

Review

Spatial Multi-Omics Advances in Osteoarthritis: From Molecular Landscapes to Precision Medicine

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Abstract

Osteoarthritis (OA) is a pervasive, multifactorial joint disorder with an escalating global prevalence, yet it remains without effective disease-modifying therapies. Current research and clinical management are hindered by a reliance on traditional bulk-omics and imaging modalities that fail to capture the profound molecular and cellular heterogeneity of the disease. While bulk-omics provides a comprehensive but averaged view, it homogenizes complex tissue microenvironments, losing critical information about cell-cell interactions and niche-specific pathogenic pathways. This review posits that the emerging field of spatial multi-omics represents a paradigm shift, transcending the limitations of previous approaches by integrating molecular profiling with native tissue context. By simultaneously mapping the transcriptome, proteome, metabolome, and other molecular layers in a spatially resolved manner, this framework can deconstruct the intricate cellular architecture of the osteoarthritic joint. This capability offers unprecedented opportunities for precision diagnostics, enabling the discovery of novel biomarkers and the molecularly precise definition of disease endotypes. The capacity to pinpoint microenvironment-specific pathways also provides a powerful foundation for the development of personalized, next-generation therapeutics that target the root cause of disease, not just its symptoms. The analysis presented here delineates the technologies, applications, and challenges of this transformative field, underscoring its potential to revolutionize OA research and pave the way for a new era of precision care.

Keywords: Osteoarthritis, precision medicine, multi-omics, biomarkers.

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Introduction

Osteoarthritis (OA) is a chronic, degenerative joint disorder that represents a major and growing public health crisis [1]. The disease is characterized by the progressive degeneration of articular cartilage, inflammation of the synovial membrane, changes in subchondral bone, and the formation of osteophytes [2–4]. The World Health Organization estimates that by 2024, OA will be the fourth leading cause of knee disability globally [5]. In 2020 alone, an estimated 595 million individuals were afflicted with OA, representing 7.6% of the global population and a staggering 132.2% increase in the total number of cases since 1990 [6].

Despite its immense prevalence and significant impact on quality of life, there is a fundamental lack of effective, Food and Drug Administration (FDA)-approved therapies that can modify the trajectory of OA progression [7,8]. Current treatments primarily focus on symptomatic relief rather than addressing the underlying disease mechanisms. A key challenge in clinical management is the late diagnosis of OA. Articular cartilage is aneural, meaning it lacks nerve innervation, so initial damage is non-symptomatic, and patients are often diagnosed at a more advanced stage when joint pain and stiffness become prevalent [9–11]. This late diagnosis makes clinical management more difficult and highlights an urgent need for new research paradigms and diagnostic tools that can enable earlier, more precise intervention.

In the past decade, omics technologies have become instrumental in investigating the complex, multifactorial pathogenesis of OA [12,13]. Initially, research focused on single-omics, such as genomics, transcriptomics, or proteomics, to provide an understanding of molecular pathways and pathophysiological changes [14]. For instance, a recent review highlighted significant advancements in the molecular mapping of joint tissues through studies that characterized non-coding RNAs (ncRNAs) and identified key OA-associated pathways [13]. While these single-omics studies have provided unique insights, their findings do not always align, hindering the definition of generally accepted disease subtypes [14].

The field has since progressed to multi-omics, which integrates data from different molecular layers—such as the transcriptome and metabolome—to gain a more comprehensive view of the disease [14]. This approach has been used to explore the regulatory correlations between plasma metabolomics and single-cell transcriptomics, for instance, revealing a shared lipid metabolic dysregulation signature in OA [14]. However, a fundamental limitation persists: these multi-omics approaches are often based on the analysis of homogenized, or “bulk” tissue samples [15]. The process of averaging molecular signals across all cell types in a sample, a common practice in bulk RNA-seq, effectively masks the true heterogeneity of the disease [13]. The signal is averaged across a diverse range of cell populations,

from chondrocytes to synovial cells, resulting in a blurred molecular picture. This loss of spatial context and cellular resolution means that critical information about cell-cell interactions and the microenvironment-specific pathways that drive disease progression is lost [13]. This explains why translating bulk-omics findings into effective, disease-modifying therapies has been a significant challenge [14].

The etiology of OA is fundamentally a spatially regulated process, yet traditional methods have failed to account for this critical dimension. To address this, a new generation of technologies, known as spatial omics, has emerged to enable the measurement of molecular characteristics of cells while preserving their native spatial context [14]. This approach allows for the high-resolution mapping of transcripts, proteins, and metabolites within affected tissues, shedding light on how specific cell populations and their molecular pathways contribute to inflammation and tissue damage [16].

By integrating spatial context, researchers can move from a correlative understanding of OA to a more causal one. For example, a traditional bulk RNA-sequencing study might identify an upregulation of a particular gene in OA cartilage. However, a spatial multi-omics analysis can pinpoint that this gene is only upregulated in a specific subpopulation of chondrocytes located within the superficial zone of the cartilage, as shown in Fig. 1 [17–19]. By layering data from other omics modalities, such as proteomics and metabolomics, onto this same spatial map, a more comprehensive picture can be constructed [20,21]. This could reveal that this specific chondrocyte subpopulation is also secreting pro-inflammatory cytokines that are driving matrix degradation in its immediate vicinity [22]. This spatially-anchored, multi-modal data provides a clear causal link between a specific cell type, a molecular signature, and a pathogenic mechanism, a level of insight that is impossible to achieve with bulk analysis alone [14]. This powerful framework is now positioned to redefine OA research and pave the way for truly personalized care.

Current Landscape of Omics in OA

Genomics and Epigenomics

Genomics and epigenomics have provided foundational insights into the genetic and non-coding risk factors for OA [23]. Recent reviews have highlighted significant advancements in the comprehensive molecular mapping of OA joint tissues [15,24–26]. The largest OA genetics study to date was published by the Genetics of Osteoarthritis Consortium, reinforcing the strong hereditary component of the disease [15]. A particularly compelling study demonstrated a direct link between human evolution and OA susceptibility [27]. Their research revealed that evolutionary mechanisms like antagonistic pleiotropy, where a beneficial allele is positively selected for along with linked deleterious alleles, led to the conservation of genetic factors that, while beneficial for knee morphology, also increased the risk for

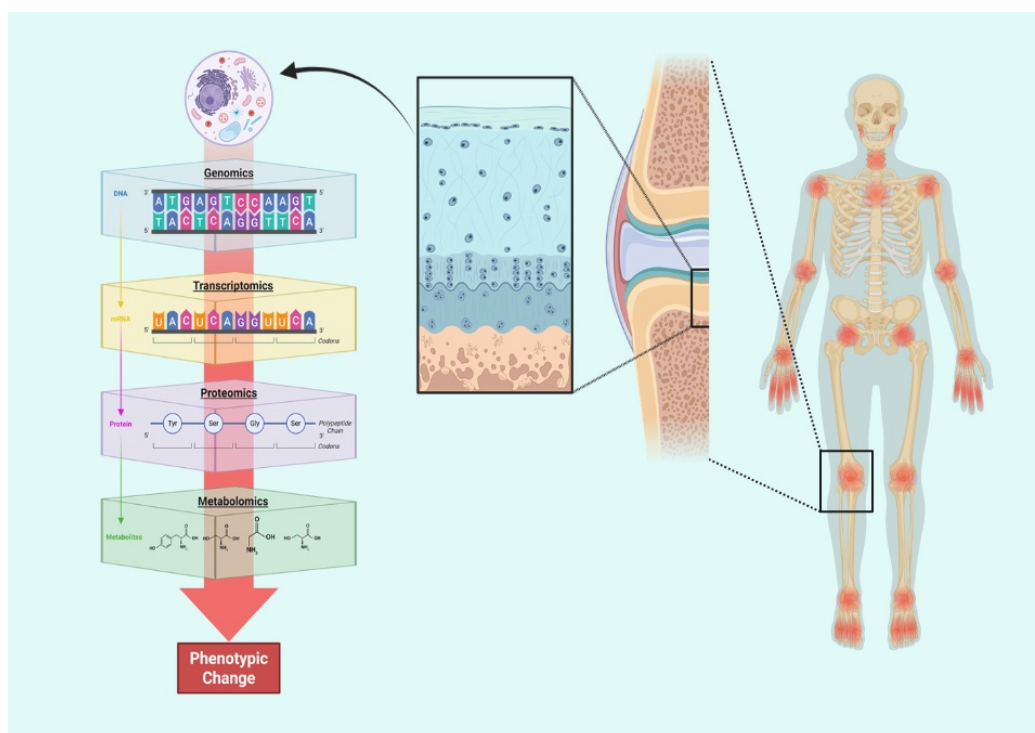


Fig. 1. Spatial multi-omics analysis to pinpoint the gene that is upregulated in a specific subpopulation of chondrocytes located within the superficial zone of the cartilage as the initial driver of osteoarthritis. Created in BioRender. Gangadaran, P. (2026) <https://BioRender.com/f47m406>.

OA [27].

The study functionally evaluated the gene *GDF5*, which is essential for knee development and also implicated in OA risk, identifying a causative variant (rs6060369) within a constrained regulatory element [28–31]. Mice lacking this element formed normal joints but developed morphological defects and OA later in life, with effects likely mediated through *PITX1* [27,28]. This finding establishes that OA's pathogenesis is not merely a degenerative process but is, in part, a side-effect of a successful evolutionary adaptation, underscoring the need for a comprehensive, systems-level understanding that integrates genetics, epigenetics, and tissue biology. Higher-impact genomics studies are already embracing this approach by integrating multiple methodologies, including chromatin accessibility mapping, single-cell RNA-sequencing, and spatial transcriptomics (ST) analyses [15].

Transcriptomics and ncRNAs

Transcriptomics, the study of the complete set of RNA molecules, provides a snapshot of a cell's function and can be used for deep phenotyping and patient stratification [32]. While gene expression data from transcriptomics is powerful, it is an incomplete picture. The analysis of RNA levels does not always correlate with the final abundance of functional proteins due to post-transcriptional regulation [13]. A major area of focus has been the role of ncRNAs, particularly microRNAs (miRNAs), in regulating

gene expression and cellular function. Over 80 miRNAs have been reported to be involved in OA pathology [33]. For example, studies have shown that miR-140 is down-regulated in osteoarthritic cartilage and targets genes such as *ADAMTS5* and *MMP-13*, which are crucial for cartilage matrix degradation [33]. However, technical challenges inherent to musculoskeletal tissues introduce significant limitations [32]. These tissues are often quiescent, with fewer cells and a dense matrix, making it difficult to obtain a sufficient amount of high-quality RNA [32]. The integrity of the RNA is a critical factor, and low-quality samples can introduce substantial noise into the data [32]. Additionally, standardizing sample collection and preparation, such as avoiding biases from enzymatic digestion during cell isolation, remains a challenge [32]. Relying solely on transcriptomics can therefore lead to an incomplete or misleading understanding of disease mechanisms, reinforcing the need for the integration of orthogonal data layers, such as proteomics and epigenomics, to provide a more robust and validated picture [14].

Proteomics and Metabolomics

Proteomics and metabolomics provide a more direct readout of the “functional” state of a cell or tissue by measuring proteins and metabolites, which are the direct executors of biological processes [13]. A study that analyzed synovial fluid and serum identified differentially expressed proteins like serine protease inhibitors, complement com-

ponents, and apolipoproteins in OA patients [34,35]. These proteins are believed to be involved in inflammation and cartilage breakdown. Simultaneously, serum metabolite profiles showed significant alterations in amino acid, lipid, glucose, and energy metabolism, with increased levels of creatinine and glutamine and reduced levels of leucine and histidine [35].

The research establishes a crucial connection between systemic metabolic state and local joint pathology. Evidence suggests that OA is not just a local disease but has contributions from systemic tissues and biofluids, such as adipose tissue and blood components [36]. Systemic changes, particularly those related to obesity and diet, can influence the local joint microenvironment through paracrine tissue interactions and the sharing of metabolites and transcripts between the systemic circulation and the joint space [36]. The identification of a shared lipid metabolic dysregulation signature in OA further supports this concept. At the molecular level, fatty acid binding protein 4 (FABP4) has emerged as a specific mediator bridging systemic metabolic dysregulation and local joint pathology. FABP4 has been shown to drive osteogenesis and angiogenesis within the joint as part of metabolic OA pathogenesis, and separately to induce chondrocyte degeneration through activation of the NF- κ B signaling pathway. These findings provide a concrete molecular example of how systemic lipid metabolism, dysregulated in conditions such as obesity, translates into spatially localized destructive processes within the cartilage and subchondral bone compartments. The conclusion drawn is that a comprehensive understanding of OA requires a multi-omics approach that spans both local and systemic levels to capture this intricate molecular dialogue [14]. The prevailing consensus is that a group of molecules, rather than a single biomarker, will be needed for effective diagnosis and prognosis due to the high variability and complexity of the disease [37].

Extracellular Vesicle Omics

Small extracellular vesicles (sEVs) have emerged as crucial mediators of intercellular communication in joint tissues [38]. These nanoscale, membrane-bound particles, including exosomes and microvesicles, carry a diverse cargo of proteins, lipids, and nucleic acids that reflect the state of their cell of origin during joint health and disease [39,40]. This makes them an ideal source for non-invasive biomarkers, bridging the gap between difficult-to-access joint pathology and easily-sampled biofluids [41].

The application of multi-omics to sEVs presents a unique opportunity to identify novel diagnostic and therapeutic targets [42]. For instance, a transcriptomic analysis of sEVs from plasma showed a significant downregulation of miR-193b-3p in OA patients, mirroring changes in cartilage tissue [43]. This suggests that circulating sEVs can serve as a non-invasive, surrogate marker for joint-specific molecular changes [41]. Similarly, proteomic studies of

sEVs have identified proteins like cathepsin F that are differentially expressed in OA patients, highlighting their potential for enhancing diagnostic accuracy [41]. The therapeutic potential of sEVs is also significant, with studies demonstrating that sEVs derived from mesenchymal stem cells (MSCs) can promote chondrogenesis and enhance cartilage repair by delivering chondroprotective factors like *SOX9* mRNA and miR-140 [44]. The dual function of sEVs as both a source for non-invasive liquid biopsies and as a therapeutic delivery system positions them as a key complement to spatially resolved tissue analysis in a precision medicine framework. The mechanistic link between circulating sEVs cargo and spatially defined tissue pathology operates through a traceable release hierarchy: spatial transcriptomic and proteomic profiling of OA joint tissue first identifies cell-type-specific molecular signatures anchored to defined anatomical compartments, such as superficial zone chondrocytes or the synovial lining layer. Cells within these niches release sEVs carrying a subset of that molecular cargo into the synovial fluid, from which they enter the systemic circulation. Cross-validating spatially derived tissue signatures against sEVs cargo isolated from matched patient biofluids therefore provides the methodological bridge that traces a circulating marker back to its tissue of origin and its precise anatomical niche, transforming a non-invasive liquid biopsy finding into a spatially interpretable signal [38]. The advances and limitations of omics technologies in OA research are summarized in Table 1.

Spatial Omics Technologies: From Maps to Mechanisms

ST

ST encompasses a variety of methods designed to quantify RNA expression while preserving the native spatial context of the tissue [45]. These technologies are broadly categorized into two main groups: imaging-based and sequencing-based approaches. Imaging-based methods, such as *In Situ* Hybridization (ISH) and *In Situ* Sequencing (ISS), allow for the direct visualization of RNA molecules in their native environment [46]. These approaches offer high RNA capture efficiency and single-cell resolution but are typically limited to profiling a few hundred unique genes at once due to the high cost and labor-intensive nature of adding more probes [45,46].

Conversely, sequencing-based methods like *In Situ* Capturing (ISC), exemplified by platforms such as 10x Visium, capture thousands of genes for transcriptome-wide profiling but at the cost of lower RNA capture efficiency and spatial resolution [46]. This creates a core technical trilemma between resolution, plex (the number of molecules detected), and throughput. For instance, the 10x Visium platform profiles gene expression on 55- μ m-diameter spots, with each spot often containing 10–20 cells, thereby averaging the signal and limiting the ability to re-

Table 1. Key advances and limitations of omics technologies in OA research.

Omics technology	Key contribution to OA research	Key limitation/Challenge
Genomics	Identification of genetic risk loci; GWAS studies pinpoint variants associated with traits/diseases.	Variants often in non-coding regions; do not directly identify causal genes.
Epigenomics	Characterization of non-coding RNAs (e.g., miRNAs); provides insight into gene silencing/activation.	Data is correlative; does not establish causality.
Transcriptomics	Provides a snapshot of gene expression; useful for deep phenotyping of heterogeneous patient populations.	RNA levels do not always correlate with protein abundance; post-translational modifications (PTMs) further complicate this correlation, as protein function is not captured at the transcript level; challenges with low RNA yield and integrity from tissue samples.
Proteomics	Direct measurement of functional proteins; reveals changes in inflammatory pathways and cartilage breakdown.	Limited by dynamic range; post-translational modifications require specialized analysis.
Metabolomics	Identifies metabolic pathways altered in disease; reveals a shared lipid metabolic dysregulation signature.	High variability due to diet and environment; metabolite identification is challenging.
Extracellular vesicle omics	Non-invasive source of biomarkers (liquid biopsy); reflects joint-specific molecular changes in biofluids.	Challenges in standardizing isolation techniques; limited understanding of underlying mechanisms.

OA, osteoarthritis; GWAS, genome-wide association study; miRNA, microRNA; RNA, ribonucleic acid; EV, extracellular vesicle; DNA, deoxyribonucleic acid; mRNA, messenger ribonucleic acid; PTM, post-translational modification; SNP, single nucleotide polymorphism; QC, quality control; MS, mass spectrometry.

solve single-cell heterogeneity [23]. While newer technologies like 10x Visium High Definition (HD) achieve single-cell resolution with 2- μ m capture tiles, this comes with a higher cost per sample [23]. The choice of platform is therefore a strategic decision based on the specific research question. A study aiming to identify a rare cell-type-specific biomarker would require a high-resolution, targeted approach, whereas an experiment designed to profile the overall gene expression landscape of a tissue would favor a high-throughput, sequencing-based method [46]. This understanding of the trade-offs is crucial for a clinical researcher when designing an experiment.

Spatial Proteomics and Metabolomics

To address the limitations of transcriptomics, which do not directly measure the functional molecules of a cell, spatial proteomics and metabolomics have advanced significantly, with spatially resolved mass spectrometry (MS) approaches, including mass spectrometry imaging (MSI), at the forefront [47]. MSI allows for the *in situ*, label-free analysis of proteins and metabolites, providing a direct readout of the functional state of the tissue.

Spatial proteomics and metabolomics offer a vital, orthogonal data layer that can validate and complement transcriptional data. For example, while transcriptomics might indicate an upregulation in the RNA of a particular gene, spatial proteomics can directly measure the abundance and localization of the protein product, revealing discrepancies due to post-transcriptional regulation [13]. Techniques like MALDI MSI and Secondary Ion Mass Spectrometry

(SIMS) can produce spatial distribution maps of metabolites with submicron to single-cell resolution, showing how different cell populations, even with similar gene expression, have unique metabolic profiles [48]. The high chemical specificity and sensitivity of these methods make them ideal for mapping critical metabolic changes in the OA joint [47]. However, challenges remain, such as the difficulty in confidently identifying all metabolites and the “Big Data” handling required due to the added spatial dimension [47].

3D and Temporal Spatial Omics

The pathogenesis of OA is a chronic, progressive process. A single 2D spatial map provides a powerful but static view of one disease stage. To overcome this, the field is progressing towards the construction of 3D and spatiotemporal maps of musculoskeletal tissues [49]. This advancement represents the ultimate goal of capturing disease dynamics.

Recent studies have successfully reconstructed the dynamic trajectories of cell populations in musculoskeletal systems [50]. For example, spatiotemporal transcriptomic mapping of acutely injured skeletal muscle revealed the dynamic organization of macrophage subtypes into functional zones during tissue regeneration [51]. In bone biology, a comprehensive cellular atlas of human bone architecture was created, which delineated the intricate molecular processes and cell-cell dependencies that coordinate bone metabolism [52]. It should be noted that, to date, published 3D spatiotemporal omics studies conducted directly in OA joint tissues remain limited. The studies cited here are drawn from adjacent musculoskeletal systems, skeletal

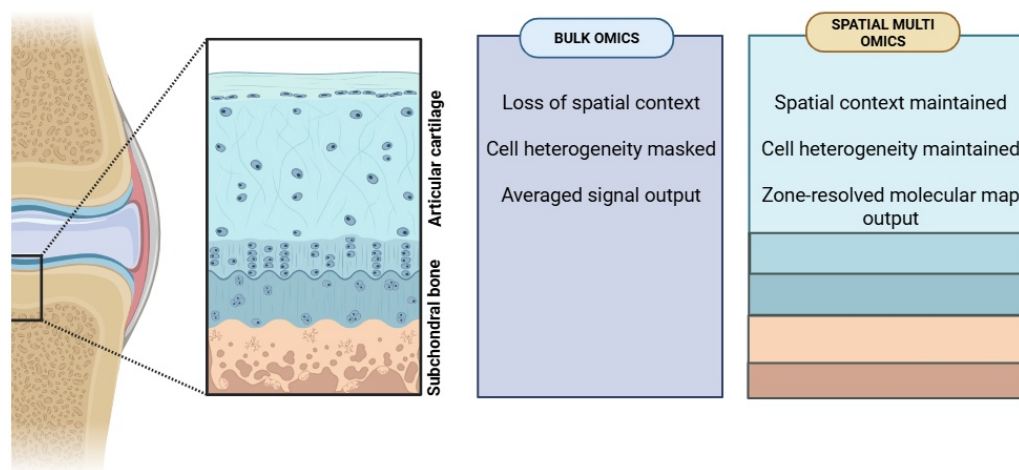


Fig. 2. Comparative analysis of spatial omics platforms. Gangadaran, P. (2026) <https://BioRender.com/zgxyfi0>.

muscle and bone, and represent the closest methodological precedents currently available. Dedicated OA spatiotemporal atlases therefore constitute a critical unmet need in the field, and their development should be a priority for forthcoming collaborative initiatives. By integrating multi-omics data over time, researchers can reconstruct the dynamic trajectories of cell populations and molecular pathways during disease progression, rather than relying on a single, static snapshot. This would enable the identification of “tipping points” or key transitions where molecular changes occur, providing a crucial window for therapeutic intervention. This progression from a static “map” to a dynamic “movie” of the disease is essential for developing timely and effective treatments for OA. The comparative analysis of spatial omics platforms is mentioned in Table 2 and Fig. 2.

The Concept of Spatial Multi-Omics

Definition and Value Proposition

Spatial multi-omics is defined as the comprehensive, joint analysis of multiple molecular layers—including the transcriptome, epigenome, proteome, and metabolome—in a spatially resolved manner within the same tissue section [16]. This approach represents a new framework for understanding the complex, multifactorial nature of OA. The disease is not a single entity but a heterogeneous collection of molecularly distinct endotypes, each with its own pathophysiological mechanisms. Traditional methods have struggled to define these endotypes because they miss the spatial context and the intricate interplay between different molecular layers [14].

The value proposition of spatial multi-omics lies in its ability to move from mere association to causality and from a blurred, bulk signal to the precise cellular niches that drive disease progression [14]. For example, a multi-omics study on end-stage hip OA patients identi-

fied two distinct endotypes that differed primarily in pro-inflammatory factors and energy metabolism [14]. By integrating metabolomic and genetic data, researchers were able to link specific metabolic markers to single-nucleotide polymorphisms (SNPs) and differential gene expression, thereby defining the endotypes with molecular precision [14]. This ability to define molecularly distinct endotypes is the key to identifying “theratypes”—subgroups of patients for whom a specific treatment would be most suitable, as shown in Figs. 3 and 4 [14]. This is the ultimate objective of personalized medicine in OA and provides a powerful alternative to the current one-size-fits-all approach to treatment. Critically, the theratype framework is not static; non-invasive biomarkers derived from circulating sEVs, cross-validated against the spatially resolved tissue signatures that define each endotype, can serve as longitudinal readouts of therapy response, enabling real-time refinement of treatment allocation.

Applications in OA

Pathogenesis Mapping and Endotype Definition

Spatial multi-omics enables the deconvolution of OA heterogeneity, revealing the intricate cellular and molecular architecture of the disease. A spatial analysis of the synovial membrane in OA patients revealed a significant alteration in cellular composition, most notably a marked infiltration and enrichment of myeloid cells, which are now understood to be a key component of the inflammatory milieu [22]. The identification of these distinct cell populations and their spatial organization transforms our understanding of OA pathogenesis, highlighting that it is not a homogeneous disease of cartilage degeneration but a complex process involving diverse, spatially-organized cell populations in multiple tissues [14].

Table 2. Comparative analysis of spatial omics platforms.

Platform/Technology	Analyte type	Resolution (μm /cells)	Plex/Throughput	Key advantage	Key limitation	Applicability in OA research
10x Visium	RNA	55- μm spots, 10–20 cells per spot	Whole transcriptome (>18,000 genes)	Unbiased, high throughput; good for landscape mapping	Lower spatial resolution, signal is averaged across multiple cells	Suitable for whole-tissue landscape mapping of OA cartilage, synovium, and subchondral bone; useful for identifying broad regional gene expression changes
10x Visium HD	RNA	2- μm capture tiles, approaching single-cell	Whole transcriptome (>18,000 genes)	High resolution, single-cell level detail; maintains spatial context	Higher cost per biopsy; requires specialized equipment	Preferred for resolving single chondrocyte heterogeneity within cartilage zones; valuable for rare cell subpopulation identification
NanoString GeoMx DSP	RNA, Protein	Variable, dependent on ROI size (50–100 cells)	Up to whole transcriptome or 100s of proteins	User-defined region-of-interest (ROI) profiling; can analyze both RNA and protein	Variable resolution; lower throughput for whole tissue analysis	Ideal for hypothesis-driven ROI analysis of pre-defined OA-relevant structures (e.g., osteophyte front, synovial lining layer)
MSI (MALDI/SIMS)	Protein, Metabolite	Submicron to single-cell resolution	Hundreds of metabolites/proteins	Label-free, direct measurement of functional molecules	Metabolite identification is challenging; data is “Big Data” due to added spatial dimension	Best suited for metabolite and lipid mapping in subchondral bone and cartilage matrix; directly relevant to lipid dysregulation in metabolic OA

RNA, ribonucleic acid; ROI, region of interest; DSP, digital spatial profiling; MSI, mass spectrometry imaging; MALDI, matrix-assisted laser desorption/ionization; SIMS, secondary ion mass spectrometry; μm , micrometer.

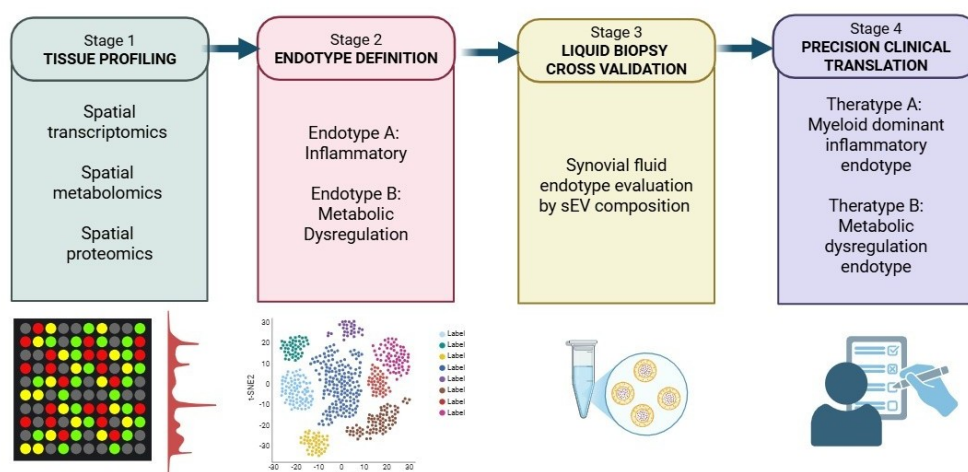


Fig. 4. Spatial multi-omics in osteoarthritis. Gangadaran, P. (2026) <https://BioRender.com/48e7200>.

tire pathological network rather than a single node [12,62]. For example, a study using a multi-omics approach on a rat model of OA demonstrated that a multi-component herbal formulation called Yograj Guggulu (YG) was effective because its various components altered protein expression in multiple key pathways related to inflammation and cartilage degradation [63]. This polypharmacological approach, guided by spatial multi-omics data, may be more effective for a complex, multifactorial disease like OA than traditional single-target drug development [63]. Analogous research in cancer biology shows that spatial multi-omics analysis of the tumor microenvironment can identify novel cellular features and predictive markers for therapeutic responses, a strategy that can be directly applied to the OA joint [64].

Precision Stratification for Clinical Trials

The lack of consensus in OA patient subtyping has been a major barrier to the successful development of effective treatments. Clinical trials for OA therapies have a high failure rate because they often treat a heterogeneous patient population as a single, homogenous group [65]. Spatial multi-omics provides a solution to this problem by enabling the molecularly precise definition of disease endotypes and the stratification of patients for clinical trials [66,67].

Multi-omics analysis can feed relevant information into computational models and machine learning for enhanced endo-phenotyping and patient stratification [14]. The ultimate goal is to identify “theratypes,” or patient subgroups for whom a specific treatment would be most suitable [14,68–71]. By designing clinical trials that recruit patients based on their specific molecular endotype rather than broad clinical symptoms, researchers can significantly reduce the noise in clinical data. This would make it easier to demonstrate the efficacy of a targeted therapy, thereby increasing the likelihood of successful drug development and

paving the way for truly personalized interventions [14]. The biomarkers and molecular alterations in OA are tabulated in Table 3.

Computational and Analytical Challenges

Data Integration

The rise of spatial multi-omics has led to an “explosive growth of data” that presents significant computational challenges for data storage and analysis [61,76,77]. The fundamental challenge lies in integrating different data modalities, such as transcriptomics, proteomics, and metabolomics, in a way that preserves and leverages spatial information [78]. Traditional data integration methods often handle this in an ad-hoc manner, with data arriving in a tabular format that discards the critical spatial connectivity and leads to the loss of true positive associations [79]. This is a major roadblock, as the most profound biological insights come from revealing causal chains across different molecular layers. If the spatial connectivity between a gene, its protein, and a downstream metabolite is lost, it becomes impossible to understand the localized pathogenic mechanism. A promising solution is the development of new computational models that transform multi-omics data into multi-channel “images,” which can then be analyzed using convolutional layers to preserve the spatial information [79].

AI and Machine Learning for Multi-Modal Spatial Data Interpretation

AI and machine learning are essential for interpreting the sheer complexity and volume of multi-modal spatial data [14]. Sophisticated algorithms, such as deep-learning-based segmentation tools like Cellpose 2.0 and Mesmer, have shown superior performance in assigning signals to individual cells, a critical step in spatial analysis [80]. AI models using convolutional layers have also been shown to

Table 3. Biomarkers and molecular alterations in osteoarthritis.

Biomarker/Molecular alteration	Omics layer	Tissue/Fluid	Biological implication	Reference
Upregulated CNTNAP2 and STMN2	Transcriptomics	Subchondral Bone	Linked to OA lesions	[13]
Downregulation of miR-140	Transcriptomics	Cartilage	Targets genes for cartilage matrix degradation	[72]
Elevated miR-16, miR-20b, miR-29c, miR-30b	Transcriptomics (miRNA)	Plasma	Potential for diagnostic biomarker discovery	[73]
Elevated Cathepsin F and reduced Ig α 2 chain	Proteomics	sEVs (serum)	Potential to enhance diagnostic accuracy	[41]
Elevated C3, C4b, C9	Proteomics	Synovial Fluid	Implicated in inflammation and cartilage breakdown	[34]
Increased Creatinine, Glutamine	Metabolomics	Serum	Indicates altered metabolism and adaptation to stress	[74]
Reduced Leucine and Histidine	Metabolomics	Serum	Reflects altered amino acid metabolism	[75]
Elevated OLR1 and CD69	Spatial Omics	Synovium	Drivers of spatial homeostasis; novel therapeutic target	[22]
Pro-inflammatory factors & energy metabolism markers	Multi-omics	Cartilage, Blood	Defines molecular OA endotypes	[17]

improve performance by decreasing the impact of noise in the data [79].

However, the use of complex deep learning models introduces a significant challenge: the “black box” dilemma [81]. While these models can accurately classify patients or predict outcomes, the logical reasoning behind the classification is often unclear [82]. This lack of interpretability poses a major regulatory hurdle in the medical field, as physicians and regulatory bodies need to understand why a particular decision was made before a tool can be deployed clinically [83–85]. The future of AI in this field will require a shift from purely predictive models to models that are both accurate and interpretable, perhaps through the use of methods like integrated gradients.

Standardization and Reproducibility Concerns

The lack of standardized frameworks, experimental protocols, and data formats is a significant challenge that hinders rapid progress and reproducibility in the field [86, 87]. The significant differences in throughput, coverage, and spatial resolution of different technologies make it difficult to develop generalizable algorithms [88]. A single experiment can generate heterogeneous data from different platforms in various formats, including images, tables, and polygons. Without a common data “language,” it is difficult for different research groups to share data or for a single lab to integrate data from different platforms. The emergence of standardized data containers and infrastructure, such as SpatialData and SpatialExperiment, is a critical step towards unifying this heterogeneous data [89]. This infrastructure will facilitate the development of “plug-and-play” analysis algorithms and is a necessary step towards building the open-access OA spatial multi-omics atlases that are needed to accelerate research [27]. The computational chal-

lenges and solutions in spatial multi-omics are tabulated in Table 4.

Translational Outlook

The translational potential of spatial multi-omics for OA is immense, offering a pathway to bridge the gap between fundamental research and clinical application [90]. The initial applications of this technology will likely be in preclinical research, where it can be used in animal models to identify and validate therapeutic targets and mechanisms of action, as demonstrated by a study on a multi-component herbal therapy [63]. The next crucial step will be to use these technologies in human clinical research to define disease endotypes with molecular precision and to stratify patients for clinical trials, thereby increasing the likelihood of therapeutic success [14]. The ultimate goal is to integrate these spatially resolved biomarkers into routine patient care for diagnostics, prognostics, and to guide treatment selection [91,92]. For example, spatial omics could help clinicians differentiate between OA and other arthritides like psoriatic arthritis, which may present with similar symptoms but have distinct molecular underpinnings [14].

However, the path to clinical deployment is not without its challenges. The rollout of AI (Artificial intelligence)-based diagnostic and prognostic tools in the medical field has been slow due to significant regulatory hurdles [93]. The “black box” nature of many complex machine learning models, where the reasoning for a prediction is unclear, poses a major barrier to regulatory approval and clinical adoption [94,95]. Ensuring the standardization and reproducibility of spatial multi-omics experiments across different labs and clinical sites will be essential to establish their reliability as diagnostic tools [14]. Several additional workflow and infrastructure barriers stand between current

Table 4. Computational challenges and solutions in spatial multi-omics.

Challenge	Description	Proposed solution/Tool
Data volume	The sheer size of high-resolution, high-plex datasets, which require substantial computing power and storage.	Cloud-based platforms, e.g., AtoMx™ Spatial Informatics Platform (SIP).
Multi-modal integration	Combining disparate data types (e.g., transcriptomics, proteomics) while preserving spatial relationships.	Data transformation into multi-channel images for analysis with convolutional layers.
Batch effects/Noise	Biases introduced by different platforms and sample preparation methods, which can obscure true biological signals.	AI models with convolutional layers; normalization methods that use spatial neighborhood information.
Data interpretability	The “black box” nature of complex deep learning models, where the reasoning behind a prediction is unclear.	Development of interpretable AI models, e.g., using integrated gradients (IG).
Standardization	The lack of common data formats and protocols, which makes data sharing and reproducibility difficult.	Standardized data containers (e.g., SpatialData, SpatialExperiment) and open-access atlases.

research applications and routine clinical use. No spatial multi-omics assay has yet received Conformité Européenne (European Conformity) (CE) marking or FDA clearance for clinical diagnostics, meaning all current applications remain research-grade. Standard clinical pathology pipelines rely on formalin-fixed paraffin-embedded (FFPE) archival tissue; most ST platforms were optimized for fresh-frozen material, and although newer platform iterations have improved FFPE compatibility, signal quality and gene detection rates remain inferior to fresh-frozen preparations. Beyond tissue handling, the computational infrastructure required—including the storage, processing, and bioinformatic interpretation of high-plex spatial datasets—is not available in routine clinical or even most academic hospital settings, necessitating specialist support that is currently a significant bottleneck to scaling these technologies toward patient care. Overcoming these computational and regulatory challenges is a prerequisite for fully realizing the transformative potential of spatial multi-omics in clinical practice. Two additional practical barriers deserve explicit discussion. First, the per-sample cost of high-resolution platforms such as 10x Visium HD and NanoString GeoMx DSP remains substantial, limiting the feasibility of large-cohort studies and routine clinical deployment without significant reductions in reagent costs or the development of cost-sharing biobank infrastructures. Second, and perhaps more fundamentally, the biology of OA creates a sampling problem that cannot be resolved by technology alone. Articular cartilage is aneural, and patients remain asymptomatic during early disease; by the time a diagnosis is made and tissue becomes accessible, it is almost invariably end-stage surgical material obtained at arthroplasty. Most existing tissue banks therefore hold specimens that reflect late-stage pathology, systematically biasing the molecular landscapes that spatial multi-omics can capture. Addressing this requires the development of arthroscopic biopsy protocols designed for research purposes, prospective biobanking initiatives that collect tissue at multiple disease stages, and international coordination to build repositories sufficiently pow-

ered for spatially resolved discovery studies.

Future Directions

Single-Cell Spatial Multi-Omics

The future of spatial multi-omics in OA research lies in achieving true single-cell resolution [96,97]. Current high-throughput methods often profile a signal averaged across multiple cells within a single spot, obscuring the heterogeneity that exists even within a seemingly homogenous cell type [98]. A 55- μm spot on a 10x Visium slide, for example, can contain multiple cells, blurring the molecular profiles of individual chondrocytes [98]. The widespread adoption of single-cell analytical techniques is already revolutionizing our understanding of cellular heterogeneity, but the spatial context is often lost [99]. The ultimate goal is to combine these two fields, enabling the characterization of the unique molecular profile of every single cell in the joint, revealing subtle intercellular differences that could be key to disease initiation or progression [100]. This would provide an unprecedented level of detail, allowing researchers to pinpoint the exact cells that are driving pathology.

Live-Imaging Coupled With Omics Readouts

A powerful future direction is the integration of live-imaging with omics readouts, moving from a static “map” of the disease to a dynamic “movie” [27]. This would involve combining real-time imaging of cellular processes with a rapid, high-resolution omics readout to capture how molecular changes unfold over time and space. This would allow for the direct observation of key pathological events as they happen in the joint, such as the initial activation of a chondrocyte in response to a mechanical stimulus or the infiltration of an immune cell into the synovium [19,101,102]. This approach would be invaluable for understanding the kinetic aspects of OA pathogenesis and identifying the optimal time window for therapeutic intervention.

Collaborative Efforts: Building Open-Access OA Spatial Multi-Omics Atlases

The high cost and complexity of spatial multi-omics experiments mean that no single research group can generate a comprehensive dataset of the OA joint. To accelerate research, collaborative efforts are essential to build shared, open-access OA spatial multi-omics atlases [32]. Initiatives to create comprehensive atlases of bone cellular architecture and the human spatial transcriptome are already underway [52]. The creation of a dedicated OA atlas would allow the entire scientific community to leverage these datasets, facilitating rapid biomarker discovery, therapeutic target identification, and the development of new computational tools to analyze the data [103,104]. This community-driven approach will be critical for realizing the full potential of spatial multi-omics for OA.

Conclusions

The shift from traditional omics to spatial multi-omics marks a paradigm change in OA research, moving beyond bulk analyses that obscure cellular heterogeneity and microenvironmental interactions. Spatial multi-omics enables high-resolution mapping of the osteoarthritic joint, allowing precise identification of causal mechanisms within specific cellular niches. This transformative approach facilitates the definition of OA endotypes and the development of “theratypes”—patient subgroups matched to targeted therapies. It also supports the discovery of spatially resolved biomarkers, validated through non-invasive liquid biopsies, advancing personalized care. Crucially, it lays the foundation for next-generation therapeutics that address entire pathological networks. However, realizing this potential demands overcoming challenges in data volume, multi-modal integration, and AI interpretability. Progress hinges on interdisciplinary collaboration among bioinformaticians, orthopaedic clinicians, and systems biologists to create open-access atlases and standardized tools. Spatial multi-omics is poised to become central to precision OA management, redefining both research and clinical strategies.

Abbreviations

AI, Artificial Intelligence; AUC, Area Under the Curve; CE, Conformité Européenne (European Conformity); DNA, Deoxyribonucleic Acid; DSP, Digital Spatial Profiling; EV, Extracellular Vesicle; FABP4, Fatty Acid-Binding Protein 4; FDA, Food and Drug Administration; FFPE, Formalin-Fixed Paraffin-Embedded; *GDF5*, Growth Differentiation Factor 5; GWAS, Genome-Wide Association Study; HD, High Definition; IG, Integrated Gradients; ISC, *In Situ* Capturing; ISH, *In Situ* Hybridization; ISS, *In Situ* Sequencing; MALDI, Matrix-Assisted Laser Desorption/Ionization; miRNA, MicroRNA; MMP, Matrix Metalloproteinase; MSC, Mesenchymal Stem Cell; MS, Mass Spectrometry; MSI, Mass Spectrometry Imaging;

mRNA, Messenger Ribonucleic Acid; ncRNA, Non-coding RNA; NF- κ B, Nuclear Factor Kappa B; OA, Osteoarthritis; PTM, Post-Translational Modification; QC, Quality Control; RNA, Ribonucleic Acid; RNA-seq, RNA Sequencing; ROI, Region of Interest; sEVs, Small Extracellular Vesicles; SIMS, Secondary Ion Mass Spectrometry; SIP, Spatial Informatics Platform; SNP, Single Nucleotide Polymorphism; ST, Spatial Transcriptomics; μ m, Micrometer.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

An AI-based tools (Wordvice AI or ChatGPT by OpenAI, GPT-5.2, accessed July 2026) was used under author supervision in a limited manner to improve English expression, clarify structure, and adjust word count in manuscript text and answer reviewers. The authors reviewed and edited the content independently and take full responsibility for the final manuscript.

Availability of Data and Materials

Not applicable. All information presented in this review is derived from previously published literature.

Author Contributions

MJ, NJ, and SR conceptualized the review theme and drafted the initial manuscript. AN and SM contributed to literature search, data interpretation, and writing of individual sections. RLR, BCA, and PG provided critical revisions, intellectual input, and structural refinement of the manuscript. NJ and PG served as corresponding authors. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable. This review article does not involve human participants or animal experiments.

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Conflict of Interest

The authors declare that they have no competing interests.

References

- [1] Ma W, Chen H, Yuan Q, Chen X, Li H. Global, regional, and national epidemiology of osteoarthritis in working-age individuals: insights

- from the global burden of disease study 1990–2021. Scientific reports. 2025; 15: 7907. <http://doi.org/10.1038/s41598-025-91783-6>.
- [2] Tong L, Yu H, Huang X, Shen J, Xiao G, Chen L, *et al.* Current understanding of osteoarthritis pathogenesis and relevant new approaches. Bone research. 2022; 10: 60. <http://doi.org/10.1038/s41413-022-00226-9>.
 - [3] Fazio A, Di Martino A, Brunello M, Traina F, Marvi MV, Mazzotti A, *et al.* The involvement of signaling pathways in the pathogenesis of osteoarthritis: An update. Journal of orthopaedic translation. 2024; 47: 116–124. <http://doi.org/10.1016/j.jot.2024.06.002>.
 - [4] He Y, Li Z, Alexander PG, Ocasio-Nieves BD, Yocum L, Lin H, *et al.* Pathogenesis of Osteoarthritis: Risk Factors, Regulatory Pathways in Chondrocytes, and Experimental Models. Biology. 2020; 9: 194. <http://doi.org/10.3390/biology9080194>.
 - [5] Courties A, Kouki I, Soliman N, Mathieu S, Sellam J. Osteoarthritis year in review 2024: Epidemiology and therapy. Osteoarthritis and cartilage. 2024; 32: 1397–404. <http://doi.org/10.1016/j.joca.2024.07.014>.
 - [6] GBD 2021 Osteoarthritis Collaborators. Global, regional, and national burden of osteoarthritis, 1990–2020 and projections to 2050: a systematic analysis for the Global Burden of Disease Study 2021. The Lancet. Rheumatology. 2023; 5: e508–e522. [http://doi.org/10.1016/S2665-9913\(23\)00163-7](http://doi.org/10.1016/S2665-9913(23)00163-7).
 - [7] Katz JN, Neogi T, Callahan LF, Block JA, Conaghan PG, Simon LS, *et al.* Disease modification in osteoarthritis; pathways to drug approval. Osteoarthritis and cartilage open. 2020; 2: 100059. <http://doi.org/10.1016/j.ocarto.2020.100059>.
 - [8] Kim JS, Borges S, Clauw DJ, Conaghan PG, Felson DT, Fleming TR, *et al.* FDA/Arthritis Foundation osteoarthritis drug development workshop recap: Assessment of long-term benefit. Seminars in arthritis and rheumatism. 2022; 56: 152070. <http://doi.org/10.1016/j.semarthrit.2022.152070>.
 - [9] Chang L-R, Marston G, Martin A. Anatomy, Cartilage. StatPearls Publishing: Treasure Island (FL). 2025.
 - [10] ur Rehman S, Iqbal S, Umair Shahid M, Soman Jahangir M, Latif Malik A. Cartilage: Structure, Function, and the Pathogenesis of Osteoarthritis. Advancements in Synovial Joint Science-Structure, Function, and Beyond. IntechOpen; 2024. <http://doi.org/10.5772/intechopen.1003264>.
 - [11] del Río E. Thick or Thin? Implications of Cartilage Architecture for Osteoarthritis Risk in Sedentary Lifestyles. Biomedicines. 2025; 13: 1650. <http://doi.org/10.3390/biomedicines13071650>.
 - [12] Wei Y, Qian H, Zhang X, Wang J, Yan H, Xiao N, *et al.* Progress in multi-omics studies of osteoarthritis. Biomarker research. 2025; 13: 26. <http://doi.org/10.1186/s40364-025-00732-y>.
 - [13] Liu Y, Molchanov V, Brass D, Yang T. Recent advances in omics and the integration of multi-omics in osteoarthritis research. Arthritis research & therapy. 2025; 27: 100. <http://doi.org/10.1186/s13075-025-03563-2>.
 - [14] Welsing PMJ, El Bouhaddani S, Zhu L, Nijhof NC, Mastbergen SC, Wen C, *et al.* Omics-driven insights into the molecular pathways driving osteoarthritis pathogenesis. Connective Tissue Research. 2025; 66: 323–330. <http://doi.org/10.1080/03008207.2025.2541291>.
 - [15] Wilson TG, Baghel M, Ali SA. Osteoarthritis year in review 2025: Genetics, genomics, and epigenetics. Osteoarthritis Cartilage. 2025; 33: 1293–1299. <http://doi.org/10.1016/j.joca.2025.08.004>.
 - [16] Liu X, Peng T, Xu M, Lin S, Hu B, Chu T, *et al.* Spatial multi-omics: deciphering technological landscape of integration of multi-omics and its applications. Journal of hematology & oncology. 2024; 17: 72. <http://doi.org/10.1186/s13045-024-01596-9>.
 - [17] Rai MF, Collins KH, Lang A, Maerz T, Geurts J, Ruiz-Romero C, *et al.* Three decades of advancements in osteoarthritis research: insights from transcriptomic, proteomic, and metabolomic studies. Osteoarthritis and cartilage. 2024; 32: 385–397. <http://doi.org/10.1016/j.joca.2023.11.019>.
 - [18] Fan Y, Bian X, Meng X, Li L, Fu L, Zhang Y, *et al.* Unveiling inflammatory and prehypertrophic cell populations as key contributors to knee cartilage degeneration in osteoarthritis using multi-omics data integration. Annals of the rheumatic diseases. 2024; 83: 926–944. <http://doi.org/10.1136/ard-2023-224420>.
 - [19] Li X, Fang L, Zhou R, Yao L, Clayton SW, Muscat S, *et al.* Current cutting-edge omics techniques on musculoskeletal tissues and diseases. Bone research. 2025; 13: 59. <http://doi.org/10.1038/s41413-025-00442-z>.
 - [20] Eicher T, Kinnebrew G, Patt A, Spencer K, Ying K, Ma Q, *et al.* Metabolomics and Multi-Omics Integration: A Survey of Computational Methods and Resources. Metabolites. 2020; 10: 202. <http://doi.org/10.3390/metabo10050202>.
 - [21] Athieniti E, Spyrou GM. A guide to multi-omics data collection and integration for translational medicine. Computational and structural biotechnology journal. 2022; 21: 134–149. <http://doi.org/10.1016/j.csbj.2022.11.050>.
 - [22] Wang C, Zeng Z, Wang T, Xie Z, Zhang J, Dong W, *et al.* Unraveling the spatial and signaling dynamics and splicing kinetics of immune infiltration in osteoarthritis synovium. Frontiers in immunology. 2025; 16: 1521038. <http://doi.org/10.3389/fimmu.2025.1521038>.
 - [23] Ramos YFM, Rice SJ, Ali SA, Pastrello C, Jurisica I, Rai MF, *et al.* Evolution and advancements in genomics and epigenomics in OA research: How far we have come. Osteoarthritis and cartilage. 2024; 32: 858–868. <http://doi.org/10.1016/j.joca.2024.02.656>.
 - [24] Roberts JB, Rockel JS, Mulders R, Capellini TD, Appleton CT, Phanstiel DH, *et al.* From mechanism to medicine: The progress and potential of epigenetics in osteoarthritis. Osteoarthritis and cartilage open. 2025; 7: 100621. <http://doi.org/10.1016/j.ocarto.2025.100621>.
 - [25] Hiruthyaswamy SP, Bose A, Upadhyay A, Raha T, Bhattacharjee S, Singha I, *et al.* Molecular signaling pathways in osteoarthritis and biomaterials for cartilage regeneration: a review. Bioengineered. 2025; 16: 2501880. <http://doi.org/10.1080/21655979.2025.2501880>.
 - [26] Katsoula G, Kreitmaier P, Zeggini E. Insights into the molecular landscape of osteoarthritis in human tissues. Current opinion in rheumatology. 2022; 34: 79–90. <http://doi.org/10.1097/BOR.0000000000000853>.
 - [27] Ratneswaran A, Kapoor M. Osteoarthritis year in review: genetics, genomics, epigenetics. Osteoarthritis and cartilage. 2021; 29: 151–160. <http://doi.org/10.1016/j.joca.2020.11.003>.
 - [28] Kim HA. Osteoarthritis—Insights From Recent Research. Journal of rheumatic diseases. 2022; 29: 132–139. <http://doi.org/10.4078/jrd.2022.29.3.132>.
 - [29] Coveney CR, Maridas D, Chen H, Muthurulan P, Liu Z, Jagoda E, *et al.* Complex Regulatory Interactions at GDF5 Shape Joint Morphology and Osteoarthritis Disease Risk. Arthritis & rheumatology. 2025; 77: 1488–1502. <http://doi.org/10.1002/art.43231>.
 - [30] Valdes AM, Evangelou E, Kerkhof HJ, Tamm A, Doherty SA, Kisand K, *et al.* The GDF5 rs143383 polymorphism is associated with osteoarthritis of the knee with genome-wide statistical significance. Annals of the rheumatic diseases. 2011; 70: 873–875. <http://doi.org/10.1136/ard.2010.134155>.
 - [31] Muthurulan P, Zhao D, Young M, Richard D, Liu Z, Emami A, *et al.* Joint disease-specificity at the regulatory base-pair level. Nature communications. 2021; 12: 4161. <http://doi.org/10.1038/s41467-021-24345-9>.
 - [32] Rai MF. Back to basics: Transcriptomics studies for deep phenotyping of osteoarthritis. Osteoarthritis and cartilage open. 2021; 3: 100166. <http://doi.org/10.1016/j.ocarto.2021.100166>.
 - [33] Zacharyasz J, Młeczko AM, Bąkowski P, Piontek T, Bąkowska-Żywicka K. Small Noncoding RNAs in Knee Osteoarthritis: The Role of MicroRNAs and tRNA-Derived Fragments. International journal of molecular sciences. 2021; 22: 5711. <http://doi.org/10.3390/ijms22115711>.

- [34] Balakrishnan L, Nirujogi RS, Ahmad S, Bhattacharjee M, Manda SS, Renuse S, *et al.* Proteomic analysis of human osteoarthritis synovial fluid. *Clinical proteomics*. 2014; 11: 6. <http://doi.org/10.1186/1559-0275-11-6>.
- [35] Rydén M, Turkiewicz A, Önnérjörd P, Tjörnstrand J, Englund M, Ali N. Identification and quantification of degradome components in human synovial fluid reveals an increased proteolytic activity in knee osteoarthritis patients vs controls. *Proteomics*. 2023; 23: 2300040. <http://doi.org/10.1002/pmic.202300040>.
- [36] Rockel JS, Potla P, Kapoor M. Transcriptomics and metabolomics: Challenges of studying obesity in osteoarthritis. *Osteoarthritis and cartilage open*. 2024; 6: 100479. <http://doi.org/10.1016/j.ocarto.2024.100479>.
- [37] Haartmans MJJ, Emanuel KS, Tuijthof GJM, Heeren RMA, Emans PJ, Cillero-Pastor B. Mass Spectrometry-based Biomarkers for Knee Osteoarthritis: A Systematic Review. *Expert review of proteomics*. 2021; 18: 693–706. <http://doi.org/10.1080/14789450.2021.1952868>.
- [38] Zhou X, Huang J, Zhang D, Qian Z, Zuo X, Sun Y. Small extracellular vesicles: the origins, current status, future prospects, and applications. *Stem cell research & therapy*. 2025; 16: 184. <http://doi.org/10.1186/s13287-025-04330-5>.
- [39] Chavda VP, Pandya A, Kumar L, Raval N, Vora LK, Pulakkat S, *et al.* Exosome nanovesicles: A potential carrier for therapeutic delivery. *Nano Today*. 2023; 49: 101771. <http://doi.org/10.1016/j.nantod.2023.101771>.
- [40] Xu G, Jin J, Fu Z, Wang G, Lei X, Xu J, *et al.* Extracellular vesicle-based drug overview: research landscape, quality control and non-clinical evaluation strategies. *Signal transduction and targeted therapy*. 2025; 10: 255. <http://doi.org/10.1038/s41392-025-02312-w>.
- [41] Wang T, Ng CY, Ng BZJ, Toh WS, Hui JHP. Multi-omics analysis of small extracellular vesicles in osteoarthritis: bridging the gap between molecular insights and clinical applications. *Burns Trauma*. 2025; 13: tkaf023. <http://doi.org/10.1093/burnst/tkaf023>.
- [42] Muthu S, Bapat A, Jain R, Jeyaraman N, Jeyaraman M. Exosomal therapy—a new frontier in regenerative medicine. *Stem cell investigation*. 2021; 8: 7. <http://doi.org/10.21037/sci-2020-037>.
- [43] Sun C, Teng F, Xia Y. Extracellular vesicles in osteoarthritis: mechanisms, therapeutic potential, and diagnostic applications. *Frontiers in immunology*. 2025; 16: 1595095. <http://doi.org/10.3389/fimmu.2025.1595095>.
- [44] González-Rodríguez A, De Toro FJ, Jorge-Mora A, Fernandez-Pernas P, Rivadulla CP, Fraga M, *et al.* Targeting osteoarthritis with small extracellular vesicle therapy: potential and perspectives. *Frontiers in bioengineering and biotechnology*. 2025; 13: 1570526. <http://doi.org/10.3389/fbioe.2025.1570526>.
- [45] Lee Y, Lee M, Shin Y, Kim K, Kim T. Spatial Omics in Clinical Research: A Comprehensive Review of Technologies and Guidelines for Applications. *International journal of molecular sciences*. 2025; 26: 3949. <http://doi.org/10.3390/ijms26093949>.
- [46] Piñeiro AJ, Houser AE, Ji AL. Research Techniques Made Simple: Spatial Transcriptomics. *The Journal of investigative dermatology*. 2022; 142: 993–1001.e1. <http://doi.org/10.1016/j.jid.2021.12.014>.
- [47] Taylor MJ, Lukowski JK, Anderton CR. Spatially Resolved Mass Spectrometry at the Single Cell: Recent Innovations in Proteomics and Metabolomics. *Journal of the American Society for Mass Spectrometry*. 2021; 32: 872–894. <http://doi.org/10.1021/jasms.0c00439>.
- [48] Alexandrov T. Spatial Metabolomics and Imaging Mass Spectrometry in the Age of Artificial Intelligence. *Annual review of biomedical data science*. 2020; 3: 61–87. <http://doi.org/10.1146/annurev-biodatasci-011420-031537>.
- [49] Wang H, Cheng P, Wang J, Lv H, Han J, Hou Z, *et al.* Advances in spatial transcriptomics and its application in the musculoskeletal system. *Bone research*. 2025; 13: 54. <http://doi.org/10.1038/s41413-025-00429-w>.
- [50] Zhang Y, Wang J, Yu C, Xia K, Yang B, Zhang Y, *et al.* Advances in single-cell sequencing and its application to musculoskeletal system research. *Cell proliferation*. 2022; 55: e13161. <http://doi.org/10.1111/cpr.13161>.
- [51] Patsalos A, Halasz L, Oleksak D, Wei X, Nagy G, Tzerpos P, *et al.* Spatiotemporal transcriptomic mapping of regenerative inflammation in skeletal muscle reveals a dynamic multilayered tissue architecture. *The Journal of clinical investigation*. 2024; 134: e173858. <http://doi.org/10.1172/JCI173858>.
- [52] Lin W, Li Y, Qiu C, Zou B, Gong Y, Zhang X, *et al.* Mapping the spatial atlas of the human bone tissue integrating spatial and single-cell transcriptomics. *Nucleic acids research*. 2025; 53: gkae1298. <http://doi.org/10.1093/nar/gkae1298>.
- [53] Huang C, Zeng B, Zhou B, Chen G, Zhang Q, Hou W, *et al.* Single-cell transcriptomic analysis of chondrocytes in cartilage and pathogenesis of osteoarthritis. *Genes & diseases*. 2024; 12: 101241. <http://doi.org/10.1016/j.gendis.2024.101241>.
- [54] Fan X, Xu H, Prasadam I, Sun AR, Wu X, Crawford R, *et al.* Spatiotemporal Dynamics of Osteoarthritis: Bridging Insights from Bench to Bedside. *Aging and disease*. 2024; 16: 3233–3267. <http://doi.org/10.14336/AD.2024.1538>.
- [55] Mukherjee A, Abraham S, Singh A, Balaji S, Mukunthan KS. From Data to Cure: A Comprehensive Exploration of Multi-omics Data Analysis for Targeted Therapies. *Molecular biotechnology*. 2025; 67: 1269–1289. <http://doi.org/10.1007/s12033-024-01133-6>.
- [56] Huang J, Liu M, Zhang H, Sun G, Furey A, Rahman P, *et al.* Multi-Omics Integrative Analyses Identified Two Endotypes of Hip Osteoarthritis. *Metabolites*. 2024; 14: 480. <http://doi.org/10.3390/metabo14090480>.
- [57] Kumar MA, Baba SK, Sadida HQ, Marzooqi SA, Jerobin J, Altemani FH, *et al.* Extracellular vesicles as tools and targets in therapy for diseases. *Signal transduction and targeted therapy*. 2024; 9: 27. <http://doi.org/10.1038/s41392-024-01735-1>.
- [58] Cao J, Li C, Cui Z, Deng S, Lei T, Liu W, *et al.* Spatial Transcriptomics: A Powerful Tool in Disease Understanding and Drug Discovery. *Theranostics*. 2024; 14: 2946–2968. <http://doi.org/10.7150/thno.95908>.
- [59] Sun C, Wang A, Zhou Y, Chen P, Wang X, Huang J, *et al.* Spatially resolved multi-omics highlights cell-specific metabolic remodeling and interactions in gastric cancer. *Nature communications*. 2023; 14: 2692. <http://doi.org/10.1038/s41467-023-38360-5>.
- [60] Farbehi N, Alinejad-Rokny H, Chtanova T, Rnjak-Kovacina J. Spatial and single-cell transcriptomics unravel the complex interplay between the body and medical implants. *Cell Biomaterials*. 2025; 1: 100099. <http://doi.org/10.1016/j.celbio.2025.100099>.
- [61] Xie P, Sun AR, Gao F, Li L, Crawford R, Prasadam I. Resolving microenvironment complexity and cellular heterogeneity in osteoarthritis via spatial transcriptomics. *Osteoarthritis Cartilage*. 2026; 34: 332–342. <http://doi.org/10.1016/j.joca.2025.07.007>.
- [62] Gao Q, Yao D, Yin Z, Yu G, Shi B, Wang J. Comprehensive multi-omics approach reveals potential therapeutic targets and agents for osteoarthritis. *Postgraduate medical journal*. 2025; 101: 464–474. <http://doi.org/10.1093/postmj/qgae176>.
- [63] Redkar AS, Surendran A, Rajdev B, Prasad N, Prakash AN, Hazarika D, *et al.* Multi Omics Driven Polypharmacology for Osteoarthritis Joint Regeneration. 2025. (preprint)
- [64] Wu Y, Shi Y, Luo Z, Zhou X, Chen Y, Song X, *et al.* Spatial multi-omics analysis of tumor-stroma boundary cell features for predicting breast cancer progression and therapy response. *Frontiers in cell and developmental biology*. 2025; 13: 1570696. <http://doi.org/10.3389/fcell.2025.1570696>.
- [65] Jenei-Lanzl Z, Maurer S, Brenner RE, Zaucke F, Fuchs M, Rieger J. Emerging concepts and challenges in the development of disease-modifying osteoarthritis drugs – a more refined perspective. *Archives of pharmacological research*. 2025; 48: 467–494. <http://doi.org/10.1007/s12272-025-01551-3>.
- [66] Fan X, Sun AR, Young RSE, Afara IO, Hamilton BR, Ong LJY, *et al.*

- Spatial analysis of the osteoarthritis microenvironment: techniques, insights, and applications. Bone research. 2024; 12: 7. <http://doi.org/10.1038/s41413-023-00304-6>.
- [67] Mobasheri A, Kapoor M, Ali SA, Lang A, Madry H. The future of deep phenotyping in osteoarthritis: How can high throughput omics technologies advance our understanding of the cellular and molecular taxonomy of the disease? Osteoarthritis and cartilage open. 2021; 3: 100144. <http://doi.org/10.1016/j.ocarto.2021.100144>.
- [68] Chin S, Collins JE. Analytic challenges in defining structural phenotypes in OA clinical trials: a perspective. Osteoarthritis imaging. 2025; 5: 100273. <http://doi.org/10.1016/j.ostima.2025.100273>.
- [69] Hunter DJ, Deveza LA. Deconstructing the “types” of osteoarthritis. Osteoarthritis imaging. 2024; 5: 100257. <http://doi.org/10.1016/j.ostima.2024.100257>.
- [70] Reihls E, Fischer A, Gerner I, Windhager R, Toegel S, Zaucke F, *et al.* Beyond symptomatic alignment: evaluating the integration of causal mechanisms in matching animal models with human pathotypes in osteoarthritis research. Arthritis research & therapy. 2025; 27: 109. <http://doi.org/10.1186/s13075-025-03561-4>.
- [71] Kim EH, Jeon S, Park J, Ryu JH, Mobasheri A, Matta C, *et al.* Progressing future osteoarthritis treatment toward precision medicine: integrating regenerative medicine, gene therapy and circadian biology. Experimental & molecular medicine. 2025; 57: 1133–1142. <http://doi.org/10.1038/s12276-025-01481-6>.
- [72] Woods S, Charlton S, Cheung K, Hao Y, Soul J, Reynard LN, *et al.* microRNA-seq of cartilage reveals an overabundance of miR-140-3p which contains functional isomiRs. RNA. 2020; 26: 1575–1588. <http://doi.org/10.1261/rna.075176.120>.
- [73] Lao TD, Le TAH. Data Integration Reveals the Potential Biomarkers of Circulating MicroRNAs in Osteoarthritis. Diagnostics. 2021; 11: 412. <http://doi.org/10.3390/diagnostics11030412>.
- [74] Arjun A, Chellamuthu G, Jeyaraman N, Jeyaraman M, Khanna M. Metabolomics in Osteoarthritis Knee: A Systematic Review of Literature. Indian Journal of Orthopaedics. 2024; 58: 813–828. <http://doi.org/10.1007/s43465-024-01169-5>.
- [75] Mehta O, Vijay A, Gohir SA, Kelly T, Zhang W, Doherty M, *et al.* Serum Metabolome Analysis Identified Amino-Acid Metabolism Associated With Pain in People With Symptomatic Knee Osteoarthritis—A Cross-Sectional Study. The journal of pain. 2023; 24: 1251–1261. <http://doi.org/10.1016/j.jpain.2023.02.023>.
- [76] Fang S, Chen B, Zhang Y, Sun H, Liu L, Liu S, *et al.* Computational Approaches and Challenges in Spatial Transcriptomics. Genomics Proteomics Bioinformatics. 2023; 21: 24–47. <http://doi.org/10.1016/j.gpb.2022.10.001>.
- [77] Sharma D. Artificial intelligence, machine learning and omic data integration in osteoarthritis. Osteoarthritis and cartilage. 2026; 34: 311–320. <http://doi.org/10.1016/j.joca.2025.10.012>.
- [78] Sanches PHG, de Melo NC, Porcari AM, de Carvalho LM. Integrating Molecular Perspectives: Strategies for Comprehensive Multi-Omics Integrative Data Analysis and Machine Learning Applications in Transcriptomics, Proteomics, and Metabolomics. Biology. 2024; 13: 848. <http://doi.org/10.3390/biology13110848>.
- [79] Sokač M, Kjær A, Dyrskjøt L, Haibe-Kains B, Jwl Aerts H, Birkbak NJ. Spatial transformation of multi-omics data unlocks novel insights into cancer biology. eLife. 2023; 12: RP87133. <http://doi.org/10.7554/eLife.87133>.
- [80] Kiessling P, Kuppe C. Spatial multi-omics: novel tools to study the complexity of cardiovascular diseases. Genome medicine. 2024; 16: 14. <http://doi.org/10.1186/s13073-024-01282-y>.
- [81] Hassija V, Chamola V, Mahapatra A, Singal A, Goel D, Huang K, *et al.* Interpreting Black-Box Models: A Review on Explainable Artificial Intelligence. Cognitive Computation. 2024; 16: 45–74. <http://doi.org/10.1007/s12559-023-10179-8>.
- [82] Xu H, Shuttleworth KMJ. Medical artificial intelligence and the black box problem: a view based on the ethical principle of “do no harm”. Intelligent Medicine. 2024; 4: 52–57. <http://doi.org/10.1016/j.imed.2023.08.001>.
- [83] Chan B. Black-box assisted medical decisions: AI power vs. ethical physician care. Medicine, health care, and philosophy. 2023; 26: 285–292. <http://doi.org/10.1007/s11019-023-10153-z>.
- [84] Isa * A, Boukari S, Aliyu M. Towards Closing The Major Barrier of Adoption of Blackbox Models in The Medical Arena Based on Human-Centered XAI Design. International Journal of Emerging Multidisciplinaries: Computer Science & Artificial Intelligence. 2025; 4: 22. <http://doi.org/10.54938/ijemdesai.2025.04.1.468>.
- [85] Muhammad D, Bendeche M. Unveiling the black box: A systematic review of Explainable Artificial Intelligence in medical image analysis. Computational and structural biotechnology journal. 2024; 24: 542–560. <http://doi.org/10.1016/j.csbj.2024.08.005>.
- [86] Alexandrov T, Saez-Rodriguez J, Saka SK. Enablers and challenges of spatial omics, a melting pot of technologies. Molecular systems biology. 2023; 19: e10571. <http://doi.org/10.15252/msb.202110571>.
- [87] Luo Y, Zhao C, Chen F. Multiomics Research: Principles and Challenges in Integrated Analysis. Biodesign research. 2024; 6: 0059. <http://doi.org/10.34133/bdr.0059>.
- [88] Huan C, Li J, Li Y, Zhao S, Yang Q, Zhang Z, *et al.* Spatially Resolved Multiomics: Data Analysis from Monoomics to Multiomics. BME frontiers. 2024; 6: 0084. <http://doi.org/10.34133/bmef.0084>.
- [89] Righelli D, Weber LM, Crowell HL, Pardo B, Collado-Torres L, Ghazanfar S, *et al.* SpatialExperiment: infrastructure for spatially-resolved transcriptomics data in R using Bioconductor. Bioinformatics. 2022; 38: 3128–3131. <http://doi.org/10.1093/bioinformatics/btac299>.
- [90] Peters H, Potla P, Rockel JS, Tockovska T, Pastrello C, Jurisica I, *et al.* Cell and transcriptomic diversity of infrapatellar fat pad during knee osteoarthritis. Annals of the rheumatic diseases. 2025; 84: 351–367. <http://doi.org/10.1136/ard-2024-225928>.
- [91] Mulholland E, Leedham S. Redefining clinical practice through spatial profiling: a revolution in tissue analysis. Annals of the Royal College of Surgeons of England. 2024; 106: 305–312. <http://doi.org/10.1308/rcsann.2023.0091>.
- [92] Liu J, Yang L, Liu D, Wu Q, Yu Y, Huang X, *et al.* The role of multi-omics in biomarker discovery, diagnosis, prognosis, and therapeutic monitoring of tissue repair and regeneration processes. Journal of orthopaedic translation. 2025; 54: 131–151. <http://doi.org/10.1016/j.jot.2025.07.006>.
- [93] Aamir A, Iqbal A, Jawed F, Ashfaq F, Hafsa H, Anas Z, *et al.* Exploring the current and prospective role of artificial intelligence in disease diagnosis. Annals of medicine and surgery. 2024; 86: 943–949. <http://doi.org/10.1097/MS9.0000000000001700>.
- [94] Hassan M, Kushniruk A, Borycki E. Barriers to and Facilitators of Artificial Intelligence Adoption in Health Care: Scoping Review. JMIR Hum Factors. 2024; 11: e48633. <http://doi.org/10.2196/48633>.
- [95] Mohammed S, Malhotra N. Ethical and regulatory challenges in machine learning-based healthcare systems: A review of implementation barriers and future directions. BenchCouncil Transactions on Benchmarks, Standards and Evaluations. 2025; 5: 100215. <http://doi.org/10.1016/j.tbench.2025.100215>.
- [96] He H, Zhao X, Zhang B, Zhao S, Wu Y. Integrated multi-omics and machine learning reveals immune-metabolic signatures in osteoarthritis: from bulk RNA-seq to single-cell resolution. Frontiers in immunology. 2025; 16: 1599930. <http://doi.org/10.3389/fimmu.2025.1599930>.
- [97] Delgado-Sanchez I, Fernandez-Pozo N, Duran I. Standardizing single-cell approaches to osteoarthritis: Toward a comprehensive cellular atlas. Osteoarthritis and cartilage. 2026; 34: 380–383. <http://doi.org/10.1016/j.joca.2025.08.016>.
- [98] Smith KD, Prince DK, MacDonald JW, Bammler TK, Akilesh S. Challenges and Opportunities for the Clinical Translation of Spatial Transcriptomics Technologies. Glomerular diseases. 2024; 4: 49–63. <http://doi.org/10.1159/000538344>.

- [99] Feng S, Li J, Tian J, Lu S, Zhao Y. Application of Single-Cell and Spatial Omics in Musculoskeletal Disorder Research. *International journal of molecular sciences*. 2023; 24: 2271. <http://doi.org/10.3390/ijms24032271>.
- [100] Matsushita Y, Noguchi A, Ono W, Ono N. Multi-omics analysis in developmental bone biology. *The Japanese dental science review*. 2023; 59: 412–420. <http://doi.org/10.1016/j.jdsr.2023.10.006>.
- [101] Hao T, Pei Z, Hu S, Zhao Z, He W, Wang J, *et al*. Identification of osteoarthritis-associated chondrocyte subpopulations and key gene-regulating drugs based on multi-omics analysis. *Scientific reports*. 2025; 15: 12448. <http://doi.org/10.1038/s41598-025-90694-w>.
- [102] Chen B, Wang L, Xie D, Wang Y. Exploration and breakthrough in the mode of chondrocyte death - A potential new mechanism for osteoarthritis. *Biomedicine & pharmacotherapy = Biomédecine & pharmacothérapie*. 2024; 170: 115990. <http://doi.org/10.1016/j.biopha.2023.115990>.
- [103] Kamya P, Ozerov IV, Pun FW, Tretina K, Fokina T, Chen S, *et al*. PandaOmics: An AI-Driven Platform for Therapeutic Target and Biomarker Discovery. *Journal of chemical information and modeling*. 2024; 64: 3961–3969. <http://doi.org/10.1021/acs.jcim.3c01619>.
- [104] Mi H, Sivagnanam S, Ho WJ, Zhang S, Bergman D, Deshpande A, *et al*. Computational methods and biomarker discovery strategies for spatial proteomics: a review in immuno-oncology. *Briefings in bioinformatics*. 2024; 25: bbae421. <http://doi.org/10.1093/bib/bbae421>.

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