Section: Anatomy

Original Research Article

HISTO-MORPHOMETRIC ASSESSMENT OF ZONAL ARCHITECTURE AND CELLULAR DISTRIBUTION IN AGING HUMAN HIP ARTICULAR CARTILAGE: A TERTIARY CARE STUDY

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Received : 07/04/2026
Received in revised form : 17/05/2026
Accepted : 03/06/2026

Keywords:
Zonal Architecture, Hip Articular Cartilage, Anatomy

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DOI: 10.47009/jamp.2026.8.3.162

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2026; 8 (3); 903-906

ABSTRACT

Background: The structural integrity of the hip joint depends on a strictly organized zonal architecture. **Objectives:** This study aims to describe the age-related shifts in these zones and the corresponding cellular density changes in a clinical population. **Materials and Methods:** Articular cartilage from 60 patients (aged 50–80 years) was analyzed using H&E staining to evaluate the superficial, middle, and deep zones. **Results:** We observed a significant reduction in cartilage thickness, dropping from an average of 2.2 µm in the 50–55 age group to 0.7 µm in the 76–80 age group ($r = -0.95$, $p = 0.003$). Topographical analysis showed a loss of distinct zonal boundaries and an increase in surface fibrillation with advancing age. **Conclusion:** The transition from a defined four-layer architecture to a disorganized, thinned matrix is a hallmark of aging hip cartilage, providing a histological baseline for early joint pathology.

INTRODUCTION

Articular cartilage of the hip joint is a highly specialized, avascular connective tissue that functions as a wear-resistant surface, crucial for smooth, pain-free joint movement. This unique durability is derived from a complex extracellular matrix (ECM) dominated by a framework of Type II collagen fibers and a high concentration of proteoglycans. The organization of this ECM is not uniform but follows a precise zonal architecture.[1]

1.1 Zonal Organization and Aging

Superficial (Tangential) Zone: Characterized by flattened, ovoid chondrocytes and collagen fibers oriented parallel to the joint surface to resist shear forces. Middle (Transitional) Zone: Contains larger, spherical chondrocytes and randomly oriented collagen fibers. Deep (Radial) Zone: Where chondrocytes are arranged in vertical columns perpendicular to the articular surface, offering maximum resistance to compressive loads.[2]

The Calcified Zone

This is the deepest layer, separating the hyaline cartilage from the underlying subchondral bone.

Structure: It contains a small number of cells embedded in a mineralized matrix. It is separated from the deep zone by a distinct boundary called the tidemark.[3]

The calcified zone acts as a mechanical transition, anchoring the soft articular cartilage to the rigid subchondral bone

As an individual ages, this intricate organization undergoes significant, yet predictable, histomorphological remodeling. This physiological process can reduce the cartilage's mechanical integrity, often serving as a precursor to pathological osteoarthritis (OA).

1.2 Staining Challenges and Objectives A primary challenge in histomorphometric studies of hyaline cartilage is that standard hematoxylin and eosin (H&E) staining has a similar refractive index for both collagen fibers and ground substance, rendering the underlying collagenous framework virtually invisible.

Using Masson's Trichrome Staining

Masson's Trichrome is used to detail the extracellular matrix (ECM) and zonal architecture. It reveals:

Collagen Distribution: The aniline blue component of this stain has a high affinity for Type II collagen, providing an exceptional visual contrast that facilitates a clear differentiation of the superficial, middle, and deep.

Zonal Boundaries: It provides a sharp color gradient that clearly demarcates the superficial, middle, and deep zones.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Matrix Integrity: the stain can highlight surface fibrillation (shredding), vertical fissures, and irregular staining patterns that indicates a loss of proteoglycan content. This study overcomes this limitation by utilizing Masson's Trichrome histochemistry. Our primary objective is to detail the histomorphological features and collagen fiber orientation of the aging hip joint (45–85 years) to establish a comprehensive descriptive standard at a tertiary care facility.

MATERIALS AND METHODS

2.1 Study Design and Sample Population

This descriptive cross-sectional study was conducted with Institutional Review Board approval. Samples of femoral head articular cartilage were obtained from 60 patients aged 45–85 years undergoing hip arthroplasty at our tertiary care centre. Exclusion criteria included patients with a prior diagnosis of rheumatoid arthritis, post-traumatic arthritis, or skeletal dysplasia.(table 1)

2.2 Histological Processing Thin sections were sliced from the femur head near the fovea. Samples were immediately fixed in 10% neutral buffered formalin, decalcified in 10% formic acid, and embedded in paraffin wax using standard pathological techniques. Serial sections were cut at (5–7 µm) thickness and mounted on glass slides.[4]

2.3 Masson's Trichrome Staining Protocol

Staining: Serial sections were stained with H&E for cellularity and Masson's Trichrome to enhance the visual contrast of the zonal matrix architecture.[5]

2.4 Histo -morphometric Analysis Stained slides were examined under a digital microscope. Digital micrographs were captured at 4x, 10x, and 40x magnifications. Measurements were taken using a tri-ocular microscope and digital imaging software. Thickness was measured from the articulating surface to the subchondral bone interface.[6]

Table 1: Age- Specimen Distribution

S.no	Age group	Specimen count
1.	45-55	6
2.	56-60	12
3.	61-65	15
4.	66-70	13
5.	71-75	9
6.	76-85	5

RESULTS

3.1 Topographical Analysis (4x and 10x Magnification)

Younger Cohort (45–60 years): Low-power examination (4x) demonstrated a remarkably smooth and continuous articular surface. The differentiation of the superficial tangential, middle transitional, and deep radial zones was distinct. The Masson's Trichrome stain provided a sharp color gradient, showing a deeper, more saturated blue intensity in the deep zone, indicating higher collagen density compared to the paler superficial zone.(fig 1&2).

(66-75 years) The four zones were clearly demarcated by variations in cell shape and matrix staining intensity.(fig 3 & 4)

Elderly Cohort (76–85 years): Slides from this cohort frequently showed signs of age-related remodeling with loss of the superficial tangential layer. Surface fibrillation, appearing as disrupted blue staining at the articulating edge, was observed. Vertical fissures often extended into the middle transitional zone.[7] (fig 5a)

The tidemark (the boundary of the calcified zone) appeared irregular and advanced. (fig 5b)

3.2 Cellular and Matrix Analysis (40x Magnification) Chondrocyte Morphology: Younger specimens exhibited a uniform distribution of single chondrocytes or simple isogenous groups (pairs). By contrast, older samples displayed clear cellular stress markers. High-power analysis revealed clear markers of cellular stress. Chondrocyte Cloning: In older specimens, chondrocytes were frequently observed in "brood clusters" (clones of >4 cells in one lacuna), a reactive attempt to repair the degrading matrix.[8]

Cellular Depletion: A significant increase in "ghost lacunae" (empty spaces) was noted in the 80-year-old samples, signifying apoptosis. Zonal Cells: Flattened cells of the superficial zone were progressively replaced by disorganized spherical cells as the tissue thinned.

Territorial Matrix: Vibrant magenta/purple "halos" were sharply defined immediately surrounding the lacunae, most prominently in the middle and deep zones. Interterritorial Matrix: The expansive matrix between cell groups stained intensely blue. In older samples, this blue staining was often "patchy" or less intense, particularly in the territorial zones (magenta fading), indicating a decrease in proteoglycan content associated with matrix degradation.(table 2)

Table 2: Age-Related Changes in Hip Cartilage zonal architecture (N=60)

S.no	Age Group (Years)	Predominant Zonal Feature
1.	50-55	Intact 4-layer architecture
2.	56-60	Minimal surface irregularities
3.	61-65	Early fibrillation; thinning superficial zone

ISSN (O): 2687-5365; ISSN (P): 2753-6556

4.	66-70	Disrupted middle zone; decreased cellularity
5.	71-75	Severe thinning; chondrocyte cloning
6.	76-85	Widespread matrix loss; ghost lacunae, Tidemark calcification

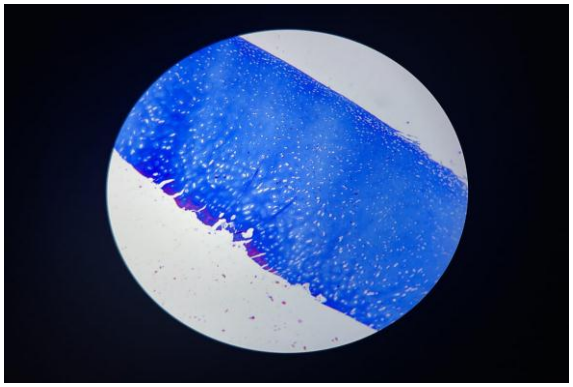

Figure 1: 10x magnification of human hip articular cartilage stained with Masson's Trichrome

Group 1: Ages 50–55 Observation: The articular surface is smooth, and the four zones (superficial, middle, deep, and calcified) are intact and clearly demarcated.

Cellular Detail: Cells in the superficial zone should appear flattened and parallel to the surface.

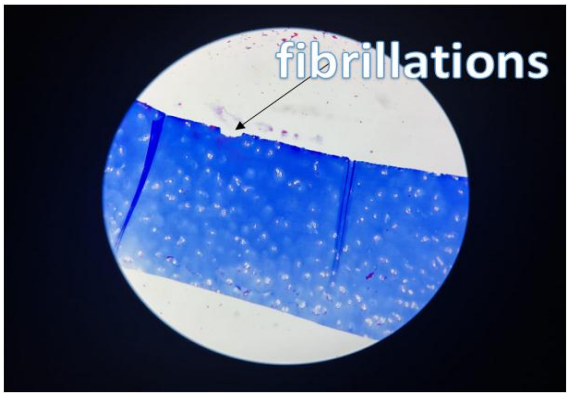

Figure 2: 10x magnification of human hip articular cartilage stained with Masson's Trichrome

Group 2: Ages 56–65 (Early Degeneration) Observation: You will see the first signs of surface fibrillation (fine fraying of the top edge) and a slight widening of the matrix space.

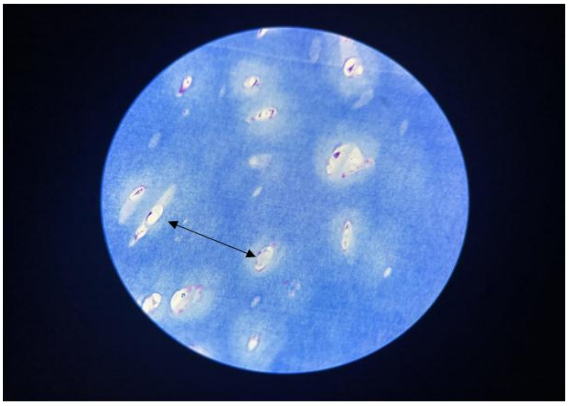

Figure 3: 40x magnification of human hip articular cartilage stained with Masson's Trichrome

Group 3: Ages 66–70 (Intermediate Stage) Observation: Chondrocyte density begins to decrease. Image showing widened interterritorial matrix spaces and fewer visible lacunae compared to Group 1.

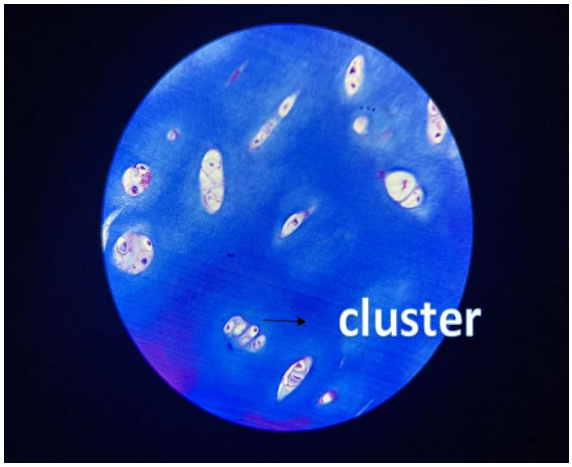

Figure 4: 40x magnification of human hip articular cartilage stained with Masson's Trichrome

Group 4: Ages 71–75 (Advanced Wear) Observation: This group often shows Chondrocyte Cloning (clusters of cells) as a reactive change to matrix loss. Image that clearly shows "brood clusters" (multiple chondrocytes squeezed into a single large lacuna).

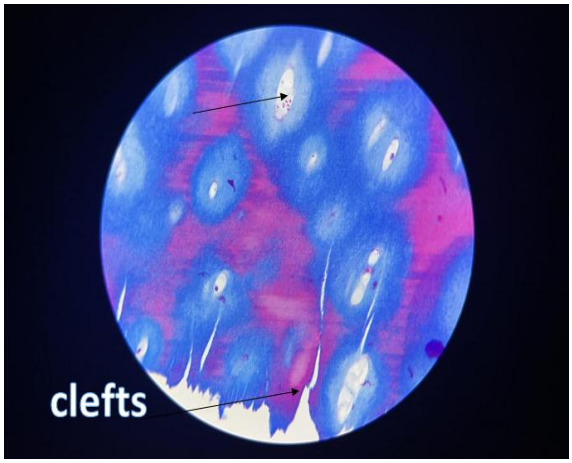

Figure 5a. 40x magnification of human hip articular cartilage stained with Masson's Trichrome

Group 5: Ages 76–85 (Severe Degeneration) Observation: Features include deep discontinuities (cracks/clefts), "ghost lacunae" (empty cell spaces), and an advanced, irregular tidemark.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

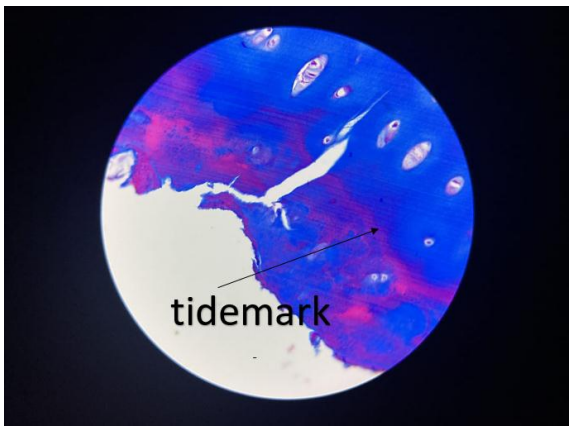

Figure 5b: 40x magnification of human hip articular cartilage stained with Masson's Trichrome

DISCUSSION

The current study demonstrates that hip cartilage aging is a multi-dimensional process involving both volume loss and architectural collapse. The negative correlation (r = -0.95) between age and thickness suggests a highly predictable degenerative timeline in the tertiary care population. The observation of chondrocyte cloning at 40x magnification is particularly significant. While younger cartilage maintains homeostasis through isolated isogenous groups, the elderly matrix exhibits these "clusters" as a hallmark of mechanical failure. Furthermore, the loss of the superficial tangential zone—the joint's primary defense against shear—explains the rapid acceleration of thinning seen after age 65.[9]

Unlike imaging studies (MRI/CT) which only show gross thickness, our histological approach allowed us to see surface fibrillation and sparse chondrocyte distribution—changes that often occur before a patient feels clinical pain. The negative correlation (r = -0.95) found in our tertiary care setting highlights that these zonal changes are highly predictable markers of biological aging.[10]

CONCLUSION

Histomorphometric changes in the articular cartilage of the hip joint follow a predictable zonal pattern that correlates significantly with advancing age. The descriptive results of this study establish that Masson's Trichrome is a vital histochemical standard for analyzing the aging joint.

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ISSN (O): 2687-5365; ISSN (P): 2753-6556