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Spatial transcriptomics: challenges and future directions in musculoskeletal diseases

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Purpose of review
This review examines recent advancements in spatial transcriptomics and its current and potential use to advance musculoskeletal (MSK) research. These insights will be vital to address the complexity of MSK diseases and will pave the way for future therapeutic developments.

Recent findings
The advent of next-generation sequencing has significantly improved our understanding of the cellular and transcriptomic heterogeneity in the MSK system. Spatial transcriptomics has revolutionized research allowing in-situ gene expression analyses directly from intact histological sections. Understanding spatial transcriptomes of cells within tissues will shed light into the biological complexity of MSK diseases. Here, we summarize the role of spatial transcriptomics in unveiling molecular mechanisms underlying MSK diseases and the challenges prohibiting its widespread application in MSK research, and opportunities to overcome these challenges.

Summary
We provide a summary of emerging techniques in spatial transcriptomic field and its use in advancing MSK research. Furthermore, challenges in its application in MSK tissues are discussed as well as potential future considerations to improve spatial transcriptomics insights.

Keywords
musculoskeletal diseases, next-generation sequencing, spatial transcriptomics

INTRODUCTION

Musculoskeletal (MSK) diseases comprise various forms of disorders affecting bones, muscles, connective tissues, and whole joints, and include wide variety of diseases such as osteoarthritis, rheumatoid arthritis (RA), psoriatic arthritis (PsA), among others [1]. These MSK diseases burden individuals with chronic pain, stiffness, and loss of function leading to poor quality of life and are a major source of disability worldwide. As such, MSK diseases represent a significant global public health and economic concern, which is projected to rise due to an increasing elderly population [2].

The advent of transcriptomic technologies, techniques to study the transcriptome or the sum of all RNA transcripts from an organism, has greatly advanced our understanding of the biological complexity in both physiological and pathological conditions [3–5]. Notably, the emergence of single cell RNA-sequencing (scRNA-seq) and single nucleus RNA-sequencing (snRNA-seq) technologies has considerably increased insights into cellular heterogeneity underlying MSK diseases and broadened our understanding of many aspects of disease pathologies. RNA sequencing has revolutionized the field by giving researchers the ability to assess contributions of single cells in homeostatic and disease states. In the past decade, there have been notable findings using scRNA-seq and snRNA-seq that have been instrumental in dissecting cellular heterogeneity in MSK tissues affected by osteoarthritis, RA, and PsA [6–8]. However, such methods require intact cells to be dissociated from their native environments and may preclude successful isolation from dense MSK tissues. Most cell dissociation methods can induce transcriptome-wide changes, such as ectopic cell death, stress and/or aggregation [9]. Importantly, these methods cannot retain the spatial organization of cell types within tissues and fall short of capturing tissue architecture complexities, including that of MSK tissues. The location of cells and their position relative to their

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KEY POINTS

- Spatial transcriptomics has revolutionized biomedical research by providing a spatial context to cellular transcriptomic profiles.
- In MSK diseases, ST has been used to investigate cellular subtypes in OA, treatment response and inflammatory signals in RA, and inflammation and immune signals in PsA.
- Technical challenges, mainly associated with samples processing of MSK tissues, have precluded wide-spread adoption of spatial transcriptomics in MSK research.
- Emerging spatial protocols for MSK tissues and other -omics technologies will provide critical insights into MSK biology and clinical research.

neighbours can better inform cell and tissue functions [10]. Within the MSK field, the ability to obtain transcriptomic information from dense MSK tissues can give a better understanding of the unique niches within affected tissues, providing useful information on cellular states and phenotypes regulating diseases. Hence, the need to perform transcriptomics on intact tissue has been a prime motivator in advancements of spatial transcriptomics.

Spatial transcriptomics, named Method of the Year 2020 by Nature Methods [11] , is a cutting-edge technology allowing in-situ gene expression analysis from intact histological sections [12]. This emerging technology has the power to transform our understanding of cellular heterogeneity within tissues. Using bioinformatic approaches, cell heterogeneity within tissues can be evaluated, enabling deeper insights into cell organization, cell-cell interactions, and their functions within intact healthy and diseased tissues [13]. Transcriptomic information within diseased microenvironments, such as regions of inflamed synovium, within degenerated cartilage, or bone marrow lesions, will be useful to gain a deeper understanding of disease pathologies. Here, we provide a discussion of recent advances in spatial transcriptomics technologies and briefly discuss their use in some studies of MSK diseases such as osteoarthritis, RA, and PsA. We also discuss the challenges and opportunities related to the application of spatial transcriptomics in MSK diseases.

SPATIAL TRANSCRIPTOMICS: SEQUENCING AND IMAGING-BASED TECHNOLOGIES OVERVIEW

ST refers to a diverse array of tools that can quantify and localize RNA expression within the spatial context of tissues and cells [12,14–16]. While methods such as in-situ hybridization (ISH) have been used for decades to interrogate spatial gene expression, it is limited to a small number of genes and needs to be manually performed [17]. Rapid advancements in spatial transcriptomics technologies coupled with decreasing costs of next-generation sequencing (NGS) over the past decade have led to significant progress in the field. Multiple technologies have emerged in the past few years, with ever increasing resolution and throughput. Broadly, they can be categorized into (1) NGS-based approaches wherein spatial information is encoded onto RNA transcripts prior to sequencing, or (2) imaging-based approaches whereby mRNAs are imaged *in situ* via microscopy (Table 1) [12,14]. Several recent reviews have comprehensively summarized current spatial transcriptomics technologies, their technical features, and avenues for advanced bioinformatics analyses [12,13,15,16].

Sequencing-based spatial platforms provide up to whole-transcriptome coverage and thus, are well suited for hypothesis-generating studies and/or differential gene expression analyses. Additionally, sequencing-based spatial transcriptomics (herein termed NGS-ST) technologies allow for larger capture areas, which is particularly beneficial to study MSK diseases with defined and compartmentalized pathology, such as in the joints of osteoarthritis and RA. Spatial transcriptomics analysis of joint tissues, such as complete capture of full-thickness cartilage, can reveal unprecedented insights underlying disease heterogeneity within discrete spaces. Notably, NGS-ST tools have higher throughput compared to imaging-based strategies. Importantly, adoption of NGS-ST techniques is more straightforward to implement as most tools do not require specialized imaging instruments nor lengthy imaging processes. Conversely, imaging-based spatial transcriptomics technologies benefit from higher sensitivity and resolution, with some techniques achieving transcriptomic profiles at a subcellular level [12]. The trade-off of this higher spatial resolution is lower throughput in transcript detection; however, its targeted profiling is apt for hypothesis-testing or clinical studies.

SPATIAL TRANSCRIPTOMICS TECHNOLOGIES: LIMITATIONS AND CHALLENGES

Despite the wide range of commercially available spatial transcriptomics technologies and their rapid evolution, no single method can address all desired parameters, namely the ability to profile at a single cell level and provide efficient mRNA recovery. Each of the available tools described above has unique capabilities and trade-offs.

Table 1. Overview and comparisons of spatial transcriptomics methods

Type	Method	Spatial resolution	Coverage	Sample compatibility	References
In-situ Capture	Stahl method of ST	Visium HD 2 µm resolution (Single Cell to Subcellular)	Gene Panel (18 000+) or Transcriptome-wide (via polyT-capture)	Fresh Frozen, Fixed, Frozen, FFPE	[18,19]
		Visium, 55 µm resolution (Multicellular)	Transcriptome-wide	Fresh Frozen, Fixed Frozen, FFPE	
In-situ Capture	Stereo-seq	200–500 nm resolution (Subcellular)	Transcriptome-wide	Fresh Frozen, FFPE	[20]
In-situ Capture	High-definition spatial transcriptomics for in situ tissue profiling	2 µm resolution (Single Cell)	Transcriptome-wide	Fresh Frozen	[21]
In-situ Capture	Slide-seq	10 µm (Single Cell)	Transcriptome-wide	Fresh Frozen	[22]
In-situ Capture	Slide-seqv2	10 µm (Single Cell)	Transcriptome-wide	Fresh Frozen	[23]
In-situ Capture	Pixel-seq	1 µm (Subcellular)	Transcriptome-wide	Fresh Frozen	[24]
Imaging-based/ISH	smFish	Subcellular	Gene Panel (18 000+)	Fresh Frozen, FFPE	[25,26]
Imaging-based/ISH	CosMx SMI	10 µm (Single Cell)	1K–6K	Fresh Frozen, FFPE	[27]
Imaging-based/ISH	RNAscope	Single molecule	up to 50K	Fresh Frozen, Fixed, Frozen, FFPE	[28]
ROI selection/In-situ microdissection	GeoMx DSP	10 µm (Single Cell within ROI)	Gene Panel (18 000+)	Fresh Frozen, Fixed, Frozen, FFPE	[29,30]
LCM and scRNA-seq	Geo-seq	Multicellular	Transcriptome-wide	Fresh Frozen	[31]
In-situ Microdissection	Tomo-seq	Multicellular	Transcriptome-wide	Fresh Frozen	[32]

DSP, digital spatial profiler; FFPE, formalin-fixed paraffin-embedded; Geo-seq, geographical position sequencing; ISH, in-situ hybridization; LCM, laser capture microdissection; Pixel-seq, Polony-indexed library-sequencing; ROI, region of interest; scRNA-seq, single-cell RNA sequencing; smFISH, single-molecule fluorescent in-situ hybridization; SMI, single-molecule imaging; ST, spatial transcriptomics; Stereo-seq, spatial enhanced resolution omics-sequencing.

(1) Single-cell resolution – The prevailing issue with spatial transcriptomics approaches is they lack the ability to capture transcripts at a true single-cell resolution. There is considerable work being done to improve spatial resolution either by increasing density of spatial probes on glass slides, like with Visium HD NGS-STplatform, or through decreasing bead diameter, as with Slide-seq v2 NGS-ST (Table 1). On a practical level, however, these emerging NGS-ST tools capture mRNA from single cell-sized areas, as cells often straddle multiple barcoded arrays or pixels. Refining the resolution down to a 'true' single cell level is necessary to gain deeper insights into the spatial gene regulation inside single cells. However, high resolution techniques are analytically challenging, as increasing resolution introduces issues such as noise, data sparsity, and difficulty inferring cell boundaries, while requiring more computational power for analyses [33]. As such, integration of spatial transcriptomics data with community driven cell segmentation tools is crucial to create a comprehensive map of cell types *in situ* and accurately quantify cell type composition [34]. The most widely used deconvolution method is based on unsupervised clustering and is derived from scRNA-seq analyses [35]. However, this does not consider mixed data from multiple cell types. There is a thriving ecosystem of community-based tools and other methods that have been developed to pare out cell types from mixed transcriptomic data from overlapping cells that are continuously evolving alongside spatial transcriptomics developments [36,37]. Moreover, commercially available spatial transcriptomics tools, such as Visium HD NGS-ST and Stereo-seq NGS-ST, provide H&E-based cell segmentation as part of their analytical pipelines [38,39]. An alternative strategy is to integrate spatial transcriptomics data with scRNA-seq to maximize resolution while preserving spatial information [40]. ScRNA-seq data compensates for lower resolution and depth in

current spatial transcriptomics methods and enables further analyses, such as cell-cell interactions and pseudo-time trajectory, to deeply characterize single cells in tissue.

(2) Species compatibility – Stereo-seq and Visium 3' gene expression assays perform spatial mapping through polyadenylated RNA transcripts offering an unbiased approach. These may be useful for the discovery of novel gene expression patterns and cellular states that targeted approaches may miss. This RNA capture approach is also well suited across a wide range of species, including humans, animals, and plants. Visium, Stereo-seq and Pixel-seq have similar mRNA recovery rates with differences in tissue size compatibility (Table 1). However, Visium 3' HD NGS-ST approach is limited to fresh-frozen tissue sections due to more stringent RNA quality requirements, which may not be compatible with hardened MSK tissues.

(3) Sample compatibility – While formalin-fixed paraffin-embedded (FFPE) tissue preservation is a widely adopted practice in pathology for long-term storage, most spatial transcriptomics methods are currently only compatible with fresh, flash-frozen tissue. This is likely due to RNA degradation during the FFPE preservation process, which impedes RNA capture [41]. Consequently, RNA detection is lower in FFPE tissue sections compared to fresh tissues due to crosslinking and RNA fragmentation during formalin fixation. Fresh, flash-frozen tissues have inherently higher RNA quality and thus can provide a more comprehensive RNA profile. This presents a challenge with MSK tissues, such as bone and cartilage, which necessitates decalcification and FFPE processing. Visium v1 and HD probe assays, in addition to Stereo-seq technology, support a myriad of sample preparations, including flash-frozen and FFPE samples [18,42]. Another challenge for nearly all spatial transcriptomics methods is the susceptibility of certain tissue sections, particularly hardened tissues like cartilage and bone, or less adherent tissues such as skin, to tearing when placed on a slide or array for spatial profiling, leading to RNA loss and lower RNA detection efficiency. Optimizing section thickness or incorporating replicates is recommended.

(4) Resolution – Overall, NGS-ST approaches have lower resolution and gene-detection efficiency than imaging-based tools [16]. Due to the limited capture size of arrays, some complex structures and lower abundant genes pose a challenge via NGS-ST methods. Depending on the efficacy of cell segmentation, study of rare cells can be difficult as they cannot be profiled individually even at a single-cell scale. It is likely that the transcriptome from these rare cells is captured and mixed with its surrounding neighbours. Of note, lower abundance transcripts, as well as noncoding and small RNAs, may not be captured without added sequencing costs. Additionally, lowly transcribed genes, such as certain transcription factors, may be difficult to detect with the relatively low detection efficiency of current era spatial transcriptomics tools. Furthermore, poly-A based capture chemistries are more inclined to detect highly expressed genes. Therefore, it is recommended to perform experiments, such as immunohistochemistry or in-situ hybridization, in parallel with spatial profiling if rare cell types or lowly expressed genes are the area of study.

(5) Tissue size – The capture area or the field-of-view dictates the tissue size and type that is feasible for study. Larger tissue sections remain a challenge, with size constraints of current spatial transcriptomics tools. Out of all NGS-ST technologies, Stereo-seq is well suited for larger tissues with capture areas of 10 x 10 or 20 x 30 mm [42]. However, underpinning this improvement is increasing sequencing and computational costs. The larger tissue sections and increasing cellular resolution require more data points, and thus more sequencing depth and higher computational power are required to visualize and interpret the data.

A comprehensive technical comparison was performed by Fu *et al.* [43] whereby current era NGS-ST technologies were evaluated based on sequencing resolution, depth, and molecular diffusion rates. They developed a benchmarking pipeline, termed cadasSTre, for cross-comparison of 11 sequencing-based spatial transcriptomics methods. Their analysis showed that different spatial transcriptomics technologies have varying capabilities with respect to capture efficiency, with Stereo-seq showing the highest capturing capability with its large array size. Notably, their analyses demonstrated the stringent requirement to mitigate blood contamination during sample preparation and tissue sectioning.

SPATIAL TRANSCRIPTOMICS OF OSTEOARTHRITIS, RHEUMATOID ARTHRITIS, AND PSORIATIC ARTHRITIS TISSUES

While spatial transcriptomics is an emergent technique, many fields in biomedical research have readily adopted spatial transcriptomics tools [13,44].

Notably, spatial transcriptomics has been used to identify spatial gene patterns involved in mechanisms of cancer, where tissue structure is significantly altered [45] or where the disease is known to be significantly heterogeneous [46]. By the same token, spatial transcriptomics is particularly useful for studying knee osteoarthritis joint tissues, whose etiology is multifactorial in nature [47]. Its heterogeneous pathology results in a wide range of clinical characteristics influencing disease progression and treatment response. Thus, effective interventions need to be curated according to disease subgroups (endotypes). Recent review articles have comprehensively summarized applications of spatial profiling to osteoarthritis [48▪▪], RA [49], and PsA [50] tissues, and their attempts to further refine the molecular complexity of these MSK diseases. Briefly, in the field of osteoarthritis, spatial transcriptomics has deepened our understanding of a number of knee joint tissues, including novel insights into chondrocyte subtypes and their organization within knee articular cartilage [51▪▪]. Spatial transcriptomics has also been utilized in RA research, primarily to understand distributions of immune cells in afflicted tissues including synovium, but also in a clinical context for the prediction of treatment response or resistance to two commonly used biological therapies for RA: rituximab (RTX) and tocilizumab (TCZ) [52]. Additionally, this technology has been used to elucidate differences in cellular niches and molecular mediators in PsA skin and synovium, providing insights into the immunopathogenesis of PsA [53]. Table 2 outlines significant findings in key spatial transcriptomics studies of osteoarthritis, RA, and PsA. Overall, these studies underscore the utility of spatial transcriptomics technologies in understanding spatial changes associated with MSK diseases for future improved diagnostic and therapeutic modalities.

To date, spatial transcriptomics has been readily applied to study mouse and early embryonic MSK tissues, but its use in adult tissues has been limited. The paucity of groups using spatial transcriptomics may be due, in part, to the technical challenges associated with MSK tissues, namely difficulties in obtaining high-quality sections. Optimization of tissue preparation is required to preserve high quality RNA. MSK tissues, such as bone, cartilage, and other joint tissues, contain dense, extracellular matrix-rich tissues, which may even be mineralized, with relatively lower cellularity, placing constraints on RNA detection rates [76]. Furthermore, in contrast to soft tissues, an extra decalcification step is often required due to the hardened nature of these samples [77]. However, this extra sample processing step often utilizes strong acids, such as hydrochloric or nitric acid, that lead to RNA degradation. Decalcification with milder reagents, such as EDTA significantly minimizes degradation of RNA quality and increases RNA recovery [78]. Of note, RNA degradation is significantly increased in cartilage samples obtained from osteoarthritis and RA patients, underlying the importance of preserving RNA quality during tissue processing [79]. Additionally, as mentioned in the previous section, these tissues are prone to detaching from slides, due to high proteoglycan content and tissue swelling [79]. FFPE tissue preservation improves tissue adhesion to slides compared to flash-frozen samples [80]. However, this widely used histological technique minimizes recovery of good quality RNA due to increased RNA cross-linking [81]. Comprehensive protocols for preparing FFPE samples from murine MSK tissues [82] and human osteochondral tissues [83] have recently been developed, and may be a transformative development for wider implementation of spatial transcriptomics in MSK research [76,82,84]. Thus, spatial transcriptomics technologies that are compatible with FFPE samples is a critical prerequisite for MSK research. Additionally, species compatibility is a consideration with some spatial transcriptomics platforms that are currently restricted to human and mouse samples, or evolutionarily similar species. Technologies which allow for species-agnostic spatial profiling, such as those that use poly-A based mRNA capture chemistry, allow for greater flexibility in research using model organisms. Another challenge is the limited capture area of current spatial transcriptomics technologies, which restricts the ability to spatially profile the entire human joint tissue. These technical constraints and the relative novelty of spatial transcriptomics tools limit a more widespread adoption of spatial transcriptomics to study MSK tissues. Adoption of modified techniques that have been successful in other challenging tissues, such as skin lesions [52,85], may also provide solutions for MSK tissues.

THE FUTURE OF SPATIAL TECHNOLOGIES FOR MUSCULOSKELETAL RESEARCH

Emergent spatial transcriptomics tools offer another layer to understand how gene expression is linked to tissue structure and unveils complexity inherent in organisms (Fig. 1). Despite the remarkable evolution in these tools, there remain avenues for growth.

(1) The ability to perform simultaneous analysis of multiple modalities, such as transcriptome, proteome, epigenome, and metabolome, using the same tissue sections or in adjacent sections, will improve our understanding of cellular structure

Table 2. Summary of spatial transcriptomics technologies used in osteoarthritis, rheumatoid arthritis, and psoriatic arthritis studies

ST type	Tissue	MSK disease	Species	Sample preparation	Key findings	References
In-situ capture; Visium	Healthy and degenerated ACL	OA	Human	FFPE	Reported 10 fibroblast subclusters in ACL in spatial proximity to endothelial and immune cells	[54]
Re-annotation (RA synovial membrane spatial profile and spatrio for single cell projection)	Knee Synovium	OA	Human	Imported Database	Identified increased immune myeloid and lymphoid cells in OA synovial lining	[55]
Imaged-based Spatial Capture (Geo-seq) with (LCM coupled with scRNA-seq)	Knee articular cartilage	OA	Human	Fresh frozen; OCT blocks	Identified 2 novel chondrocyte populations (pre-inflammatory and inflammatory subpopulations)	[51▪▪]
Re-annotation of [51▪▪]	Knee cartilage	OA	Human	Fresh Frozen; OCT blocks	ST analysis identified distinct cell populations in inflamed OA cartilage	[56]
In-situ capture; Visium	Knee Infrapatellar Fat Pad	OA	Human	Fresh frozen; OCT blocks	Reported 5 subclusters of fibroblasts within KOA fat pad	[57]
GeoMx-DSP	Knee Synovium	OA	Human	Fresh frozen; OCT blocks	Identified association of immune exhaustion and loss of immune regulatory macrophages with worse pain	[58▪▪]
In-situ capture; Visium	Hip synovium	OA	Human	FFPE	Putative role of sublining FLS and fibrotic chondrocytes in molecular mechanisms by which synovial cells promote hip OA pathogenesis from FAI	[59]
In-situ capture; Visium	Bone/Femoral Head	OA	Human	FFPE	Identified spatial gradient of distinct gene expression and signalling pathways stemming from trabecular bone	[60]
In-situ capture; Visium	Hind limbs	OA	Mouse	Fresh frozen; OCT blocks	Identified three chondrocyte subclusters in E18.5 mouse embryonic knee cartilage	[61]
In-situ capture; Visium	Knee joint Synovial fluid	OA	Mouse	FFPE	Demonstrated fat-secreted factors are required for KOA development	[62]
In-situ capture; Visium	Knee joint	OA	Mouse	FFPE	ST of 5-week mice post DMM surgery, Top GO terms were associated with DNA organization, particularly histone-related functions, suggesting potential changes in chromatin structure and transcriptional regulation	[63]
In-situ spatial barcoding (Stahl method)	Knee or Hip Synovium	RA	Human	Fresh frozen; OCT blocks	ST of RA and SpA synovium revealed an abundance of central memory T cells in RA synovium and effector memory T cells in SpA synovium	[64]

Table 2 (*Continued*)

ST type	Tissue	MSK disease	Species	Sample preparation	Key findings	References
In-situ capture; Visium	Synovium	RA	Human	Fresh frozen; OCT blocks	Spatial analysis revealed a combination of TNF, IFN-γ, and IL-1β exposures drive 4 distinct FLS subtypes in RA synovium	[65]
In-situ capture; Visium	Knee Synovium	RA	Human	Fresh frozen; OCT blocks	ST analysis revealed high expression of the IFN-α response pathway lining FLS within relapsed RA patients	[66]
In-situ spatial barcoding (Stahl method)	Knee or Hip Synovium	RA	Human	Fresh frozen; OCT blocks	Synovial tissue from two patient groups, seropositive and seronegative RA, underwent ST. Identified regions where infiltrating leukocytes organize into TLO cell-dense areas. Leukocyte migration pathways are enriched within TLOs	[67]
In-situ capture; Visium	Synovium	RA	Human	Fresh frozen; OCT blocks	ST analysis revealed novel subset of activated sublining ITGA5+, which potentially modulates pro-inflammatory response in RA synovium	[68]
GeoMX-DSP	Synovium	RA	Human	FFPE	Investigated the efficacy of two commonly used biological therapies for RA: RTX and TCZ. ST analysis revealed that the expression of FAP, a fibroblast marker, was increased in the synovial sublining of nonresponders	[52]
In-situ capture; Visium	Synovium	RA	Human	Fresh frozen; OCT blocks	Identified S-TPH within TLS in synovial tissue where they interact with B-cells promoting antibody generation	[69]
In-situ capture; Visium	Synovium	RA	Human	FFPE; Formalin	Use of Deep Topic Modelling to identify disease-relevant cellular communities	[70]
Imaged-based spatial capture	CosMx	RA	Human	FFPE	Identified inflammatory DC3s in the hyperplastic lining of RA promoting synovitis	[71]
In-situ capture; Visium	Knee or Wrist Synovium	RA	Human	Fresh frozen; OCT blocks	ST analysis revealed autoreactive plasma cell differentiation in early RA synovium	[72]
In-situ capture; Visium	PLN	RA	Mouse	Fresh frozen; OCT blocks	Identified PLN regions with increased Ig production in TNF-Tg mice with advanced RA	[73]
In-situ capture; Visium	Synovium	PsA and RA	Human	Fresh frozen; OCT blocks	ST analysis revealed CD200+ fibroblasts form mesenchymal network regulating inflammation and tissue repair	[74]
In-situ capture; Visium	Skin	PsA	Human	Fresh frozen; OCT blocks	ST analysis revealed repositioning of immune cells into upper skin layers within Pso lesions	[53]

Table 2 (*Continued*)

ST type	Tissue	MSK disease	Species	Sample preparation	Key findings	References
In-situ capture; Visium	Skin	PsA	Human	Fresh frozen; OCT blocks	Identified HIF1α is activated in PsA epidermis	[75▪▪]

ACL, anterior cruciate ligament; DC, dendritic cell; DMM, destabilization of the medial meniscus; FAI, femoroacetabular impingement; FAP, fibroblast activation protein; FFPE, formalin-fixed paraffin-embedded; FLS, fibroblast-like synoviocytes; Geo-seq, geographical position sequencing; GO, Gene Ontology; HIF1α, hypoxia-inducible factor 1α; IFN, interferon-gamma; Ig, immunoglobulin; IL, interleukin; KOA, knee osteoarthritis; LCM, laser capture microdissection; MMP, matrix metalloproteinase; MSK, musculoskeletal; OA, osteoarthritis; OCT, optimal cutting temperature; PLN, popliteal lymph node; PsA, psoriatic arthritis; PsO, psoriasis; RA, rheumatoid arthritis; RTX, rituximab; scRNA-seq, single-cell RNA sequencing; SpA, spondylarthritis; ST, spatial transcriptomics; S-TPH, stem-like peripheral helper cell; TCZ, tocilizumab; TLO, tertiary lymphoid organs; TLS, tertiary lymphoid structures; TNF, tumour necrosis factor; TNF-Tg, tumour necrosis factor transgenic mouse model.

and function [86]. In fact, spatial multiomics was named one of the seven technologies to watch by Nature in 2022 [87]. One such example is DBiT-seq, which allows simultaneous profiling of the transcriptome and proteome from the same tissue section [88]. This technology is still in development, with spatial resolution limited to 10 μm capture areas. Likewise, spatial-CITE-seq and STARmapPLUS also allow for mapping of transcriptomes and proteomes, albeit with lower resolution and coverage than current spatial transcriptomics techniques [89,90]. Of note, spatial proteomics can provide a more practical option for studying bone and cartilage, which as stated above, require processing that often results in RNA degradation [91]. However, decalcification does not generally affect the antigenicity of proteins and thus research using

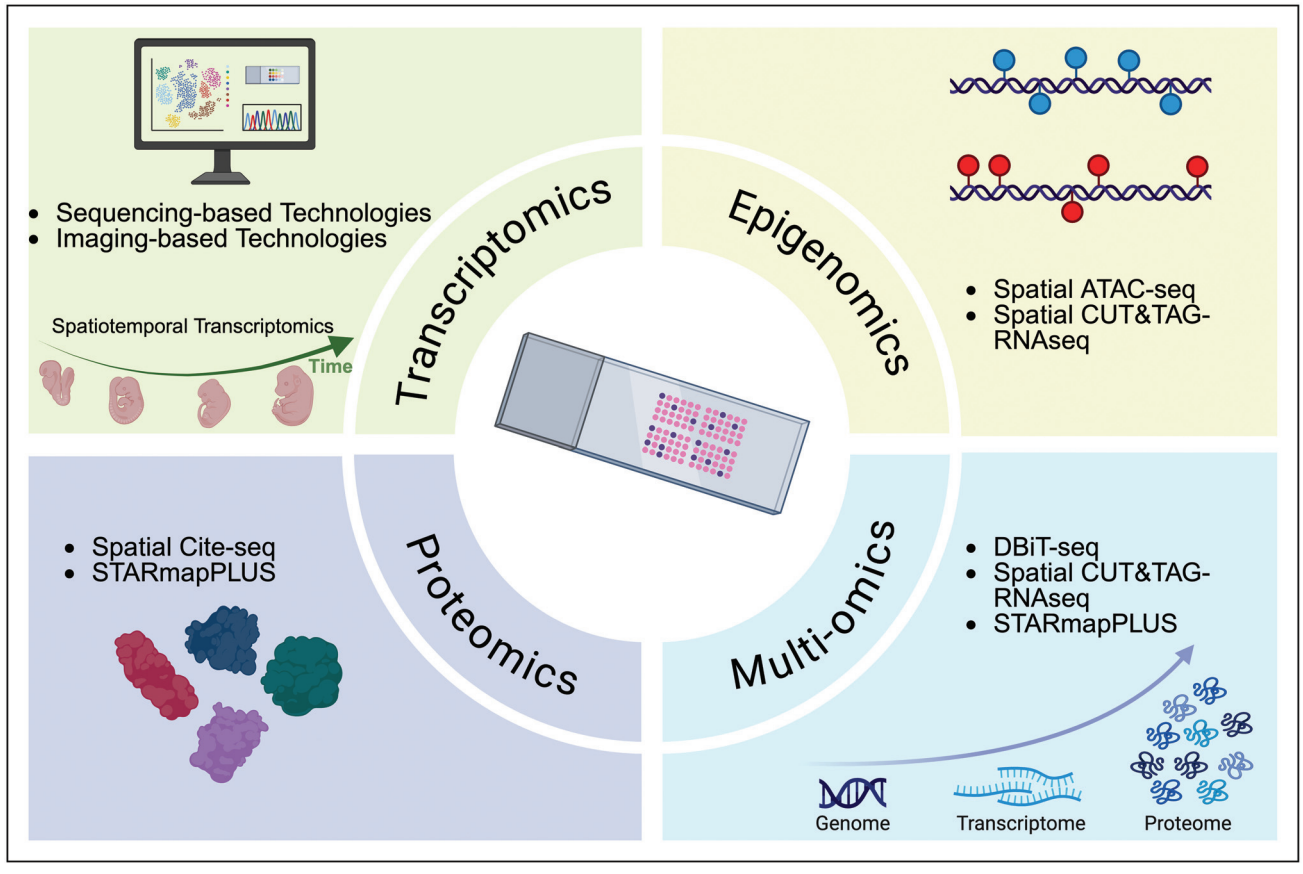

FIGURE 1. Applications of spatial –omics in musculoskeletal diseases. The emergence of Spatial –Omics technologies and methods have enabled the study of transcriptomes, proteomes, epigenomes, and multiomes, among others (such as the metabolome), whilst preserving tissue context at a spatial level. Current methods to perform Spatial Transcriptomics are described in detail in Table 1. Created in BioRender (https://BioRender.com/wgabpzd).

antibody-based spatial proteomics may be more feasible [92]. Comprehensive profiling of transcriptomes combined with epigenomic measures, such as chromatin accessibility using Spatial ATAC-RNAseq, or histone modifications using Spatial CUT&Tag-RNAseq, will enable further insights into the complexity of gene regulatory networks [93,94]. Linking transcription with upstream epigenetic regulatory mechanisms may reveal cell-type specific interactions driving further insights into disease heterogeneity and pathology. One such study used scRNA-seq and spatial proteomics to comprehensively profile human bone marrow niche unveiling novel cellular interactions driving haematopoiesis [95]. While integration of multiple modalities presents practical bioinformatic challenges, novel tools, such as SpatialData, have been developed to analyse spatial multiomics data [96].

(2) Currently, spatial transcriptomics technologies can only capture transient states of tissues. The transcriptomic snapshot belies the molecularly dynamic nature of cells. Spatiotemporal transcriptomic studies have been conducted using independent samples from different time points, which presents a challenge in heterogeneous tissues, or more commonly through mathematically inferring temporal information using computational approaches [16]. The models generated by these tools should be interpreted as statistical assumptions rather than an authentic transitional path of cells. Live-seq is a novel technique providing a temporal dimension to scRNA-seq, allowing a continuous molecular analysis of live cells [97]. Further developments to add spatial context will be necessary to capture spatiotemporal transcriptomics.

(3) Tissues and their microenvironment exist in three-dimensional structures [98,99]. However, current spatial transcriptomics methods acquire transcriptomic data in two-dimensional planes, masking the complexity of the 3D dynamic cellular assembly and organization regulating cell and tissue function. In fact, most spatial transcriptomics technologies can only profile thin sections, typically 5–20 μm thick. Joint cartilage is composed of a dense extracellular matrix (ECM) with varying chondrocyte density and collagen fibre arrangement among its four layers [100]. Deep-STARmap and Deep-RIbomap are two novel techniques enabling volumetric multicell layer in-situ quantification of the transcriptome and proteome, respectively, in 200 μm thick tissue sections [101]. Capturing the 3D transcriptomic profile across multiple cell layers or through the zonal organization of cartilage may reveal further insights into spatial chondrocyte function and regulation.

CONCLUSION

Understanding the underlying mechanisms driving MSK disease pathogenesis and progression has become increasingly crucial for the development of therapeutic strategies, which are essential for mitigating the individual and societal burdens posed by this group of diseases. There is a pressing need for additional research leveraging spatial transcriptomics technologies to improve the understanding of MSK disease mechanisms, advance target identification, and develop novel treatments. This article has highlighted novel spatial transcriptomics technologies and how they can be used to elucidate spatially localized cell types and mechanisms driving MSK disease pathology. While spatial transcriptomics holds great promise to provide a deeper understanding of MSK biology, improvements to spatial resolution and efficiency of RNA capture will be crucial for future development of clinical diagnostic or therapeutic approaches.

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Conflicts of interest

M.K. is an officer and board member for the Osteoarthritis Research Society International and serves on the scientific advisory board for Chiron Inc. and GlaxoSmithKline. Other authors declare no competing interests.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

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