

# Refixation of the Excised Acetabular Labrum can Affect the Structural Changes of Cartilage Surface in the Hip Joint

## La Refijación del Labrum Acetabular Extirpado Puede Afectar los Cambios Estructurales de la Superficie del Cartilago en la Articulación Coxal

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**SUMMARY:** Refixation of the damaged acetabular labrum is a method of surgical treatment of the hip joint that can promote the repair of joint function after injury and prevent premature osteoarthritis. We sought to determine the condition of the hip joint in rabbits 4 months after excision of the acetabular labrum and the condition of the joint after labral refixation. The articular cartilage of the femoral head and acetabulum was examined by histological methods, multipoint measurement of cartilage thickness, and the ratio between cartilage matrix and chondrocytes lacunae, and the condition of cartilage according to the OARSI grading scale was carried out. On this model, a correlation analysis was performed between the results of the OARSI grading scale and the data of linear morphometry. All these parameters made it possible to better assess changes in articular cartilage. The ratio between matrix and chondrocyte lacunae turned out to be a method that allows establishing early cartilage damage when erosion, fibrosis or deformation did not occur. We found significant differences between the condition of the cartilage after excision of acetabular labrum and after labral refixation, which give hope to confirm that this surgical technique can delay or prevent progressive changes in the cartilage of the damaged hip joint.

**KEY WORDS:** Acetabular labrum; Hip joint; Femoral head; Acetabulum; Cartilage; Model of labrum excision; Refixation

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## INTRODUCTION

Injury of the hip joint adversely affects the function of the lower limb and can cause disability (Da Rocha *et al.*, 2012). It is known about the connection between damage to the acetabular labrum and changed biomechanics of the joint (Meermans *et al.*, 2010), inflammation due to femoroacetabular impingement (Haneda *et al.*, 2020), pain syndrome and progressive arthrosis (McCarthy *et al.*, 2001). Labral injury causes hypermobility (Philippon *et al.*, 2007), reduces hip joint stability and synovial fluid retention (Cadet *et al.*, 2012). Therefore, preserving the integrity of the acetabular labrum is important for adequate biomechanics, joint stability and joint protection (Song *et al.*, 2012). A

disorder of the hip labrum causes contact tension between the cartilages of the femoral head and the acetabulum. Ignoring and not treating this condition can ultimately lead to premature osteoarthritis (Philippon *et al.*, 2012; Domzalski *et al.*, 2017).

Some observations indicate that the result of arthroscopic surgery of the hip joint directly depends on the level of saving of the labrum (Harris, 2016; Carton & Filan, 2017). After labral refixation a tendency to delay progressive degenerative changes in the joint was observed (Larson & Wulf, 2009; Kollmorgen & Mather, 2017; Maldonado *et al.*, 2021), although some negative consequences are known (labral

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tear, degeneration, detachment) (Abdelaal *et al.*, 2023). Considering this, the aim of the study was to investigate the changes in the articular cartilage of the hip joint after excision of the acetabular labrum and after its refixation.

## MATERIAL AND METHOD

**Animals.** Experiments were conducted on rabbits (*Chinchilla lanigera*) weighing 4.5-5.0 kg. All animal manipulations were performed under anesthesia (sodium thiopental at a dose of 60 mg/kg, i.v.) and according to European Convention for the protection of vertebrate animals used for experimental and other scientific purposes", "Guide for the care and use of Laboratory Animals" (Strasbourg, 1986), with recommendations of the bioethics commission of State Institution "Institute of Traumatology and Orthopedics of the National Academy of Medical Sciences of Ukraine", Kyiv, Ukraine.

**Experimental design.** The model of the experiment consisted in unilateral excision of the acetabular labrum of the hip joint (n=4), and in other animals refixation of the labrum immediately after excision (n=3). Anesthetized animals had access to the joint on the dorsal surface of the hind-limb, cut off the muscles from the greater trochanter, dissected the capsule, and dislocated the head of the femur. Transosseous acetabular labral refixation was carried out with non-absorbable braided thread No. 2-0 to the greater trochanter. The wound was sutured in layers. As a control, the cartilage of intact animals (n=3) was examined.

**Histological examination.** After 4 months, the rabbits were removed from the experiments by administering a lethal dose of anesthesia. The articular complex was fixed in a 10 % formalin solution (NBF, pH 7.4). The femoral head and the acetabulum with a thickness of about 2 cm were sawn off, decalcified (OsteoFast 1, BioGnost, Croatia), dehydrated in isopropanol, compacted in paraplast (Leica Surgipath Paraplast Regular). Sections were cut at 6 µm with a Thermo Microm HM 360 microtome. The sections were stained with hematoxylin and eosin (H&E), picro-sirius red (PSR), safranin O with fast green (SOFG). Micropreparations were examined using an Olympus BX51 microscope.

**Quantitative assessment and morphometry.** The cartilage surface of the femoral head and acetabulum were assessed by changes in the staining of the cartilage with safranin, the presence of fibrosis, and cartilage erosion. The Osteoarthritis Research Society International grading scale (OARSI) was used as the most appropriate for the assessment of cartilage condition in this work. The principle of cartilage assessment

is described in the article (Pritzker *et al.*, 2006). In order to increase the accuracy of determining the grade of changes, each sample was evaluated in 4 zones (2 in the center and 2 peripheral zones of the cartilage) and then the average score was recorded for the sample. Linear morphometry was performed using AxioVision SE64 Rel.4.9.1 software (Carl Zeiss), which consisted in measuring the distance from the cartilage surface to the tidemark between mineralized and non-mineralized cartilage. We determined the ratio of lacunae in the cartilage to its matrix. Microphotograph were taken at ×100 and contained an area of cartilage approximately 1.2-1.5 mm<sup>2</sup> in size. The cartilage area and the total area of all lacunae per image (with chondrocytes and empty lacunae) were determined, and further mathematical calculations were carried out.

**Statistical analysis.** Statistical data processing was carried out using one way ANOVA Bonferroni post-hoc test and Spearman's rho correlation.

## RESULTS

Four months after the operation, the rabbits showed a reduction in the normal range of motion of the femoral joint, reluctance of the animals to active movements and signs of hip muscle hypotrophy. According to the results of histological studies, structural changes were established in the articular cartilages, from the side of the femoral head and the acetabulum. Some differences in the level of development of changes were found between the cases, from almost intact or weakly expressed changes (Grade 0 and 1) to violations with signs of cartilage erosion, deformation of the cartilage surface (Grade 3 and 4) (Fig. 1). The degree of changes in both joint surfaces was usually similar. A strong correlation was established between the condition of the articular cartilage of the femoral head and the acetabulum according to the OARSI grading scale (n=7; r=0.88; p=0.01). According to the OARSI grading scale, no significant difference was found between the state of joint cartilage after labral excision and after labral refixation (p=0.80; p=0.15) (Fig. 2).

We concluded that the assessment of the state of cartilage should not be carried out only by signs of cartilage erosion, the appearance of signs of fibrosis and the level of chondromucoid staining. We observed a relationship between cartilage erosion, inflammation, and fibrous changes on the cartilage surface (in this triad, resorption is replaced by fibrous connective tissue). But initial cartilage changes (Grade 1), or cartilage changes without erosion but with altered normal chondromucoid staining (heterogeneous SOFG staining occurs) (Grade 1-2) have differences. These differences are characterized by the reduction of chondrocytes, the appearance

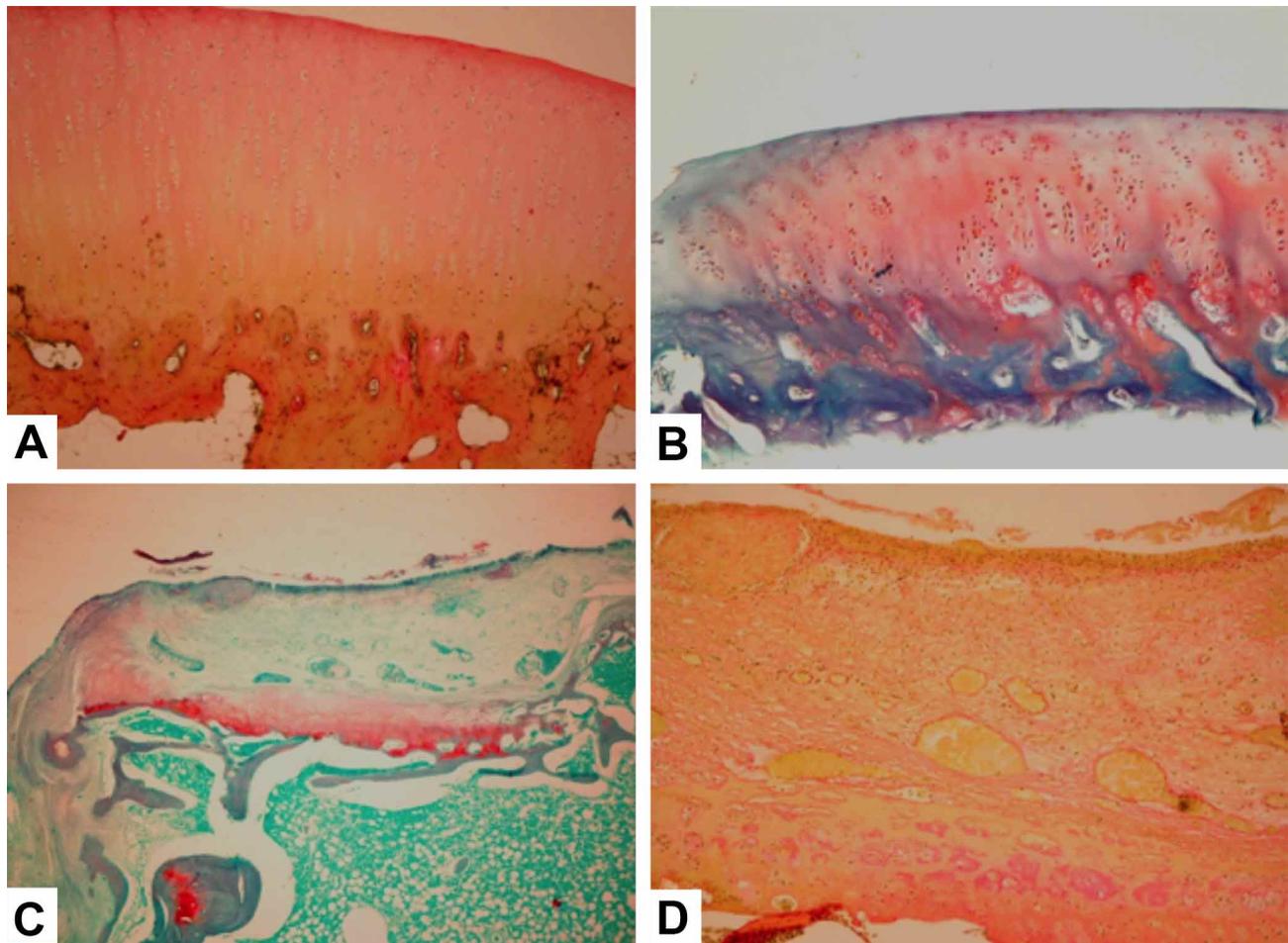


Fig. 1. Structural changes in the cartilage of the femoral head and the acetabulum: a – almost intact of femoral head cartilage, PSR staining,  $\times 100$ ; b – chondrocyte clustering and heterogeneity of cartilage staining in the femoral head after labrum refixation, SOFG staining,  $\times 100$ ; c and d – erosion of cartilage and fibrosis in the acetabulum after labrum refixation, SOFG and PSR staining,  $\times 40$  and  $\times 100$ .

of empty lacunae. Therefore, additional methods of morphometry can be used in cartilage studies, which in the complex give more numerical data for an objective assessment.

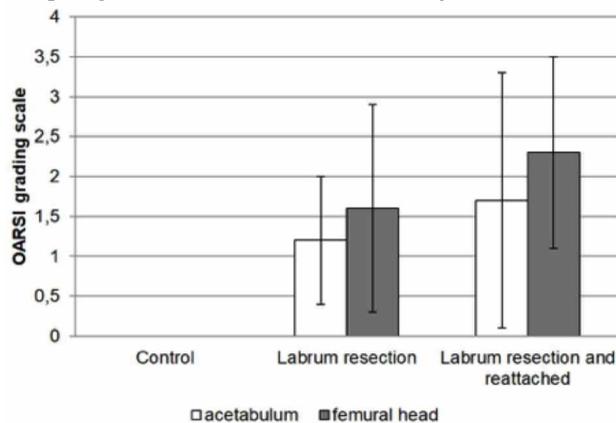


Fig. 2. The results of the assessment of the state of the articular cartilage according to the OARSI grading scale. Data presented as Mean  $\pm$  SD

The results of linear cartilage morphometry ( $n=7$ ) showed a significant decrease in the thickness of the articular surfaces (Fig. 3). At the same time, the results of multiple measurements (15 measurements for 4 sectors in the sample) were more expressed in the cartilage of the femoral head. After labral refixation the thickness of the articular cartilage of the femoral head was greater compared to the group after labral excision ( $p=0.01$  vs  $p=0.97$ ). We noted the impact on the results of measurements of individual characteristics of cartilage thickness in animals, but there were no doubts about its reduced thickness caused labral excision. A good indicator for additional assessment of cartilage condition is the ratio of chondromucoid to lacunae with chondrocytes. We found that the specific density of lacunae (including those empty as a result of chondrocyte apoptosis) increases through erosion of the cartilage surface, or swelling of lacunae. Chondrocyte clustering is also reflected in a decrease in the ratio of chondrocytes to chondromucoid. The acetabular cartilage in the refixation group had a significantly better ratio of

chondrocytes to chondromucoid, but not in the femoral head (Fig. 4). We established a strong correlation between the clustering factor (absence 0; small groups 1; larger cells 2) and the grade according to the OARSI grading scale in the general sample of observations (n=7; r=0.77; p=0.01). At the same time, the indicator has not correlate with the thickness of the studied cartilage (n=7; r=-0.06; p=0.84). The better degree of cartilage preservation of the acetabulum after labral refixation is an encouraging result, but it requires further studies with a larger number of observations.

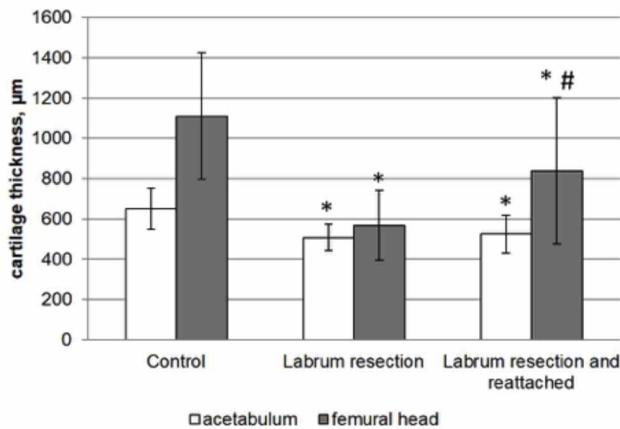


Fig. 3. Changes in the thickness of articular cartilage according to the results of linear morphometry. Data presented as Mean±SD, \* p< 0.05, compared with the control group; \*\* p< 0.05, compared with the experimental group after labral excision.

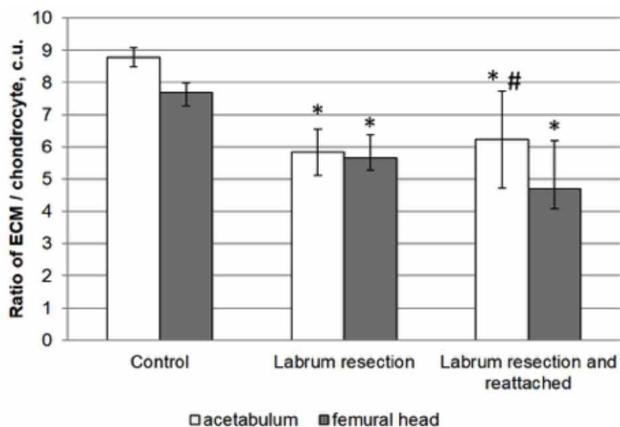


Fig. 4. The results of the assessment of the ratio of the specific density of lacunae with or without chondrocytes in relation to the cartilage matrix. Data presented as Mean±SD, \* p< 0.05, compared with the control group; # p< 0.05, compared with the experimental group after labral excision

## DISCUSSION

A successful model of damage to the capsule of the hip joint and a multifaceted quantitative assessment of the condition of the articular cartilage are the key to objectivity in

the analysis of research results (Ponce *et al.*, 2018). In this work, we have demonstrated the results of a rabbit labral excision model and the consequences after refixation. Few morphological studies have been devoted to this joint problem, but they are important for understanding the patterns of joint disorders, the development of premature osteoarthritis, and the potential for joint tissue recovery (Gonzalez *et al.*, 2021). Cartilage erosion and osteoarthritis were observed after 24 weeks in a labral resection in a porcine model. A significant reduction in the progression of osteoarthritis and biomechanical recovery was noted compared to labral resection after acetabular labral reconstruction using autografts (Shi *et al.*, 2019). The level of recovery of the biomechanics of the hip joint after labral reconstruction remains controversial, ranging from insufficient (Lee *et al.*, 2015) to an almost intact joint (Capurro *et al.*, 2023).

In another work, 12 weeks after partial excision of the labrum and repair (repaired via a minimally invasive arthroscopic technique), fibrovascular repair tissue in labrum is shown (Philippon *et al.*, 2012). Remodeling of the extracellular matrix of the graft (autologous tendons) and collagen expression progresses from 12 to 24 weeks (Shi *et al.*, 2016; Wu *et al.*, 2022).

Morphological evidence was obtained that labral refixation has a potentially beneficial effect on joint condition 4 months after surgery and possible progressive cartilage damage. Some differences in the changes in the articular cartilage of the femoral head and the acetabulum were also noted. In the second, reactions of chondrocytes in the form of clustering were observed much less often, and fibrous changes with the appearance of collagen occurred against on the background of cartilage erosion. Such changes in cartilage morphology occurred more often in the femoral head. Chondrocyte clustering differs sharply from the "normal cellularity" and is considered as a manifestation of regeneration and proliferation of chondrocytes (Karim *et al.*, 2018; Muldrew *et al.*, 2001). But these changes were observed with significant damage to the surface of the cartilage and histochemical changes of the chondromucoid after labral excision and after labral refixation. In this condition, focal cellular regeneration has no prospects in cartilage repair. This can explain the occurrence of clustering in case of significant damage, while early changes in morphology do not have changes in cartilage cellularity (Lotz *et al.*, 2010). And although a number of variables may have influenced these results (such as the difficulty of transosseous fixation, the number of sutures for sufficient fixation, potential inflammation), our data indicate a difference in the results obtained after labral excision and after labral refixation. We also observed the reorganization of collagen fibers in the restored labrum (in this work, we do not present the results of capsule morphology).

## CONCLUSION

In conclusion, we demonstrated in this work the development of articular cartilage damage due to labral excision of the hip joint, as well as labral refixation can potentially affect the delay of degenerative changes in the cartilage. An objective indicator in the study of cartilage condition is the ratio of chondrocytes in the lacunae to the extracellular matrix, this indicator correlates with the values that can be obtained according to the OARSI grading scale, and does not necessarily have a relationship with the thickness of the cartilage. The latter should be measured with caution, since individual differences and the presence of local erosions complicate the interpretation of results and the formation of objective conclusions.

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**RESUMEN:** La refijación del labrum acetabular dañado es un método de tratamiento quirúrgico de la articulación coxal, que puede promover la reparación de la función articular después de una lesión y prevenir la osteoartritis prematura. Intentamos determinar el estado de la articulación coxal en conejos de 4 meses después de la escisión del labrum acetabular y observar el estado de la articulación después de la refijación del labrum. El cartílago articular de la cabeza femoral y el acetábulo se examinó por métodos histológicos, se midió a través de multipunto el grosor del cartílago y se realizó la relación entre la matriz del cartílago y las lagunas de condrocitos, y se llevó a cabo la condición del cartílago según la escala de clasificación OARSI. Sobre este modelo se realizó un análisis de correlación entre los resultados de la escala de calificación OARSI y los datos de la morfometría lineal. Todos estos parámetros permitieron evaluar mejor los cambios en el cartílago articular. La relación entre la matriz y las lagunas de condrocitos resultó ser un método que permite establecer temprano el daño del cartílago cuando no se presentó erosión, fibrosis o deformación. Encontramos diferencias significativas entre la condición del cartílago después de la extirpación del labrum acetabular y después de la refijación del labrum, lo que da la esperanza de confirmar que esta técnica quirúrgica puede retrasar o prevenir cambios progresivos en el cartílago de la articulación coxal dañada.

**PALABRAS CLAVE:** Labrum acetabular; Articulación coxal; Cabeza femoral; Acetábulo; Cartílago; Modelo de escisión del labrum; Refijación.

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