagen.

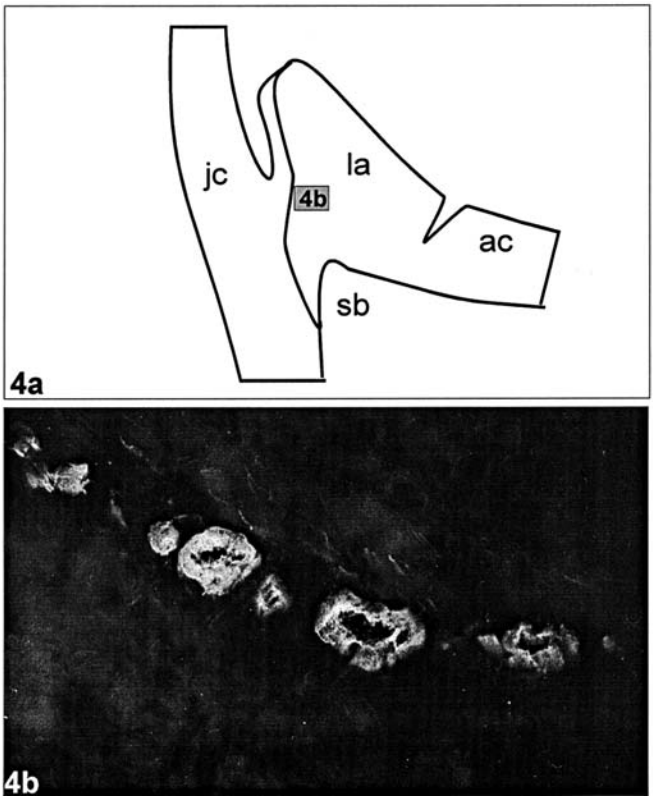

Fig. 4a, b Vascularization. **a** Schematic drawing of the acetabular labrum that indicates the origin of the detail (**b**); *jc* joint capsule, *la* labrum acetabulare, *ac* articular cartilage, *sb* subchondral bone. **b** Immunostaining of laminin as a component of the basement membrane of blood vessels. Blood vessels could be detected in the external one-third of the labrum, x100, 55 years, male

Blood supply

In comparison to controls of human skeletal muscle, immunohistochemical proof of laminin was positive in the wall of blood vessels in all investigated sections.

The acetabular labrum is nourished by the well-vascularized joint capsule. From the joint capsule blood vessels enter the peripheral part of the labrum. In contrast to the well-vascularized capsular tissue, the density of vessels within the labrum is greatly reduced (Fig. 4). Laminin immunostaining was only positive in the external one-third of the labrum. In the inner two-thirds the laminin immunostaining was negative, and the tissue was avascular (Fig. 4).

Discussion

Tears of the actetabular labrum may impair the function of the hip joint. The direction of tears and lesions of intraarticular structures such as joint lips or menisci depends highly on the architecture of the collagen fibrils [19, 20] because they are always directed along the greatest tensile strain [24]. By following Ohtani's procedure [15], it is possible to expose collagen fibrils after removing cells

and non-fibrillar extracellular matrix. Thus, the path of the fibrils can be studied directly under the scanning electron microscope [20]. A systematic analysis of the tissue architecture became possible by uncovering the acetabular labrum layer-by-layer in a graduated maceration process using hypochlorous acid (HClO) [20]. The present study reveals that the acetabular labrum is composed of three layers. The surface of the acetabular labrum is formed by a network of delicate fibrils (layer 1). Beneath the superficial network there is a layer composed of lamellar-like collagen fibril bundles (layer 2). The fibril bundles intersect at different angles. The chief portion of the collagenous fibrils runs in the external main part (layer 3) and is arranged in a circular fashion in all segments of the acetabular labrum.

According to Pauwel's theory of 'causal histogenesis' [17], tissue structure is directly related to the stress on it. Regardless of their genetic type, collagen fibrils are always oriented in the direction of the greatest tension [24]. Every point of a joint surface is stressed by a normal force that is directed perpendicular to the surface [12]. In contrast to the adjacent articular cartilage, the labrum has no bony bearing, and the normal force tends to extrude the labrum from the acetabular rim because of the lack of an adequate fixation to the articular cartilage. Circular traction develops because the labrum cannot yield to a radial shift due to the fact that its horns are fixed to the transverse acetabular ligament. Biomechanical examinations have shown that under tension, the labrum is much stiffer than the adjoining articular cartilage [4]. These mechanical observations agree with our morphological findings, which reveal that the main portion of the collagen fibrils in the acetabular labrum run circularly.

In the clinical literature different aetiological factors for labral lesions have been identified [9, 10, 13, 25]. Traumatic lesions are observed mainly in younger or middle-aged patients. The traumatic type A lesion according to Tschauer [25] occurs because the labrum is stressed by high radial forces that occur when the femoral head slides around the joint lip in traumatic dislocation of the hip. Fitzgerald [7], however, has shown that labral lesions may also occur due to minor twisting injuries of the leg and hip. Another important aetiological factor is hip dysplasia [25]. The mechanism for the development of this type of lesion, called the type B lesion [25], is different. Type B lesions occur without an adequate trauma in patients with hip dysplasia as the femoral head slides against the labrum which serves as a slide barrier for it [25]. The majority of both types of lesion have a circular orientation [2, 3, 7, 9, 10, 16, 25]. Since collagen fibrils absorb traction, the acetabular labrum tissue shows the least tensile strength in the transverse direction to the circular main path of the collagen fibrils [6]. This observation agrees with clinical findings that labral tissue tears along the circular main course of the collagen fibrils in the majority of labral lesions (longitudinal tears, bucket handle tear [8]).

The transverse acetabular ligament serves to connect the anterior and posterior horns of the labrum, and the circular fibrils of layer 3 radiate directly into this ligament. The primary function of the transverse acetabular ligament is to act as a restraint against minimal motions of the acetabulum due to incongruity of the hip joint [14]. A further function of this ligament may be to suspend the circular collagen fibrils of the acetabular labrum.

Anatomical textbooks state that the acetabular labrum consists exclusively of fibrocartilage [23, 26]. In contrast, the present study reveals two tissue phenotypes: fibrocartilage and dense connective tissue. In the external circumference there is dense connective tissue. In the inner region which is directed towards the articular surface, there is a thin layer of fibrocartilage. The histological results are in accordance with the immunohistochemical analysis of collagen. The dense connective tissue contains types I and III collagen. In the fibrocartilaginous zone, immunostaining for the cartilage-specific type II collagen is also positive. According to the theory of 'causal histogenesis' [17], the stimulus for the development of fibrocartilage is provided by intermittent compressive and shearing forces, while dense connective tissue is formed as a functional adaptation to tensional stress. The discussion about the load-bearing role of the acetabular labrum is controversial. Konrath et al. [11] have shown that removal of the acetabular labrum does not significantly increase the pressure or load in the acetabulum. These authors concluded that excision of the acetabular labrum may not predispose the hip to premature osteoarthrosis. However, recent studies using poroelastic finite element models have demonstrated that the labrum can seal against fluid expression from the joint space. In the absence of this sealing, strains within the matrix of the cartilage were significantly higher [5]. Current treatment strategies such as excision of the acetabular labrum might result in immediate pain relief but will also compromise the physiological function of the labrum such as enhancing joint stability [4, 5, 6], preserving congruity and the sealing mechanism. With increasing knowledge about the function and the importance of the labrum, new surgical strategies such as repair or refixation must be developed to maintain the function of the hip joint.

The healing capacity of intraarticular structures such as menisci and joint lips is highly associated with their vascular pattern [1, 18, 19, 22]. Laminin is a basic component of the basement membrane, and immunostaining reliably detects blood vessels in dense connective tissue and fibrocartilage [18, 19]. Immunostaining for laminin demonstrated blood vessels only in the peripheral third of the labrum. These findings show that peripheral tears of the acetabular labrum such as bucket handle tears have the biological potential to heal.

In conclusion, the result of our study demonstrates that the structure of the acetabular labrum is highly significant for the pathogenesis of traumatic, dysplastic and degenerative lesions of the acetabular labrum. The load-bearing role of the labrum should not be overestimated, but excision or removal may alter the physiological functions such as enhancing joint stability and the sealing mechasm. The vascular pattern identified should encourage surgeons to develop repair strategies of peripheral labral tears to maintain its functions in the hip.

Acknowledgements We thank Mrs. R. Worm, Mr. C. Franke and Mrs. H. Waluk for their help and expert technical assistance.

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