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Dynamic shear analysis: a novel method to determine mechanical integrity of normal and torn human acetabular labra

IMPLICATIONS FOR PREDICTION OF OUTCOME OF REPAIR

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Objectives
The aim of this study was to investigate the structural integrity of torn and non-torn human acetabular labral tissue.

Methods
A total of 47 human labral specimens were obtained from a biobank. These included 22 torn specimens and 25 control specimens from patients undergoing total hip arthroplasty with macroscopically normal labra. The specimens underwent dynamic shear analysis using a rheometer to measure storage modulus, as an indicator of structural integrity.

Results
There was a significant difference in the storage modulus between torn (mean modulus = 2144.08 Pa) and non-torn (3178.1 Pa) labra (p = 0.0001).

Conclusion
The acetabular labrum of young patients with a tear has significantly reduced structural integrity compared with a non-torn labrum in older patients with end-stage osteoarthritis. This study contributes to the understanding of the biomechanics of labral tears, and the observation of reduced structural integrity in torn labra may explain why some repairs fail. Our data demonstrate that labral tears probably have a relatively narrow phenotype, presenting a basis for further investigations that will provide quantifiable data to support their classification and a means to develop a standardized surgical technique for their repair. This study also demonstrates the value of novel biomechanical testing methods in investigating pathological tissues of orthopaedic interest.

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Keywords: Biorheology, Acetabular labral tears, Osteoarthritis, Femoroacetabular impingement, Biomechanics

Article focus
- What is the structural integrity of torn and non-torn human acetabular labral tissue?

Key messages
- We present a new method for testing the biomechanics of hip tissue.
- The fundamental structural integrity of a labrum with a tear is reduced.
- Better understanding of biomechanics of tears can guide repair strategies.

Strengths and limitations
- Strength: demonstration of the effective use of dynamic shear analysis to test biomechanical properties of human tissues.
- Limitation: lack of ethically obtainable perfect control.
- Limitation: limited study size.

Introduction
Tears in the acetabular labrum are being diagnosed with increasing frequency using MRI and magnetic resonance arthrography (MRA)[1,2] and improved arthroscopic techniques (Fig. 1).[3,4] The prevalence of labral tears is now estimated to be between 22% and 55% in patients with pain in the hip or groin,[5-8] and they are consistently found in more than 85% of patients with femoroacetabular impingement (FAI).[9-11] It is generally accepted that there are at least five

Fig. 1a Fig. 1b Fig. 1c Fig. 1d Fig. 1e

Intraoperative and radiological imaging of the acetabular labrum. a) An intact and b) a torn human labrum viewed through an arthroscope; c) a torn labrum undergoing arthroscopic repair; coronal T2 MRI images of a human hip with d) an intact and e) a torn labrum (tear highlighted with an arrow).

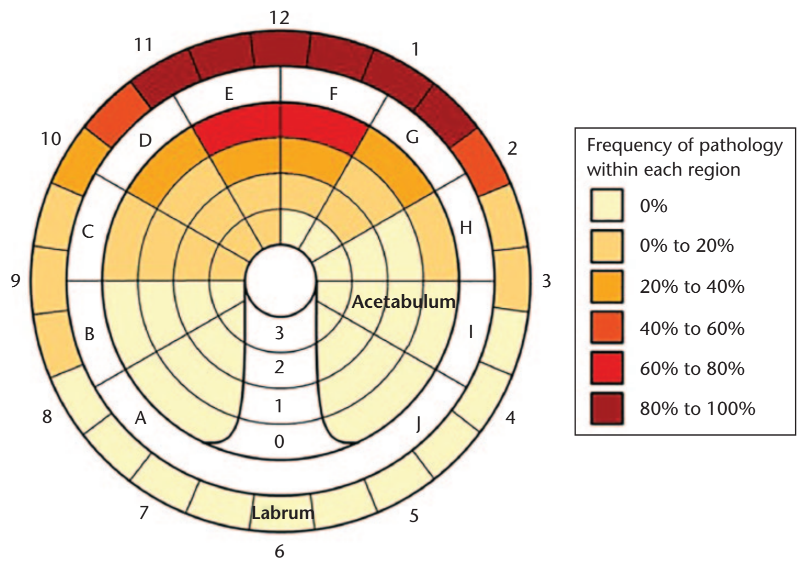


Fig. 2

The arthroscopic or radiological clock-face orientation of the acetabulum and frequency of tears in each region. Reproduced with permission from Mr Antony Palmer, Oxford, United Kingdom (previously unpublished).

aetiologies of labral tears: trauma, FAI, capsular laxity/hip hypermobility, dysplasia, and degeneration.[12] Regardless of the aetiology, the literature suggests that once a tear is present, an alteration of the biomechanical environment of the hip occurs. Typically, this results in a loss of stability, and FAI[13] and labral tears are now believed to influence the development of degenerative changes in the hip.[7,14-16]

The labrum is triangular in cross section, with its base attached to the acetabular rim. Many stages, states, and classifications of labral tears have been described.[17,18] Tears most commonly occur in the anterosuperior region (Fig. 2)[19-21] and usually involve the articular margin (so-called 'watershed' lesions), as opposed to the capsular margin.[22] However, both posterior and posterosuperior tears have been recognized.[23,24]

The aim of surgery is to restore the normal mechanics of the hip and to repair or debride damaged labral tissue.[4] It is logical to attempt repair as the labrum has a load-bearing function.[25,26] However, there is also a biomechanical argument for debridement.[27] Which technique provides a better outcome, however, remains unclear,[28,29] although there is growing support for repair over debridement.[30-33]

It is possible that different patients will benefit from different forms of surgical treatment for labral tears, including debridement, repair, and reconstruction. Importantly, little is known about the biomechanical impact of damage to labral tissue and its integrity. A better understanding of the effect of tears on integrity has implications on all surgical options. Understanding the exact relationship between the structural integrity and load tolerance of the labrum is, therefore, essential if we are to provide the most appropriate surgical treatment based on the type of damage, and improve the methods of fixation or replacement that are currently available. This remains a key issue given the advances in therapeutic applications of arthroscopy.[3]

Dynamic shear analysis (DSA). In order to obtain a more comprehensive understanding of the mechanics of the labrum, attention needs to be paid to the fact that it is

Table I. Classification according to morphology[18] and localization,[17] with associated pathologies of tear samples

Gender	Morphological classification	Location	Comments
Male	Radial fibrillated	Lateral	FAI and grade IV changes seen
Female	Longitudinal peripheral	12 o'clock to 3 o'clock	FAI, cam/osteophyte and degeneration
Female	Longitudinal peripheral	Anterosuperior labral detachment	Full thickness cartilage defect, no femoral head lesion
Male	Longitudinal peripheral	Circumferential	Severe degeneration
Male	Longitudinal peripheral	Superior	FAI and grade II changes seen
Female	Longitudinal peripheral	10 o'clock to 12 o'clock	Capsular scarring due to previous trauma
Male	Longitudinal peripheral	Anterosuperior labral detachment	Irreparable
Female	Longitudinal peripheral	12 o'clock to 2 o'clock	
Male	Longitudinal peripheral	Circumferential	Massive macerated tear
Female	Longitudinal peripheral	7 o'clock to 5 o'clock	OA, K/L grade 3 or 4 in over 50% of joint
Male	Longitudinal peripheral	Anterosuperior labral detachment	Massive macerated tear with areas of OA, K/L grade 3 or 4
Male	Longitudinal peripheral	Circumferential	Massive macerated tear
Female	Radial flap	11 o'clock to 1 o'clock	Small carpet lesion
Female	Radial flap	Anterior	
Male	Radial flap	Anterosuperior labral detachment	FAI, cam
Female	Radial flap	Chondrolabral junction	FAI, cam
Female	Radial flap	Superior	Labrum appeared thin and atrophic

FAI, femeroacetabular impingement; OA, osteoarthritis; K/L, Kellgren–Lawrence

subjected to multidirectional forces involving shear and compression.

Viscoelastic behaviour can be quantified in oscillation, and almost all biomaterials, including bone, sit within a viscoelastic spectrum of soft condensed matter and can be tested using DSA. This novel technique is based on rheometry, the study of flow and material deformation of samples in response to shearing mechanical stress, and offers insight into their mechanical properties. It was developed and used successfully in the analysis of the mechanics of rotator cuff tendons.[34] Analyzing samples of torn and non-torn acetabular labra in this way allows calculation of the bulk storage modulus (G prime),[35] which can act as an indicator of mechanical integrity.

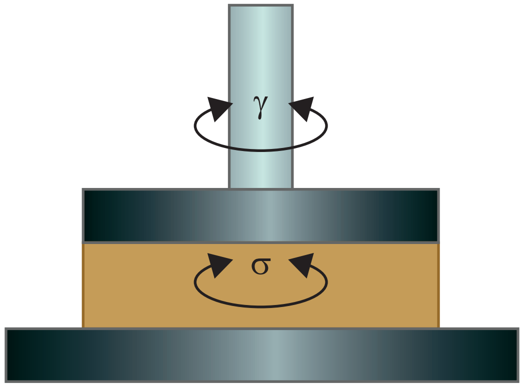
Hence, the aim of this study was to describe the mechanical integrity and behaviour of torn acetabular labral tissue, using DSA, with the intention of providing a basis for improving future technology and patient selection for surgery.

Materials and Methods

Patients and specimen collection. After approval by a local ethics committee, 47 full-thickness, flash-frozen labral specimens, collected intraoperatively by the senior author (SG-J) between 2010 and 2015 and stored at -80°C, were obtained from the Oxford Musculoskeletal Biobank. The specimens remained stored at -80°C when not in use and were thawed thoroughly prior to dissection and testing. A total of 22 specimens represented biopsies from 17 patients, nine women and eight men with a mean age of 34.35 years (21 to 49), who were diagnosed pre- and intraoperatively with labral tears, and who underwent arthroscopic repair or debridement. MRA reports and operation notes written by the senior author described the position and morphology of the tears and any associated pathology (Table I). Specimens were taken from the most damaged part of the labrum. A total of 25 control specimens were taken from 11 patients, seven women and four men with a mean age of 68.7 years (48 to 78), who had undergone total hip arthroplasty (THA) for osteoarthritis, without associated FAI, who had macroscopically intact labra with no evidence of damage. Specimens in this group were fully resected labra that were subsequently divided for storage in the biobank.

A pre-study power calculation[36] indicated that eight torn and non-torn labral specimens were needed to detect an effect size of one standard deviation with a certainty of 80% (power, 0.8) and a 5% level of error ($\alpha < 0.05$). It is known that a substantial area within the labrum is made up of a core region that can be identified by the naked eye, and that represents the most homogeneous layer in which most fibres are co-oriented.[37-39] All biopsies were dissected to give a standard 3 mm × 3 mm length and breadth, and care was taken to ensure that the specimens contained this core region. Furthermore, due to the high prevalence of anterosuperior labral tears in the study group, biopsies were taken from this region of the control specimens in order to provide consistency and the best possible comparison.

Rheological assessment. A Bohlin Gemini 200 HR Nano rheometer (Malvern Instruments Ltd, Malvern, United Kingdom) was used to collect the DSA data. A minimum of two readings were obtained from each biopsy, from which a mean was calculated for each specimen. Assessment of the test-retest variability found that the correlation coefficient (r) was 0.90. The specimens were loaded between two parallel plates pre-warmed to 37°C and maintained at 100% humidity to keep the water content constant. Prior to the study, the criterion for valid modulus readings was set as a thrust of approximately 20 g, in line with previous studies.[34] Specimens were then subjected to a standard oscillatory test (0.623 rad/s to 39.6 rad/s, 0.001 strain) (Fig. 3). The upper plate moves



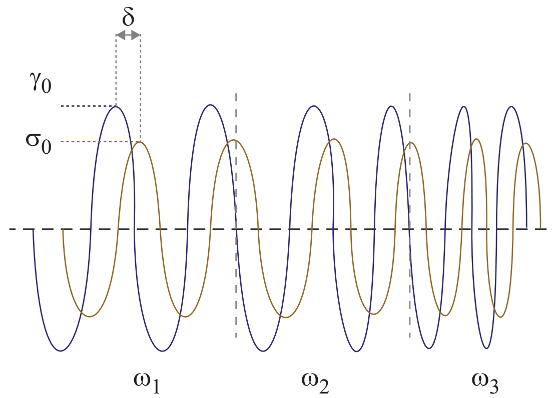


Fig. 3a

Fig. 3b

Dynamic shear analysis. a) Operation: specimens were compressed to a known force between two parallel plates. The upper plate moves at a fixed strain and records the stress response. b) Deformation: specimens were subjected to a sinusoidal strain wave over a range of frequencies (ω). The resulting strain peak (γ_0), stress peak (σ_0), and difference in phase (δ) are measured for each frequency.

sinusoidally at a fixed strain and records the stress response from the labral samples over a range of frequencies (ω) by measuring the torque on the drive motor. The digitally mapped bearing of this motor is used to record the absolute displacement, which is converted to strain. The resulting strain peak (γ_0), stress peak (σ_0), and difference in phase (δ) were measured for each frequency. These values were used to calculate the elastic (storage) modulus (G'), using the equation $G'\omega_1 = (\sigma_0/\gamma_0)\cos\delta$ (Fig. 3). The mean storage modulus across all frequencies was used to indicate the mechanical integrity of the specimens.

Statistical analysis. An unpaired Student's *t*-test was used to assess differences between normal and torn labra. F-test confirmed that the sample sets were of equal variance. The effects of gender were studied using an unpaired Student's *t*-test. Any association between the modulus and age was analyzed using a Pearson's rank correlation. All tests were two-tailed and a p-value < 0.05 and $\alpha < 0.05$ were considered significant. All statistical analysis was performed in Microsoft Office Excel 2013 for Windows (Microsoft, Redmond, Washington).

Results

Effect of tears. Six specimens were excluded from the final analysis due to initial machine/user error resulting in their over-compression. These included three from patients with labral tears and three control samples. Thus, the final analysis only included 19 labral tears and 22 control specimens. There was a significant difference between torn (mean modulus = 2144.08 Pa, SD 640.58) and normal labra (mean modulus = 3178.1 Pa, SD 853.07), showing that non-torn labra have a higher modulus and hence greater mechanical integrity than torn labra (unpaired Student's *t*-test, p=0.0001).

Influence of preoperative factors. Due to the limited availability of specimens stored appropriately within the tissue bank, it was not possible to match the mean age and gender of those with torn labra and controls. There was only a mild positive correlation between age and modulus (Pearson's rank correlation = 0.48, Fig. 4a); however, the study was not powered to detect an effect of age on modulus. There was no obvious correlation between gender and modulus (Student's *t*-test p=0.82, Fig. 4b).

Within the specimens of labral tear, there was no significant difference for the mean storage modulus of tears between men and women (2357 Pa *vs* 1851 Pa, respectively, p=0.09, Table II). The breakdown of specimens into morphological and location-based classifications (Table II) resulted in groups too small for meaningful statistical comparison. However, we observed that the structural integrity of anterosuperior labral and superior labral tears is lower than in circumferential tears (1943.75 Pa, 1519.04 Pa and 2488.73 Pa, respectively).

Discussion

This study presents novel insights into the mechanical integrity of torn and non-torn human acetabular labra from fresh-frozen, non-degenerative specimens under multi-axial and compressive conditions. We found that the healthy labral material has a significantly higher shear modulus than tissue from a torn labrum, indicating that a torn labrum has inferior mechanical performance characteristics (p=0.0001). The finding that torn labra from young patients have a lower mechanical integrity than degenerative but intact labra from patients undergoing THA for end-stage osteoarthritis reflects a significant mechanical, and possibly biological, process. The labrum is under shear stress normally and following repair. Specifically, the free sections of labrum between adjacent sutures used for the repair are likely to be the most vulnerable to shearing. It is, therefore, important that the labrum can resist any force placed on it following repair. These findings may offer some insight into both the aetiology of tears and the postoperative failure in some patients by identifying reduced structural integrity in these sections of labrum.

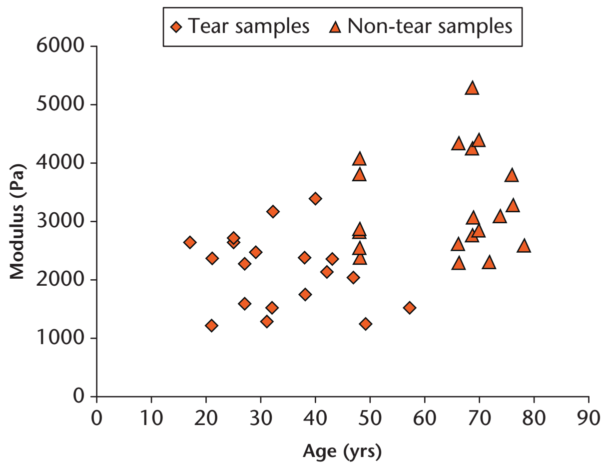


Fig. 4a

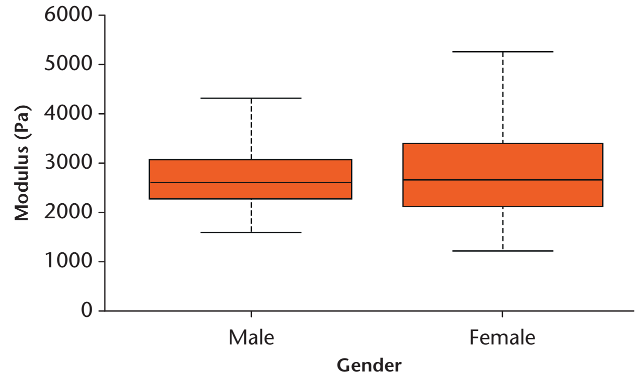


Fig. 4b

The influence of preoperative factors on modulus, with a) a scatter plot showing the influence of age, and b) a box plot showing the influence of gender. Labral modulus shows a mild positive correlation with patient age (r=0.48), and is unaffected by gender (p=0.82). In b), the whiskers represent the maximum and minimum, and the boxes represent the median and the interquartile range.

Table II. Comparison of the mean storage modulus (Pa) of tear specimens when classified by gender, morphology, and location

Classification (n)	Mean storage modulus (Pa)
Gender	
Men (11)	2357 (SD 453)
Women (9)*	1851 (SD 769)
Morphological classification	
Radial fibrillated (1)	2367
Longitudinal peripheral (14)	2084 (SD 618)
Radial flap (5)*	2297 (SD 863)
Location	
Anterior (1)*	4484
Superior (3)	1822 (SD 461)
Anterosuperior (8)	1944 (SD 577)
Circumferential (5)	2489 (SD 594)
Chondrolabral junction (1)	3392
Posterior (1)	1519
Lateral (1)	2367

*Anterior specimen excluded from analysis due to incorrect testing frequencies used with rheometer

While trauma might affect any part of the labrum, hypolaxity of the anterior capsule and the iliofemoral ligament increases its tensile loading and possibly shear loading.[40] Furthermore, bony impingement and dysplasia can both cause increased compressive loading. Any of these pathologies, therefore, combined with the reduced biomechanical properties, could lead to failure of the labrum. We observed that anterosuperior tears had particularly reduced mechanical integrity compared with controls. The significance of these findings is hard to interpret with small data sets; however, it does correlate with some previous findings. Smith et al[39] found that the anterosuperior quadrant of the labrum has the weakest biomechanical properties, and agreed with others suggesting that this area of the hip is subsequently the most susceptible to damage.[26,41,42]

In support of this, Beck et al[41] showed that, where damage to the acetabular articular cartilage is located anterosuperiorly, it has a mean depth of 11 mm, while where damage is restricted to a narrow circumferential band, it has a mean depth of 4 mm. This may account for the higher structural integrity we observed in circumferential tears. Furthermore, Walker et al,[42] in a study of foetal development of the human hip, noted mechanically weak areolar tissue in 13.7% of foetal hips, which was always found in the anterosuperior region of the labrum. This may weaken the labrum, accounting for the reduced structural integrity in this area.

Krych et al[32] performed a level 1 prospective randomized study comparing arthroscopic labral repair and selective labral debridement in women undergoing arthroscopy for FAI. They followed up 36 patients for a mean of 32 months and showed that the mean Hip Outcome Score (HOS) for activities of daily living was significantly better in the repair group (91.2 (73 to 100)) than in the debridement group (80.9 (42.6 to 100)) ($p < 0.05$). The mean postoperative sports HOS was significantly greater in the repair group (88.7 (28.6 to 100)) than in the debridement group (76.3 (28.6 to 100)) ($p < 0.05$). The patient subjective outcome was also significantly better in the labral repair group (p=0.046). Likewise, Larson and Giveans[43] reviewed two groups undergoing arthroscopy for treatment of tears associated with pincer or combined pincer–cam lesions. A total of 94 hips were reviewed and, at a mean 3.5 years' follow-up, good to excellent results were noted in 68% of the debridement group and 92% of the re-fixation group (p=0.004). The number of unsuccessful arthroscopic procedures was also found to be greater in the debridement group. However, Menge et al[4] reported the outcomes and survival of the hip ten years after arthroscopy for FAI. They reported on 79 patients who underwent labral repair and 75 who underwent debridement. The primary outcome measure was the HOS activities of daily living subscale. They found that there was no significant difference in the outcome scores and the hazard ratio (HR) for THA between the groups (HR 1.10, 95% confidence interval 0.59 to 2.05, p=0.762).

As with all biopsy specimens, the use of an analytical technique for the limited number of small labral samples that can be ethically collected is critical to understanding acetabular labral pathology. Using a rheometer, instead of the traditional tensile tester, minimized destructive and potentially misleading clamping effects by subjecting the specimens to oscillatory deformation under gentle compression. In tensile testing, there is a wide variation in the method of gripping the specimens, owing to the difficulty of achieving a uniform and effective hold on the collection of the partially independent subfibres. Any failure to obtain consistent grip at the interface of the grip with the tissue will result in a decrease in measured stiffness and strength.[44] DSA has been shown to be an effective answer to this problem in small specimens of rotator cuff tendons.[34]

The study has limitations. Although the sample size is small, the study is adequately powered. Variability between specimens is relatively large, as is to be expected in biological tissues. The results are robust enough to produce significant differences. Although the optimal control specimen is a normal labrum, such specimens are difficult to obtain, especially in comparatively young patients, to match the demographics of those sustaining a labral tear. It would be unethical to obtain labral biopsies from healthy patients. The two main sources for young non-torn labra would be patients who have suffered trauma or who have avascular necrosis, both of which may prove equally compromised controls to those that we used due to the aetiology of the injury. Instead of a normal labrum, therefore, control samples were obtained from patients who underwent THA. These labra cannot, however, be truly considered to be normal. Thus the study is potentially limited by the pathological characteristics of the control specimens, and by the possible effects of age, although the mild correlation which we observed may be due to inadequate power to measure this particular association. However, as most of the studies dealing with the biomechanical properties of articular soft tissues have shown a decrease in strength with age,[45] the fact that the damaged labrum in the young person seems to be weaker than the intact labrum in older patients with end-stage osteoarthritis is a strong indicator of the significance of the mechanical effects of tearing on the tissue. Although the data were valid, it would be desirable to investigate the normal labrum.

Characterizing and comparing the shear modulus of torn and non-torn human labra is relevant, as the hip is a complex joint subjected to many forces, including shear. We only investigated shear responses and did not seek to quantify uniaxial tensile properties. However, previous studies of heterogeneous materials have shown that tensile properties are related to mechanical parameters such as shear and bulk moduli.[46] Previous authors have also shown the benefits of testing tissues at ranges that are sub-failure and more consistent with *in vivo* loading, rather than focusing on the linear region of loading and at failure.[34,47]

In order to obtain uniformity of sample size and shape, the specimens in this study were surgically dissected, which might lead to changes in the material properties of the tissues. However, both control and tear specimens were prepared in this way and relative differences should not be affected. The use of this method allowed us to include all layers of the labrum and not just core samples as in previous studies. Environmental factors were controlled in this study, as both hydration status[48,49] and temperature[50] have been shown to alter the biomechanical properties of intra-articular tissue.

Further studies are required to explore, for example, the effects of other hip pathologies and their locations on the integrity of the labrum, to explore the importance of patient demographics, and to elucidate the clinical implications of differences in the material properties of the labrum. If differences in labral shear moduli have a relationship with aetiology or failure rates, it may be possible to use DSA to assess the strength of different forms of repair and to guide the development of a standardized technique. Such data may also allow the early identification of mechanically weakened tissue which might not be able to support a simple sutured repair, and hence require debridement. As the variability within the torn samples is similar to that seen in the intact labrum, it is likely that labral tears represent a relatively narrow phenotype, which is promising when seeking a standardized surgical technique for repair. If further studies were able to provide a threshold of integrity of the articular tissue, DSA, with labral size and volume, might help to delineate diseased tissue which appears macroscopically normal, in order to differentiate between which partial tears should be debrided and those that need to be repaired, and which method of repair should be used.

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Author Contributions

- A. K. Woods: Designing the original experiments, Collecting and preparing samples, Analyzing the data, Preparing the manuscript.
- J. Broomfield: Collecting samples intraoperatively, Preparing samples for testing, Writing and revising the manuscript.
- P. Monk: Collecting samples intraoperatively, Preparing samples for testing, Writing and revising the manuscript.
- F. Vollrath: Providing the equipment for sample testing, Contributing to experimental design and analysis of results, Writing and revising the manuscript.
- S. Glyn-Jones: Providing samples for testing, Collecting samples intraoperatively, Contributing to analysis of the results and expert analysis of their context in the field, Writing and revising the manuscript.

Conflict of Interest Statement

- None declared