

Reviewer #1:

**Specific Comments to Authors:** Comments about the manuscript: “Proliferation and differentiation patterns of mesenchymal stem cells under stimulating factors” Mesenchymal stem cells (MSCs) have multipotent differentiation capacities, the process of which is known to depend on specialized stimulating factors. However, other factors such as mechanical morphology and exosomes involved in their differentiation have been discovered recently. Some regulatory mechanisms must therefore be better understood. In addition, limitations hamper the clinical application of MSC therapy. The purpose of this article is to review recent discoveries of these stimulatory factors. This review concerning the methods of differentiation of embryonic stem cells is useful and should be of interest to specialists but also more broadly to biological researchers and teachers. The manuscript is well written and I have only one minor remark to make. Given that the text contains many abbreviations, a list of these abbreviations with their meaning could simplify reading.

**Response:** Thanks for your kind and supportive suggestions regarding our manuscript. We provided a list of the abbreviations in the revised paper.

Reviewer #2:

**Specific Comments to Authors:** \*The authors summarized the current knowledge regarding differentiation patterns of MSCs under specific stimulating factors.

**Response:** Thanks for your kind and supportive suggestions towards our manuscript. Our point-to-point responses are listed below.

I think some issues should be addressed before further consideration of the manuscript. I've listed my comments below:

\*You have mainly focused on the osteogenic and chondrogenic differentiation of MSCs, while the title selected has a general concept. So, you should revise the title and include osteogenic and chondrogenic differentiation specifically.

**Response:** Agree. We revised the title as "Stimulating factors for regulation of osteogenic and chondrogenic differentiation of mesenchymal stem cells".

\*There are several missing references. For example, the first paragraph of the "Growth factors" section has no references. So, recheck the manuscript meticulously and fix this issue.

**Response:** We are sorry for this issue, and we rechecked the manuscript meticulously and revised it accordingly.

\*Your manuscript should be rechecked for English language, grammar, punctuation, spelling, and overall style. In several places, you have used past tense, while using present verbs is correct. I have mentioned some of the errors below: -Mesenchymal stem cells (MSCs) initially identified in the bone marrow "were" adult stem cells with multilineage differentiation potential. -Therefore, more studies "were" needed to clarify the mechanisms of action of bFGF at different stages of osteogenic differentiation, aiming to find a combination of growth factors to promote the osteogenic differentiation of MSCs effectively. -Therefore, it "was" crucial to figure out how to prevent hypertrophy during TGF- $\beta$  promoted cartilage differentiation. -Small non-coding RNAs (about 20–25 nucleotides)

called miRNAs “were” a subclass that could bind to complementary target sites in mRNA molecules to inhibit translation or decrease mRNA stability, controlling gene expression. -In this regard, further studies “were” needed to determine the appropriate SMG treatment time for regulating the specific lineage differentiation of MSCs. -It has been demonstrated that the strength, timing, and rate of FSS significantly “influenced” the proliferation and differentiation of MSCs. -More research “was” required to confirm the impact of FSS on MSC proliferation and the appropriate stimulus parameters for osteogenic differentiation and MSC proliferation. -These findings suggested that further studies “were” needed to determine the effects of different loads of HP on the spectral differentiation of MSCs and their complex mechanisms. This error can be seen in several places else. So, you should recheck the manuscript meticulously and fix this issue.

**Response:** We are regretful about the language problems.

We rechecked the English language, grammar, punctuation, spelling, and overall style of the manuscript, and the above-mentioned errors have been corrected. In addition, the language has been polished by MDPI company.

\*“Rowlands, A.S. found that osteogenic differentiation of MSCs occurred mainly on polyacrylamide gels of 80 kPa stiffness and that RUNX2 was also expressed at high levels.” should be changed to ‘Rowlands et al. found that ...’.

**Response:** We revised accordingly.

\*You should use the full name in the first usage of words instead of abbreviations. -In a concentration-dependent manner, bFGF might promote the proliferation of MSCs from several tissue sources, including “BM-PACs, SMSC, hASC, UC-MSC, and BM-MSCs”. -There are several unnecessary sentences which should be deleted. I have mentioned some examples below: -In this article, we discussed how growth factors, cytokines, and miRNAs affect the proliferation and differentiation of MSCs. -The research advancement on the significant growth factors that triggered MSCs proliferation in vitro is discussed below. -Here is a summary of several miRNAs' impacts on MSC destiny and their action targets.

**Response:** Thanks for your advice. In the revised manuscript, we used the full names of the abbreviations and deleted unnecessary sentences.

\*Drawing some tables which summarize the text is recommended.

**Response:** We added Tables 1-5, which summarize the text in the revised manuscript.