

Response to Reviewer 2 Comments

In this review article, the authors tried to summarize the utility, present knowledge and future perspectives of single-cell and spatial omics in the fields of bone, cartilage, muscle and tendon research and relevant clinical sciences. This is a unique attempt to overview a number of studies belonging to a wide range of medical and biological category and thus precious.

We thank the reviewer for this comment.

However, several points need to be addressed.

Major points

1) The title does not correctly represent the contents of the article. Application of something to disorders suggests therapeutic utility of something for disorders. For example, application of single-cell and spatial omics in musculoskeletal disorder research may be better. Relevant phrases in Abstract should be reconsidered as well.

Response:

We thank the reviewer for this suggestion. We have modified the title, abstract and several phrases in the body part.

Title:

“Application of Single-Cell and Spatial Omics in Musculoskeletal Disorder Research”

On line 20:

“However, the application of these techniques in research about musculoskeletal disorders is still limited.”

On line 28:

“Finally, the application potential and future development directions of single-cell and spatial omics in research about musculoskeletal diseases are discussed.”

On line 176:

“As the new single-cell protocols continuously developed, more studies applied the protocol to characterize healthy or diseased human samples”

On line 249:

“3. Single-cell and spatial omics were applied to the characterization of various musculoskeletal diseases”

On line 263:

“Application of single cell and spatial omics to decode bone-related diseases”

On line 267:

“Table 3. Application of single cell and spatial omics to decode cartilage-or-joints-related diseases”

On line 273:

“Table 4. Application of single cell and spatial omics in muscles-or-tendons-related diseases”

On line 340:

Current single-cell studies applied to the characterization of mice hindlimb and calvarium focused on molecules, pathways, and even temporal and spatial nature that cause malformations.

On line 396:

“Future researches can apply the single-cell methods to the characterization of other developmental diseases in other organs.”

On line 553:

“...spatial transcriptomics was applied to profile synovial samples from RA and spondyloarthritis (SpA) patients [20].”

On line 625:

“Single-cell analysis recognized several muscle cell types, especially in MuSCs [151], and this technique was applied to decode muscle injury and regeneration.”

On line 729:

“It is also crucial to apply single-cell studies to the characterization of higher animals like dogs, sheep, and pigs.”

On line 830:

“Besides, therapy-related single-cell research also has a huge scope for exploration. With the wide application of bioactive materials to cure the musculoskeletal disorders,...”

2) Figure 1 needs reconsideration. First, a number of abbreviations in the legend, such as FCs, preFCs, RegCs, RepCs, preHTCs and so on, do not appear in the figure body. Second, the three categories shown in Prussian blue should be represented by the words in the same category: for example, Bone, Cartilage or joint, Muscle or tendon. Third, all of the words below "Disorders" and "Cells" should be enlarged.

Response:
 Thanks for your comment. We have modified the legend, words and word sizes in Figure 1 according to your suggestion.
 You can see these revisions on line 287 of the manuscript.

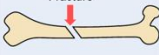
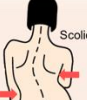
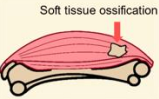
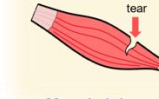
Musculoskeletal System Conditions					
Bone		Cartilage or joints		Muscles or tendons	
Disorders	Cells	Disorders	Cells	Disorders	Cells
 Bone injury	MSCs Osteoblast Osteoclast SSPCs Chondrocytes	 Cartilage injury	Fibroblast Lgr5+ interzone cells	 Tendon injury	TSPCs FAPs
 Skeletal dysplasia	MSCs Osteoblast Osteoclast CPCs	 Osteoarthritis	Special Chondrocyte clusters	 Tendinopathy	Microfibril associated tenocytes Endothelium T cells
 Heterotopic ossification	SSPCs TSPCs	 Rheumatoid Arthritis	Fibroblasts Immune cells	 Muscle injury	FAPs MuSCs Immune cells
 Osteoporosis	Osteoclast Immune cells	 IVD degeneration	CPCs HomCs NPCs NPPCs	 Muscular dystrophy	FAPs

Figure 1. Musculoskeletal system conditions and their involved cell populations have been studied by single-cell or spatial omics techniques. MSCs: mesenchymal stem cells; SSPCs: skeleton stem/progenitor cells; CPCs: Cartilage progenitor cells; TSPCs: tendon stem/progenitor cells; HomCs: homeostatic chondrocytes; FAPs: fibro-adipogenic progenitors; MuSCs: muscle stem cells.

3) The table also contains multiple problems. Although this is the only table in this article, it is labeled Table 2. Involvement of too many columns and lines makes this table hard to follow. This table needs to be split into 3 pieces; the first one for "osteogenic (or bone)", the second one for "chondrogenic or joint (or cartilage or joint)", and the third for "Muscle and tendon"; then the first column can be removed. Columns for "Resources of samples" and "Methods" may be omitted, since details are described in the text. As a result, three concise tables that are easy to follow can be obtained.

Response:

Thanks for your comment. In fact, we have "Table 1. Common cell types and markers of the musculoskeletal system". Table 2 has been divided into Table 2~4 for easy reading.

Table 2. Application of single cell and spatial omics to decode bone-related diseases

Disease	Animal	Location	Phenotype driving gene/materials in mice models	Important affected cell types	Reference
Bone injury and regeneration	Mice	Rib	Smo	Cxcl12-expressing SSPCs	Serowoky et al., 2022 [65]
	Mice	Frontal bones	p75 (<i>Ngfr</i>)	Itgb1 mesenchymal, Il1a, Il10, and Tnf-expressing mesenchymal, immune cells	Xu et al., 2022 [70]
	Mice	Long bone	-	Osteoblast lineage cells, chondrocytes, fibroblasts, Fabp5+ Mmp9+ Septoclasts	Sivaraj et al., 2022 [74]
Congenital Skeletal dysplasia	Mice	Hindlimb	-	HC	Wang et al., 2022 [79]
	Mice	Calvarium	TrkA	Mesenchymal progenitor cells	Tower et al., 2021 [19]
Adolescent idiopathic scoliosis	Human	Spinal cancellous bone tissues	-	MSC-IGFBP5, CPC-PCNA, OC-BIRC3	Yang et al., 2021 [52]
Heterotopic ossification	Mice	Muscle	rhBMP2-Matrigel mixtures preimmune antibody (referred to as BMP2/IgG) or neutralizing activin A antibody (BMP2/nActA.Ab)	Sox9-expressing skeletal progenitors, Acan- and Col2a1-expressing clusters	Mundy et al., 2021[92]
	Mice	Achilles tendon	-	Prg4+ TSPC	Tachibana et al., 2022 [93]

Osteoporosis	Human	Femoral head	-	Osteoclasts and immune cells	Wang et al., 2022 [54]
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SSPCs: skeleton stem/progenitor cells, HC: hypertrophic chondrocytes, MSC: mesenchymal stem cell, CPC: chondrocyte progenitor cell, OC: osteoclast, TSPCs: tendon stem/progenitor cells.

Table 3. Application of single cell and spatial omics to decode cartilage-or-joints-related diseases

Disease	Animal	Location	Phenotype driving gene/materials in mice models	Important affected cell types	Reference
Chondrogenic regeneration	Mice	Digit tip	Ectopic BMP9	Fibroblasts	Yu et al., 2022 [113]
	Human	Cartilage	-	ProC, FC, preHTC, CPC	Ji et al., 2019 [56]
	Human	Cartilage	-	FC, preFC, RegC, RepC, and preHTC	Chou et al., 2020 [57]
Osteoarthritis	Human	Cartilage	-	RegC	Wang et al., 2021 [120]
	Human	Cartilage	-	StrC (containing a ferroptotic cluster), RegC	Lv et al., 2022 [122]
	Human	Synovitis	-	Immune cells and fibroblasts	Chou et al., 2020 [57]
Kashin-Beck disease	Human	Cartilage	-	HomC, MTC	Wang et al., 2021 [120]
	Human	Synovitis	-	CD55 ⁺ fibroblast, CD90 ⁺ fibroblast	Stephenson et al., 2018 [29]
Rheumatoid arthritis	Human	Synovitis	-	THY1(CD90) ⁺ HLA-DRAhi sublining fibroblasts	Zhang et al., 2019 [135]
	Human	Synovitis	-	Immune cells fibroblasts	Carlberg et al., 2019 [20]

IVD degeneration	Human	IVD	-	CPC, HomC, and other chondrocyte subsets	Zhang et al., 2021 [138]
	Human	IVD	-	IR NPC, FC NPC	Ling et al., 2021 [139]
	Human	IVD	-	-	Cherif et al., 2022 [140]
	Human	IVD	-	NP progenitor cells	Gan et al., 2021 [55]

proC: proliferative chondrocytes, FC: fibrochondrocytes, preHTC: prehypertrophic chondrocytes. CPC: cartilage progenitor cells, preFC: prefibrochondrocytes, RegC: regulatory chondrocytes, RepC: reparative chondrocytes, StrC: stressed chondrocytes, HomC: homeostatic chondrocytes, MTC: mitochondrial chondrocytes, NP: nucleus pulposus, IR NPC: Inflammatory response NP cells, FC NPC: fibrocartilaginous NP cells.

Table 4. Application of single cell and spatial omics in muscles-or-tendons-related diseases

Disease	Animal	Location	Phenotype driving gene/materials in mice models	Important affected cell types	Reference
Muscular dystrophy	Mice	Single-cell analysis: hind limb, further experiment: diaphragms	mdx	Tie2 ^{high} FAP, Vcam ⁺ FAP	Malecova et al., 2018 [159]
	Mice	Hindlimb muscle	notexin	MuSC, myogenic progenitors, FAP, tendocytes	De Micheli et al., 2020 [131] [152]
Injury of muscle	Mice	Hindlimb muscle	notexin	MuSC, PM	Dell'Orso et al., 2019 [132] [153]
	Mice	tibialis anterior (TA) muscles	notexin	MuSC expressing immune genes, immune cells, activated FAP	Oprea et al., 2020 [154]

	Mice	Patellar tendons	PDGF-AA protein	Tppp3 ⁺ Pdgfra ⁺ tendon stem cells. Tppp3 ⁺ Pdgfra ⁺ FAP	Harvey et al., 2019 [142]
Injury of tendon	Mice	Diseased Achilles, toe extensor or Diseased peroneus longus	-	IL33-expressing endothelium, microfibril associated tenocytes	Kendal et al., 2020 [15]
Tendinopathy	Human	Supraspinatus and subscapularis tendon	EGF, GAS pathway	T cells	Garcia-Melchor et al., 2021 [145]

FAP: fibro-adipogenic progenitor, MuSC: muscle stem cells, PM: primary myoblasts, PDGF-AA: platelet-derived growth factor-AA.

4) Several abbreviations are used undefined or defined after the first use in the text. What does "CITE" stand for? As a latter example, IVD is defined on page 14.

Response:

We thank the reviewer for this suggestion. We have added the definition of CITE-seq and IVD after the first use in the text.

In line 75:

"While single-cell multi-omics (specifically Cellular Indexing of Transcriptomes and Epitopes by Sequencing, CITE-seq) [15,16],..."

In line 215:

"...unaffected tissues like Intervertebral Disc (IVD) or cartilage are also accessible as control groups."

We also added the definition of OA after the first use in the text. On line 40:

"They can be either short-term (fractures, sprains, and strains), which cause pain and functional restrictions, or long-term (osteoarthritis (OA) and chronic primary low back pain)."

5) The last paragraph of section 4 and the first paragraph of section 5 are exactly the same. One of them should be removed and the corresponding section should be rewritten.

Response:

We thank the reviewer for pointing this out. The extra paragraph in section 4 is deleted.

Minor points

3.2.4. IVD degradation (IVDD), fourth sentence from the last: Do the authors mean "cell lineages" rather than "cell lines"?

Response:

We thank the reviewer for pointing this out. We intended to use "cell lineages" instead of cell lines. Please see the revision on line 579:

"In addition, using healthy samples, Gan et al. [55] identified potential cell lineages in healthy IVD tissue that might promote the regeneration process which might rescue degeneration."

3.1.2. Skeletal dysplasia: This subsection may not necessarily be divided into further subsections.

Response:

We thank the reviewer for this suggestion. The subtitles were deleted.

4.1.2. Polymer Implants, second paragraph, the last sentence: What is "B cell receptor sequencing"? Please explain it.

We thank the reviewer for this suggestion. Please see the revision on line 722:

"B cell receptor (BCR) sequencing is a high-throughput RNA-sequencing method targeting the highly variable antibody (derived from BCR) repertoire, allowing the characterization of the adaptive immune response against the implants."

4.2. Drugs, fifth paragraph, second sentence: What this sentence "However, single-cells studies still need to be improved in the study of quite a few diseases of the skeletal-muscular system." is not clear. Please rephrase it.

We thank the reviewer for this suggestion. Please see the revision on line 805:

"However, studies have only been carried out in a limited number of musculoskeletal disorders, and experimental design requires further improvements according to the

characteristics of the diseases."

References: The ones after No. 61 are not exactly following the style of this journal.

We thank the reviewer for this suggestion. Please see the revisions in the reference section.