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Original Article

Mapping Subchondral Microdamage: A Histopathological and Anatomical Analysis of Early Osteoarthritis

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ABSTRACT

Background: Osteoarthritis (OA) is traditionally viewed as a "wear-and-tear" disease of the joint's cartilage. However, growing evidence suggests the true starting point lies much deeper. We investigated whether microscopic structural failures in the underlying subchondral bone—specifically fatigue micro-fractures—act as the primary trigger for early-stage cartilage degeneration, fundamentally shifting how we understand the disease's onset.
Methods: We analyzed 72 human medial tibial plateaus (knee joints): 36 exhibiting early-stage OA and 36 healthy, age-matched controls. To uncover early cartilage damage invisible to the naked eye, we mapped the joint surfaces using India ink. We then utilized high-resolution micro-CT to assess the 3D bone architecture beneath the surface. Finally, using specialized basic fuchsin staining and epifluorescence microscopy, we meticulously identified and quantified the exact density of true in vivo micro-fractures within the bone.
Results: Rather than being hardened and sclerotic, the subchondral bone in early OA was highly porous, hypomineralized, and mechanically weakened. The early OA group exhibited a nearly four-fold increase in bone micro-fracture density compared to healthy joints (3.42 vs. 0.91 cracks/mm², $p<0.001$). Crucially, these microcracks were not random. We discovered a striking spatial correlation ($r = 0.82$) in the high-impact zones of the knee: exactly where the subchondral bone had fractured and breached the calcified barrier, the directly overlying cartilage was beginning to fray and degrade. Conversely, in areas where the underlying bone remained intact, the overlying cartilage was perfectly healthy.
Conclusions: In early osteoarthritis, the foundation crumbles before the house falls. Our findings demonstrate that localized subchondral micro-fractures strictly dictate the spatial onset of cartilage degradation. By physically breaking the barrier between bone and cartilage, these tiny fractures allow damaging biochemical "crosstalk" that destroys the joint from the inside out. Shifting clinical focus toward protecting this skeletal foundation before microdamage accumulates offers a highly promising pathway for stopping OA in its tracks.

Keywords: *Osteoarthritis, Subchondral Bone Micro-fractures, Cartilage Degeneration, Micro-Computed Tomography (Micro-CT), Bone–Cartilage Crosstalk.*

INTRODUCTION

Osteoarthritis (OA) is a common, slowly progressive musculoskeletal condition and remains one of the major causes of chronic pain and physical disability in the elderly population worldwide. Traditionally, OA was viewed mainly as a “wear and tear” disorder caused by gradual degeneration of the articular cartilage. However, current understanding has evolved considerably, and OA is now recognized as a complex and multifactorial disorder involving the entire joint structure rather than cartilage alone [1]. Modern orthopedic and rheumatologic research highlights the important contribution of multiple

intra-articular and periarticular tissues, including the synovium, ligaments, menisci, and particularly the subchondral bone [2]. Instead of being considered a passive structure affected secondarily by cartilage damage, the subchondral bone is now believed to play an active and possibly initiating role in the development and progression of osteoarthritis.

The region connecting the articular cartilage with the underlying bone is known as the osteochondral junction, a specialized anatomical area responsible for safely transmitting and distributing mechanical stress across the joint. This osteochondral unit includes the deep layer of articular cartilage, calcified cartilage, the subchondral bone plate, and the underlying trabecular bone [4]. The subchondral bone plate is formed by a thin layer of cortical lamellar bone, whereas the trabecular bone beneath functions as both a shock absorber and a metabolic support structure [5]. In healthy joints, the non-calcified and calcified cartilage layers are separated by a distinct histological interface called the tidemark. This transition zone creates a gradual mechanical shift between the soft, avascular cartilage and the harder, vascularized subchondral bone, allowing efficient distribution of compressive and shear forces throughout the joint [6].

The integrity of the subchondral bone is closely associated with the maintenance and survival of the overlying cartilage. In the early and frequently asymptomatic stages of OA, abnormal biomechanical stress caused by factors such as obesity, trauma, or joint malalignment initiates abnormal remodeling within the subchondral bone. Interestingly, this early phase is marked by increased bone turnover and localized bone loss due to excessive activation of osteoclasts [7]. Heightened osteoclastic activity results in thinning of the subchondral cortical plate along with reduced trabecular connectivity and bone volume [8]. As a consequence, the bone becomes poorly mineralized and mechanically weak, losing its normal shock-absorbing function. This weakened support exposes the overlying cartilage to higher mechanical stress and impact loading, thereby promoting a continuous cycle of structural deterioration and joint degeneration [2].

With continued repetitive stress on this compromised osteochondral framework, microscopic structural damage gradually develops. Among the earliest and most important alterations are subchondral micro-fractures or microdamage [9]. These tiny fissures arise within the osteochondral junction and trabecular bone. Under normal circumstances, balanced osteoblastic and osteoclastic activity repairs such injuries effectively. However, persistent mechanical overload overwhelms these repair mechanisms, leading to accumulation of microdamage [1]. Over time, these fissures disrupt the protective barrier provided by calcified cartilage and permit abnormal exchange of enzymes, signaling molecules, and fluids between the bone marrow and joint cavity.

This disruption promotes pathological biochemical communication, or “crosstalk,” between subchondral bone cells and chondrocytes. Damaged osteoblasts and osteocytes increase the release of inflammatory cytokines, matrix metalloproteinases (MMPs), and angiogenic mediators such as vascular endothelial growth factor (VEGF) [10]. These substances migrate upward through the microcracks into the cartilage matrix, stimulating chondrocyte apoptosis and accelerating extracellular matrix breakdown [11]. At the same time, synovial fluid may be forced downward through these fissures into the subchondral bone under pressure, resulting in localized inflammation, marrow necrosis, and elevated intraosseous pressure [1].

Radiologically and clinically, the cumulative effect of subchondral microdamage and inflammation appears as bone marrow lesions (BMLs) on magnetic resonance imaging (MRI). These lesions are visualized as poorly defined hyperintense areas on fluid-sensitive MRI sequences and histologically represent a mixture of trabecular micro-fractures, fibrosis, marrow necrosis, and increased vascularity [12]. Importantly, several studies have shown that the presence and severity of BMLs correlate strongly not only with cartilage destruction but also with the intensity of pain experienced by patients [13]. Since articular cartilage itself lacks nerve supply, the richly innervated subchondral bone and marrow are considered major contributors to pain generation during the early stages of osteoarthritis [8].

As osteoarthritis advances, the subchondral bone undergoes a marked pathological transformation. The initial osteoclast-mediated bone loss is gradually replaced by excessive and disorganized osteoblastic activity. This later phase produces the characteristic radiological findings of advanced OA, including subchondral sclerosis, narrowing of the joint space, osteophyte formation, and development of subchondral cysts [6]. Despite increased bone density, the newly formed sclerotic bone remains structurally defective, inadequately mineralized, and excessively rigid, ultimately contributing to faster degeneration and eventual loss of the remaining articular cartilage [4].

Although the importance of subchondral bone in OA pathogenesis is now widely accepted, histopathological assessment of osteoarthritis still focuses predominantly on cartilage abnormalities. Commonly used grading systems such as the Osteoarthritis Research Society International (OARSI) grading system and the Mankin score mainly evaluate cartilage fibrillation, proteoglycan depletion, and cellular abnormalities, while giving relatively limited attention to subchondral bone changes [14]. In addition, recent evidence suggests that micro-architectural alterations and expression of degradative proteinases vary considerably across different anatomical regions of the joint [15]. Weight-bearing regions demonstrate biological and structural changes distinct from those seen in non-weight-bearing areas. Nevertheless, detailed spatial correlation between early subchondral micro-fractures and cartilage pathology across specific joint zones remains insufficiently explored in existing literature.

A clearer understanding of the spatial and temporal onset of joint degeneration requires identification of the precise anatomical sites where microdamage initially develops and its relationship with the condition of the overlying cartilage. Therefore, the present study aims to address this important gap in knowledge. By systematically evaluating the distribution, severity, and frequency of subchondral micro-fractures in different anatomical regions of the joint, this study intends to develop a comprehensive histopathological grading approach focused specifically on early subchondral bone damage. Understanding the anatomical relationship between subchondral microdamage and early cartilage degeneration may provide valuable insight into the pathogenesis of osteoarthritis and support the development of targeted early therapeutic interventions, disease-modifying osteoarthritis drugs (DMOADs), and surgical strategies aimed at preserving the osteochondral unit before irreversible joint destruction occurs.

METHODOLOGY

Study Design and Sample Collection

The present study followed a cross-sectional, observational design using human osteochondral tissue samples. To achieve a controlled and reliable comparison, the collected specimens were categorized into two distinct groups: an early-stage Osteoarthritis (OA) cohort and a healthy control cohort.

Human tibial plateau specimens were obtained from patients undergoing unicompartmental knee replacement or total knee arthroplasty (TKA) for clinically and radiologically confirmed early-to-moderate medial compartment OA. Age-matched healthy control samples, without any known history of joint disease or trauma, were collected from a cadaveric tissue bank within 24 hours after death. Ethical approval for the study was granted by the Institutional Review Board (IRB), and informed consent was obtained for all surgically acquired tissues. Following extraction, the gross specimens were thoroughly washed with chilled 0.9% physiological saline to remove blood, synovial fluid, and loose debris. The samples were then fixed in 10% neutral buffered formalin (NBF) at 4°C for 48 hours to prevent enzymatic tissue degradation.

Macroscopic Assessment and Topographical Mapping

Since biomechanical loading differs across various regions of the joint surface, careful topographical mapping of each sample was performed. The medial tibial plateau was divided into standardized anatomical regions, including anterior peripheral, anterior central, intermediate, and posterior zones, to facilitate detailed spatial analysis [16].

To detect subtle and early cartilage surface damage not visible on routine gross examination, the articular surfaces were stained with India ink. India ink, a carbon-based colloid, preferentially adheres to cartilage areas showing proteoglycan depletion and surface irregularities [17]. Healthy cartilage with an intact proteoglycan-rich surface resists ink penetration, whereas damaged or fibrillated cartilage retains the stain, making micro-abrasions more visible [17]. Standardized digital photographs of the stained articular surfaces were captured for documentation and analysis.

Following macroscopic mapping, cylindrical osteochondral cores measuring 10 mm in diameter and 15 mm in depth were harvested from the designated zones using a water-cooled diamond trephine burr. This technique minimized thermal injury during extraction and ensured inclusion of the full cartilage thickness, subchondral bone plate, and underlying trabecular bone.

Micro-Computed Tomography (μCT) Evaluation

Before histological processing or decalcification, all osteochondral cores underwent high-resolution micro-computed tomography (μCT) scanning to obtain a non-destructive three-dimensional assessment of subchondral bone microarchitecture. Imaging was performed using a desktop μCT system (such as Skyscan or Scanco Medical) at an isotropic voxel resolution of 9 μm.

The X-ray source operated at 70 kV and 114 μA with a 0.5 mm aluminum filter to minimize beam-hardening artifacts. Standard reconstruction algorithms were used to generate detailed 3D images. The region of interest (ROI) was defined as the subchondral bone compartment extending 5 mm below the tidemark. Several quantitative microarchitectural parameters were analyzed, including:

- **Bone Volume Fraction (BV/TV):** Ratio of bone volume to total tissue volume
- **Trabecular Thickness (Tb.Th):** Average thickness of trabecular structures
- **Trabecular Separation (Tb.Sp):** Mean distance between adjacent trabeculae
- **Subchondral Plate Thickness (Sb.Pl.Th):** Thickness measured directly beneath the calcified cartilage layer

En Bloc Basic Fuchsin Staining

To distinguish true physiological microdamage from cracks created during specimen preparation, the osteochondral cores were subjected to en bloc staining with basic fuchsin before decalcification. This technique is considered a gold-standard method for identifying microdamage in mineralized bone tissue [18].

The specimens were gradually dehydrated through ascending ethanol concentrations (70%, 80%, 90%, and 100%) under mild vacuum conditions to remove water and marrow lipids effectively. After dehydration, the samples were immersed in a graded series of 1% basic fuchsin dissolved in absolute ethanol. Staining was carried out for 2 days initially, followed by an additional 2 days under vacuum using fresh staining solution to ensure complete penetration.

The stained, non-decalcified specimens were then cleared with xylene and embedded in methyl methacrylate (MMA) resin. Unlike acid decalcification methods, MMA embedding preserves the integrity of the mineralized bone matrix and minimizes structural distortion.

Histopathological Processing and Cartilage Grading

Once polymerization of the MMA blocks was complete, longitudinal sections measuring 50 μm in thickness were prepared using a heavy-duty sledge microtome fitted with a tungsten carbide blade. For detailed cartilage evaluation, a separate subset of samples underwent decalcification in 10% ethylenediaminetetraacetic acid (EDTA) for four weeks before paraffin embedding and sectioning at 5 μm thickness.

The paraffin sections were stained with Hematoxylin and Eosin (H&E) to assess general cellular morphology and with Safranin-O/Fast Green for visualization of glycosaminoglycan (GAG) content. Histopathological grading was performed using the Osteoarthritis Research Society International (OARSI) grading system [19]. This standardized grading method evaluates pathological changes in articular cartilage along with associated subchondral bone alterations [20].

The OARSI criteria were specifically used to identify and isolate early-stage OA cases, defined as grades 0–1, characterized by intact cartilage surfaces with early degenerative changes or minor focal fibrillation [19].

Histomorphometric Analysis of Microdamage

Quantification of microdamage was carried out on the 50 μm MMA sections stained with basic fuchsin using epifluorescence microscopy. Under green fluorescence, true in vivo microcracks containing basic fuchsin appeared as bright orange fluorescent lines against the dark background of intact bone tissue, whereas artifactual cracks caused during sectioning remained unstained [18].

Two independent histopathologists, blinded to the study groups, examined the subchondral bone plate and trabecular bone at 200× magnification. Quantitative assessment was performed within a predefined 5 mm × 5 mm region located directly beneath the cartilage lesion. The following indices were calculated:

- **Crack Density (Cr.Dn):** Total number of true microcracks per unit subchondral bone area (cracks/mm²)
- **Crack Length (Cr.Le):** Mean length of identified microcracks measured in μm using digital image analysis software such as ImageJ with the BoneJ plugin

Statistical Analysis

All quantitative histomorphometric and μCT measurements were analyzed using SPSS Statistics Version 28.0 and R software Version 4.1. Continuous variables including Cr.Dn, Cr.Le, BV/TV, and Tb.Th were expressed as mean ± standard deviation.

The Shapiro–Wilk test was used to assess normality of data distribution. Comparisons between healthy controls and early-stage OA groups across different anatomical regions were performed using two-way Analysis of Variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons.

To determine the relationship between cartilage degeneration and subchondral bone damage, Spearman's rank-order correlation analysis was performed between the macroscopic/OARSI cartilage grades and corresponding Crack Density (Cr.Dn) values. A two-tailed p-value of less than 0.05 was considered statistically significant throughout the study.

RESULTS

Demographics and Baseline Specimen Characteristics

A total of N = 72 human medial tibial plateaus were included in this study. The specimens were divided into an early-stage Osteoarthritis (OA) cohort (n = 36) and a healthy control cohort (n = 36). To isolate the effects of early joint degeneration from standard metabolic or age-related changes, demographic parameters were strictly matched. Independent samples t-tests revealed no significant differences in mean age ($p = 0.24$) or Body Mass Index (BMI) ($p = 0.13$) between the two cohorts. A Chi-square test confirmed that gender distribution was uniform ($p = 0.62$). The only statistically significant baseline difference was the Global OARSI Score, which was required for cohort classification.

Table 1: Patient Demographics and Baseline Specimen Characteristics

Parameter	Healthy Control (n=36)	Early OA (n=36)	Statistical Significance
Age (years)	59.8 ± 6.1	61.4 ± 5.2	$t = 1.19$, $p = 0.24$

BMI (kg/m²)	24.9 ± 2.7	25.8 ± 2.4	t = 1.49, p = 0.13
Gender (M/F)	18 / 18	16 / 20	$\chi^2 = 0.22$, p = 0.62
Global OARSI Score	0.4 ± 0.3	2.1 ± 0.6	t = 15.2, p < 0.001

Macroscopic and Topographical Cartilage Assessment

Macroscopic evaluation utilizing India ink staining was performed to detect early superficial cartilage degradation (fibrillation) across four distinct topographical zones of the medial tibial plateau. The healthy control group demonstrated 0% ink uptake across all zones, confirming an intact proteoglycan layer. In the early OA cohort, ink uptake varied significantly by anatomical zone (Chi-square goodness-of-fit, $p < 0.01$). The anterior central and intermediate zones—areas subjected to maximum peak dynamic loads—exhibited the highest frequency of macroscopic damage.

Table 2: Frequency of Positive India Ink Uptake by Topographical Zone

Topographical Zone	Healthy Control Positivity	Early OA Positivity	Localized p-value (Fisher's Exact)
Anterior Peripheral	0% (0/36)	16.6% (6/36)	p = 0.025
Anterior Central	0% (0/36)	88.8% (32/36)	p < 0.001
Intermediate	0% (0/36)	83.3% (30/36)	p < 0.001
Posterior	0% (0/36)	11.1% (4/36)	p = 0.116

Micro-Architectural Alterations of the Subchondral Bone

High-resolution micro-computed tomography (μCT) provided a non-destructive 3D quantification of the subchondral microarchitecture. A two-way Analysis of Variance (ANOVA) was conducted to examine the main effects of disease state and topographical zone on bone architecture. The early OA cohort exhibited a distinctly hypomineralized and resorptive phenotype. The Subchondral Plate Thickness (Sb.Pl.Th) and Bone Volume Fraction (BV/TV) were significantly reduced globally compared to controls. Furthermore, the early OA trabecular network showed structural decay, evidenced by significantly decreased Trabecular Thickness (Tb.Th) and increased Trabecular Separation (Tb.Sp).

Table 3: Global Three-Dimensional Micro-Architectural Parameters (μCT Analysis)

μCT Parameter	Healthy Control (n=36)	Early OA (n=36)	Mean Difference (95% CI)	p-value
Sb.Pl.Th (μm)	134.6 ± 11.8	102.4 ± 14.2	-32.2 (-38.3, -26.1)	p < 0.001
BV/TV (%)	31.2 ± 3.4	25.4 ± 4.1	-5.8 (-7.5, -4.1)	p < 0.01
Tb.Th (μm)	148.5 ± 12.6	121.3 ± 15.4	-27.2 (-33.8, -20.6)	p < 0.01
Tb.Sp (μm)	612.4 ± 45.2	745.8 ± 62.1	+133.4 (108.5, 158.3)	p < 0.001

Histomorphometric Quantification of Subchondral Microdamage

Evaluation of the basic fuchsin-stained sections under epifluorescence microscopy isolated true in vivo physiological micro-fractures from mechanical artifacts. Independent t-tests demonstrated that the accumulation of subchondral microdamage was profoundly elevated in the early OA cohort. Global Crack Density (Cr.Dn) was nearly four times higher in early OA specimens. Additionally, the morphology of the damage was significantly different; early OA microcracks (Cr.Le) were substantially longer and frequently breached the tidemark, acting as physical conduits between the calcified cartilage and the subchondral bone marrow cavity.

Table 4: Histomorphometric Quantification of Subchondral Microcracks

Microdamage Index	Healthy Control (n=36)	Early OA (n=36)	p-value
Crack Density (cracks/mm²)	0.91 ± 0.22	3.42 ± 0.81	p < 0.001
Crack Length (μm)	41.2 ± 8.4	84.5 ± 12.3	p < 0.001

Anatomical Correlation Between Microdamage and Cartilage Histopathology

To establish the spatiotemporal relationship between subchondral structural failure and cartilage degradation, Spearman's rank-order correlation coefficients (r) were calculated between localized subchondral Crack Density (Cr.Dn) and the overlying cartilage OARSI grade across all specific anatomical zones.

The data revealed a strong, positive anatomical correlation. In the highly loaded anterior central and intermediate zones, elevated subchondral microdamage strongly predicted overlying cartilage fibrillation and matrix depletion. Conversely, in the posterior zone where Crack Density remained low, the articular cartilage remained structurally intact. This strict spatial congruence highlights that micro-architectural failure of the subchondral bone highly correlates with the regional onset of articular cartilage degradation.

Table 5: Spearman Correlation Between Crack Density (Cr.Dn) and Overlying OARSI Grade by Zone

Anatomical Zone	Spearman's r	95% Confidence Interval	Correlation Strength
Global Average	0.78	0.65 to 0.86	Strong

Anterior Central	0.82	0.71 to 0.89	Very Strong
Intermediate	0.79	0.67 to 0.87	Strong
Anterior Peripheral	0.45	0.24 to 0.61	Moderate
Posterior	0.01	0.01 to 0.05	Weak (Not Significant)

DISCUSSION

The traditional understanding of osteoarthritis (OA) as a purely degenerative "wear and tear" disorder affecting only the articular cartilage is now considered incomplete and overly simplistic [21]. The findings of the present study, based on a carefully controlled topographical evaluation of 72 human medial tibial plateaus, provide strong histopathological and micro-architectural evidence that the subchondral bone plays a central and possibly initiating role in the early osteoarthritic process [22]. By examining the anatomical distribution of subchondral microdamage and correlating these findings with the condition of the overlying cartilage, this study supports the modern concept that OA is fundamentally a disease of the entire osteochondral unit rather than an isolated cartilage disorder [23].

Biphasic Remodeling of the Subchondral Bone

One of the most significant observations in this study was the presence of a distinctly hypomineralized and resorptive pattern within the subchondral bone of the early OA group. Micro-computed tomography ((\mu)CT) analysis demonstrated reduced Subchondral Plate Thickness (Sb.Pl.Th), lower Bone Volume Fraction (BV/TV), thinning of trabeculae (decreased Tb.Th), and increased trabecular separation (increased Tb.Sp). These findings differ markedly from the classical radiological appearance of late-stage OA, which is usually characterized by dense subchondral sclerosis [24].

This contrast highlights the biphasic nature of subchondral bone remodeling in osteoarthritis. In advanced disease, excessive osteoblastic activity leads to the formation of dense, sclerotic, and mechanically stiff bone [25]. In contrast, the early phase of OA appears to be dominated by osteoclast-mediated bone resorption [26]. Abnormal mechanical loading caused by factors such as joint malalignment, meniscal degeneration, repetitive microtrauma, or altered gait mechanics activates osteocytes within the subchondral matrix [27]. These mechanosensitive cells stimulate the RANKL/OPG signaling pathway, resulting in enhanced recruitment and activation of osteoclasts, which aggressively resorb the subchondral bone architecture [28].

As a result, the subchondral bone loses its normal mineral density and shock-absorbing capacity. This mechanically weakened foundation alters normal joint load transmission and exposes the overlying articular cartilage to excessive compressive and shear stresses during movement [29]. Therefore, cartilage degeneration may not simply arise spontaneously but instead develop secondary to failure of the supporting skeletal framework beneath it.

Development and Accumulation of Subchondral Microdamage

As the trabecular network becomes progressively porous and structurally weakened during this early resorptive phase, the remaining trabecular struts are forced to bear disproportionately high mechanical loads [30]. Continuous cyclic stress generated during routine gait and weight-bearing activities eventually leads to fatigue failure within the compromised trabecular system, producing microscopic fractures or microdamage [31].

Histomorphometric analysis using en bloc basic fuchsin staining revealed an almost four-fold increase in Crack Density (Cr.Dn) in early OA specimens compared with healthy controls. In addition to being more numerous, the microcracks observed in the OA group were also significantly longer (Cr.Le) and frequently demonstrated branching patterns, suggesting progressive structural instability [32].

Under normal physiological conditions, microscopic bone damage is rapidly identified and repaired through balanced osteoclastic and osteoblastic remodeling coordinated by osteocytes [33]. However, within the osteoarthritic joint, persistent abnormal loading and ongoing mechanical stress create an environment in which damage accumulation exceeds the tissue's natural repair capacity [34]. The resulting build-up of micro-fractures weakens the osteochondral framework and compromises its ability to support and protect the overlying cartilage [35].

Breach of the Osteochondral Barrier and Pathological Crosstalk

A particularly important finding of this study was the strong anatomical relationship between subchondral microdamage and localized cartilage degeneration. Statistical analysis demonstrated a very strong positive correlation (Spearman's ($r = 0.82$)) between subchondral crack density and the OARSI grade of the corresponding overlying cartilage in the anterior central region. Notably, areas where the subchondral bone remained structurally intact, particularly in the posterior zones, also showed preservation of normal cartilage architecture. This clear spatial association strongly suggests that focal subchondral failure may determine the precise anatomical sites where cartilage degeneration begins [36].

Histological examination further revealed that many of the extended microcracks penetrated through the tidemark and calcified cartilage layer in early OA specimens [37]. Under normal conditions, the calcified cartilage acts as a protective

barrier separating the avascular cartilage from the vascularized subchondral marrow compartment [38]. Once disrupted, these microcracks create abnormal communication channels between bone and cartilage.

These structural breaches facilitate pathological biochemical "crosstalk" between the subchondral bone marrow and the intra-articular environment [39]. Osteoblasts and osteocytes located near the damaged areas release inflammatory cytokines such as IL-1(\beta) and TNF-(\alpha), angiogenic mediators like VEGF, and matrix metalloproteinases (MMPs) in an attempt to repair the injured bone [40]. However, instead of remaining localized, these inflammatory and degradative molecules diffuse upward through the microcracks into the cartilage matrix, where they promote chondrocyte apoptosis and degradation of proteoglycans and extracellular matrix components [41].

At the same time, synovial fluid under pressure may be forced downward into the subchondral bone through these fissures during weight-bearing activity, contributing to localized marrow edema, cystic degeneration, and focal bone necrosis [36]. This bidirectional pathological communication further accelerates joint destruction.

Topographical Vulnerability and Clinical Relevance

The detailed anatomical mapping performed in this study demonstrated that osteoarthritic damage was not evenly distributed across the medial tibial plateau. The anterior central and intermediate regions showed the highest frequency of India ink staining and basic fuchsin-positive microdamage. These observations correspond closely with the biomechanics of normal gait.

During the stance phase of walking, especially during heel strike and terminal extension, peak compressive and shear forces are concentrated predominantly in the anterior-central portions of the tibiofemoral joint [32]. The preferential development of microdamage within these high-load zones strongly supports the concept that early OA is fundamentally a mechanically driven disorder originating at the weakest regions of the load-bearing subchondral framework [29].

Clinically, accumulation of subchondral micro-fractures, marrow edema, fibrosis, and inflammatory changes appears on MRI as Bone Marrow Lesions (BMLs) [24]. Numerous studies have demonstrated that BMLs are strongly associated with accelerated cartilage loss and increased severity of joint pain in OA patients [27]. Because articular cartilage lacks sensory innervation, pain in early OA is believed to arise primarily from the richly innervated subchondral bone and marrow, where inflammatory mediators surrounding micro-fractures sensitize nociceptive nerve fibers [38].

Therapeutic Implications

Recognition of subchondral bone as a major driver of early OA pathogenesis has important therapeutic implications. For decades, most treatment strategies focused almost exclusively on protecting or regenerating cartilage, yet many of these approaches produced limited long-term clinical success [41]. One possible explanation is that such therapies failed to address the mechanically compromised subchondral foundation supporting the cartilage.

If early OA is primarily characterized by increased osteoclastic activity and fatigue-related microdamage accumulation, then early intervention with anti-resorptive agents such as bisphosphonates or Denosumab may theoretically interrupt disease progression [28]. By reducing excessive bone resorption and preserving subchondral structural integrity, these therapies could potentially prevent microcrack formation, reduce pathological biochemical crosstalk, and ultimately protect the overlying cartilage from secondary degeneration [34].

In addition, future therapeutic strategies may benefit from combining biomechanical correction, targeted pharmacologic therapy, and advanced imaging surveillance to identify patients during the earliest reversible stages of disease progression.

Limitations and Future Directions

Despite the robust sample size (N = 72) and comprehensive multimodal spatial analysis used in this study, several limitations should be acknowledged. Most importantly, the cross-sectional and ex vivo nature of the study prevents direct observation of disease progression over time. Although the strong spatial correlations observed strongly suggest that subchondral microdamage precedes advanced cartilage degeneration, definitive temporal causality cannot be established. Furthermore, while basic fuchsin staining remains the gold-standard method for identifying in vivo microdamage, it provides only a static representation of accumulated injury at the time of tissue harvesting and cannot differentiate between actively progressing cracks and older stabilized lesions [33].

Future studies should focus on translating these histological observations into clinically applicable, non-invasive imaging techniques. Advances in ultra-high-field MRI and development of targeted PET tracers capable of detecting early osteoclastic activity or microdamage could significantly improve early diagnosis of OA [24]. In addition, emerging molecular techniques such as spatial transcriptomics may help map the precise distribution of cytokines and MMPs surrounding subchondral microcracks, thereby providing deeper insight into the mechanisms underlying bone-cartilage biochemical communication [39].

CONCLUSION

In conclusion, the present study provides substantial histopathological and micro-architectural evidence that subchondral bone failure is an early and highly localized event in osteoarthritis. The accumulation of subchondral micro-fractures appears to represent a mechanically driven process that closely predicts the anatomical onset of cartilage degeneration. By disrupting the osteochondral barrier and promoting pathological biochemical crosstalk between bone and cartilage, subchondral microdamage acts as a critical initiator of progressive joint destruction.

These findings support a major shift in the current understanding of OA pathogenesis. Preserving the integrity of the subchondral bone and osteochondral junction before irreversible structural failure occurs may represent one of the most promising strategies for the development of true disease-modifying therapies for osteoarthritis.

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