Reviewer's code: 03197771 **Reviewer's country:** Spain

The review article by Li and Yue, entitled: "Effects of various antimicrobial agents on the multi-directional differentiation potential of bone marrow-derived MSCs" consists of an update on the effects that antibiotics and other common therapeutic agents have on the differentiation potential of BMSCs. The structure of the paper seems appropriate as the authors classify the effects according to the 3 main differentiation cell types potential and the type of compound, which may facilitate the readers the access to a particular section of interest.

1. To improve this point it is recommended that section 3 is restructured accordingly. Also Table 2 needs to be redone including a similar format to Tables 1 and 3, indicating if Agents are antibiotic. etc.

Responses: Since few antimicrobial drugs have been reported to regulate chondrogenic differentiation, and several drugs have been mentioned in the previous section, we classify drugs according to promotion or inhibition effects in the section 3. Table 2 has been redone including a similar format to Tables 1 and 3.

2. The abstract seems truncated in the last sentence. It seems that the purpose of the review is missing. The addition of a short sentence explaining the aim of the review will clarify to readers the main message of the manuscript.

Responses: The aim of the review has been added (lines 50-51).

3. It will be of interest that the authors include the usual physiologic concentrations of antibiotics used in therapy and how the in vitro study-based evidence relates with them as a means of estimating the clinical relevance of the in vitro data on treated individuals.

For example, references 14 and 15 are reporting conc. of rifampicin above 32 ug/mL to have a negative effect on MSCs. What is the conc. range in individuals treated with rifampicin? It is important that the authors add drug bioavailability information and how does this fit with in vitro information for each compound included, as long as there is information available.

Responses: First, most of the research on the usual physiologic concentrations of antibiotics has focused on plasma, while the concentration of drugs in the bone marrow and in the joint cavity has rarely been reported. Second, the in vitro study-based evidence is more instructive for topical medication. For example, an antibacterial drug can be directly released to the infected site by some route or carrier, and the drug dosage and drug loading process can be adjusted according to the in vitro study-based evidence.

4. In this line, it is not clear what is the meaning of "blood concentration" in Table 1.

Responses: The "blood concentration" in Table 1 and the corresponding parts in the manuscript have been modified to specific concentrations.

5. It is also recommended that authors review the meaning of "significant inhibition", "severely inhibited" etc. As they are arbitrary terms, it would be more appropriate to set numeric values (above 30% etc).

Responses: By reviewing the entire manuscript, we have modified the wording and added numeric values.

6. Also "differentiation" and "viability" are two different terms. They should not go together (page 10).

Responses: "Osteogenic differentiation viability" has been modified to "osteogenic differentiation potential" (line 207).

7. Also, the whole manuscript has to be reviewed to avoid vague and subjective meaningless terms such as "good" when referring to antibacterial properties…etc; it should just read "antibacterial properties". Repetitive terms should also be avoided.

Responses: We have reviewed the whole manuscript and made corrections to the above problems.

8. The meaning of the following sentences is not clear in their present form. Please review: Section 2.1 "..and inhibit the effects of intracellular bacteria secreted in cells a" "Since, penicillin cannot tolerate the enzymes produced by a variety of bacteria, the results are more likely for it to be destroyed, increasing the probability"

Responses: These two sentences have been modified (lines 116-117 and 140-143).

9. The sentence "azithromycin does not produce cytotoxicity in the concentration range of 0-200 μg/mL; however, it inhibits the differentiation potential of osteoblasts at very low concentrations" seems controversial.

Responses: According to the reference 14, azithromycin does not produce cytotoxicity in the concentration range of 0-200 μg/mL; but under the osteogenic induction environment, the addition of 100-200 μg/mL azithromycin inhibits osteoblast differentiation comparing with control group (above 75%). This may be reasonable because cell proliferation and differentiation are different processes.

10. The cell line C2C12 does not undergo osteogenic differentiation. Please review the following sentence: “A similar inhibition was also reported in the C2C12 cell line [18].”

Responses: This sentence has been modified (lines 194-195).

11. Section 2.2 “induce pathogenic bacteria to cleave” A peptide cannot proliferate. Please review: “LL-37 has also been shown to be capable of proliferating,…” -

Responses: These two sentences have been modified (lines 298 and 303).

12. Reference 11 it is not appropriate on its first appearance. To describe common differentiation criteria for MSCs, it is recommended that the authors use the following reference: Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8(4):315-7.

Responses: New references have been cited.

13. -References 9, 10 and other contain over 6 co-authors. Generally only the 6 first are cited followed by et al., Please review specific journal (WJSC) format for cites.

Responses: We have examined the previous review of WJSC, where the references listed the names of each author.

14. -Check italics for: Pseudomonas, in vitro etc -Abbreviations have to be fully described in its first appearance i.e. ATDC5 cells

Responses: The problems of italics and abbreviations have been modified.

15. Please correct section after 4.3, cannot be labeled 2.4 Rephrase last sentence in the Conclusion section for clarity.

Responses: We are sorry for the mistake and correct the number (lines 473).

16. Please review punctuation and other minor English grammar throughout the manuscript.

Responses: The entire manuscript has been carefully examined to correct punctuation and grammar problems.

Reviewer's code: 02495033 **Reviewer's country:** South Korea

In the present review, the authors reviewed the inhibitory and promoting effects many anti-microbials on the differentiation of MSCs into bone, chondrocyte, and adipocyte. The review is very extensive and sound, so may provide readers and investigators with good information. It is believed that antibiotics added to the culture medium also may affect the proliferation of MSCs. So, it is recommended that the authors should emphasize the significance of antibiotics in the medium.

Responses: Thank you for your review. Most of the articles we reviewed are about the effects of drugs on the differentiation potential of MSCs in vitro, and the drugs are mostly added to the osteogenic, chondrogenic or adipogenic induction medium according to the type of differentiation.

Reviewer's code: 03712811 **Reviewer's country:** Italy

In this study, the Authors aim at providing a review analysis of the effects elicited by a number of antimicrobial agents on three types of differentiation patterns, osteogenic, chondrogenic, and adipogenic, forming the multilineage repertoire of bone marrow-derived mesenchymal stem cells (BM-hMSCs). This is an interesting, and exhaustive review tracing an important background for a comprehensive dissection of the ability of both natural and synthetic antimicrobial agents to preserve, or conversely hamper, the differentiating potential of BM-hMSCs. This is also a remarkable area of inquiry, since the pharmacological treatment of bone infections is placed within the context of understanding to what extent this treatment may impair or favor joint tissue recovery based upon the drug effect on local tissue resident stem cells (or stem cells transplanted as a tool for local cell therapy).

Responses: Thank you for your review.

Reviewer's code: 00567975 **Reviewer's country:** Austria

In the present manuscript, the effect of different antimicrobial drugs on the multi lineage differentiation potential of bone marrow MSCs is reviewed. Review is good structured and see to cover all aspects of this topic. Several issues may be further considered by Authors:

1. Chapter Introduction. Natural penicillin is produced by fungi, not by pathogenic microorganism. Moreover, modern antibiotics are mostly synthetic ones, this should be clearly stated in the introduction.

Responses: We have modified the description in the chapter Introduction (lines 38-41).

2. Chapter 2.1 Subheading in this chapter are somewhat confusing: why did Author use sometime groups of antibiotics (e.g. beta-lactam) and sometimes their therapeutic effects (e.g. anti-tuberculosis drug)?

Responses: The groups of antibiotics and natural peptides correspond to each other and contain numbers (X.X) before the title. Due to the variety and number of antibiotics reviewed, we further classified antibiotics. The categories including beta-lactam and anti-tuberculosis drug are subtitles of antibiotics. We believe that it is more in line with the clinician's thinking habit to classify anti-tuberculosis drugs, so we will list them separately.

3. Author described in details the effect of different antibiotics on osteogenic differentiation and sometimes there are contradiction in the effect of antibiotics of the same group (e.g. cephalosporins) on the osteogenic differentiation. It would be interesting how these contradictory finding can be explained?

Responses: The effect of each drug on osteogenic differentiation is unique, and even if some drugs produce the same effect, the mechanism and the concentration may be different. Therefore, if the antibiotics of the same group have different effects on osteogenic differentiation, it is likely that the molecular mechanism and drug concentration are different.

4. Whole Review It would be important to emphasize on the clinical importance of the effect of antimicrobial drug. For example, how the concentration used in in vitro experiment are related to the clinical situation, serum concentration, minimal inhibitory concentration?

Responses: Most of the articles we reviewed are about the effects of drugs on the differentiation potential of MSCs in vitro. Because the concentration of drugs in the bone marrow and joint cavity is difficult to control after systemic administration, these in vitro study-based evidences are more instructive for clinical topical medication. For example, an antibacterial drug can be directly released to the infected site by some route or carrier, and the drug dosage and drug loading process can be adjusted according to the in vitro study-based evidence.

5. Are there any studies showing the effect of antimicrobial drugs described by Author on MSC differentiation in vivo?

Responses: References 38 and 79 describe the effects of Baicalin on promoting osteogenesis and inhibiting adipogenesis in vivo, respectively. References 46 describe the effects of extract of Piperaceae on promoting osteogenesis in Sprague-Dawley Rats.

Reviewer's code: 02446229 **Reviewer's country:** Japan

The author summarize various knowledge about antimicrobial agents on the multi-directional differentiation of bone marrow-derived mesenchymal stem cells (MSCs). The collective information for osteogenic, adipogenic and chondrogenic differentiation of MSCs in Table 1, 2 and 3, respectively in this review are important to think about therapeutic strategy and are worth to publish. I have some questions as described below. Please answer the question and possibly add your opinion in the manuscript.

1. I suppose that antimicrobial agents can be administrated either locally or systemically in various orthopedic therapies. Local concentration of the antimicrobial agents in the bone marrow where many MSCs exist should be regulated by the controlled-release technology. Please mention about this problem and show author's opinion?

Responses: Most of the articles we reviewed are about the effects of drugs on the differentiation potential of MSCs in vitro. Because the concentration of drugs in the bone marrow and joint cavity is difficult to control after systemic administration, these in vitro study-based evidences are more instructive for clinical topical medication. For example, we can use certain nanoparticles, such as mesoporous silica, as drug carriers, and cover the surface of the drug-loaded particles with biological response valves to achieve drug control release and intelligent release. According to the in vitro study-based evidence, we can optimize the drug loading dosage and carrier synthesis process to achieve the local optimal drug concentration.

2. In case of therapy based on the tissue engineering of bone and cartilage, MSCs can be cultured to be differentiate into osteoblast or chondrocyte with some adequate 3D scaffold in vitro. Which antimicrobial agents are preferable for such purpose, considering about the large scale of culture, antimicrobial spectra (species of microorganisms), risk of emerging drug-resistant microorganisms, etc.? Please mention about this point.

Responses: Seed cells, scaffolds and cytokines are three indispensable elements for the bone and cartilage tissue engineering. To find the best antimicrobial agents to promote

tissue regeneration, a comprehensive evaluation of the effects of drugs on seed cells, scaffolds and related cytokines is needed. The effect of drugs on cell differentiation is only part of it, not enough to determine the best drug choices, and a large number of in vitro and in vivo experiments are needed.

3. Recently, MSCs are used for cell transplantation therapies not only for bone regeneration and cartilage regeneration in orthopedics, but also for recovery of stroke in brain, spinal cord injury, nerve repair, reduction of graft-versus host disease (GVHD) various diseases, etc. Growth factors and cytokines produced by MSCs can accelerate repair and regeneration in those cases. Collective knowledge about antimicrobial agents on MSCs are also useful for such clinical application of MSCs. How does the author think about those issues?

Responses: The widespread use of MSCs in diseases other than bone and joint suggests a more versatile differentiation potential. In addition to the effects on osteogenesis, chondrogenesis, and adipogenesis, various antibacterial drugs may also affect the differentiation potential of MSCs into muscles and neurons, which may be confirmed in future studies.

Reviewer's code: 02446191 **Reviewer's country:** India

Authors briefly describe about various antimicrobial drugs/ agents and their potential role in osteogenic, cartilage, and adipogenic differentiation of bone marrow-derived mesenchymal stem cells. Molecular mechanisms of antimicrobial agents towards regulation of multiple differentiation potentials of MSCs must be described in detail.

Responses: Since a considerable number of articles do not address the molecular mechanisms by which antimicrobial drugs work, we have not covered them in detail. The molecular mechanisms of protein drugs and Chinese traditional drug extracts have been studied more deeply, while the mechanism of common antibiotics on the multi-directional differentiation potential of MSCs is rarely involved. We have listed the molecular mechanisms involved in the literature we reviewed in Supplementary Table 1.

Supplementary Table 1 Molecular mechanisms of antimicrobial agents towards regulation of multiple differentiation potentials

Effect	**Agent**	**Reference**	**Molecular mechanisms**
osteogenesis	Bacitracin	23	BMP/Smad pathways
	Lactoferrin	29	PKA and p38 MAPK pathways
	Hepcidin	30	BMP/Smad and p38 MAPK pathways
	LL-37	31	ERK and JUK MAPK pathways
	KR-12	32	BMP/Smad pathways
	Cordycepin	33	NF-κB pathways
		34	Wnt pathway
	Tanshinone IIA	35	BMP and Wnt pathway
		36	BMP/Smad and p38 MAPK pathways
	Andrographolide	37	Wnt/ β-catenin pathways
	Baicalin	39	Wnt/ β-catenin pathways
	Costunolide	40	ATF4-dependent HO-1 expression
	extract of Lithospermum	41	Runx2 and Osterix-dependent manner
		42	Runx2 and Osterix-dependent manner
	Naringin	43	Wnt pathway
	Curcumin	44	Wnt/ β-catenin pathways
	Limonene	45	p38 MAPK and Akt pathways

	Saikosaponin-A	48	Wnt/ β-catenin pathways
	Licochalcone A	49	ERK MAPK pathways
	Trichostatin A	50	Runx2 and BMP-dependent manner
	Voriconazole	53	Fluoride-Independent Mechanism
chondrogenesis	Oxytetracycline	58	BMP-dependent manner
	Cordycepin	59	PI3K/Bapx1 and Notch pathway
	Lactoferrin	60	Smad2/3-Sox9 pathway
	Trichostatin A	62	TGF- β1/ Sp1 Pathways
adipogenesis	Isoniazid	67	ARE signaling Pathways
		67	ARE signaling Pathways
	Spiramycin	69	PPARγ, C/EBPα, SREBP1c and AMPK Pathways
	Geldanamycin	70	MR, GR and PPARγ Pathways
	Lactoferrin	71	PPARγ Pathways
		72	insulin signaling; RB1 and AMPK Pathways
	Cordycepin	74	mTORC1-C/EBPb–PPARg pathway
	Tanshinone IIA	75	C/EBP-α, PPAR-γ, FAS, perilipin A, and STAT-3/5 Pathways
		76	11-HSD1 enzyme Pathways
	Andrographolide	77	PPARγ pathway
	Baicalin	78	Wnt/ β-catenin pathways
		79	NF-κB and p38 MAPK pathways
	Oleuropein	81	Wnt10b-mediated signaling Pathways
	Shikonin	85	ERK MAPK pathway
	Ursolic acid	86	LKB1/AMPK Pathway
	Alpinia officinarum	87	PPARγ, C/EBPα, and SREBP1c Pathways
	Dioscin	88	AMPK/MAPK pathway
	Methyl cinnamate	89	CaMKK2−AMPK Pathway
	Tetrandrine	90	C/ EBP-α, PPAR-γ, FAS, perilipin A, and STAT-3 Pathways

	Licochalcone A	92	PPARγ and SREBP Pathways
	Nelfinavir	98	C/ EBP-α and PPAR-γ Pathways
	Indinavir	103	PPARγ and SREBP-1 Pathways
	Amodiaquine	105	PPAR-γ Pathways
	Quinine	106	ERK/S6 Pathway
	Artemisinic Acid	107	C/EBP δ Pathway

Dear editor,

Thank you for your letter and for the comments concerning our manuscript entitled "Effects of various antimicrobial agents on the multi-directional differentiation potential of bone marrow-derived mesenchymal stem cells". Those comments and suggestions are all valuable and very helpful for improving the manuscript. Our point-by- point responses to the reviewers' comments are as follows.

Best regard!

Bing Yue, MD, PhD

May 17, 2019

Responses to EDITOR-IN-CHIEF (ASSOCIATE EDITOR):

1. The current Abstract gave enough Background (problems, significance, Challenge); however; the Abstract should be incorporated with the following 3 essential elements in the end to be a Descriptive Abstract, which informs of readers what specific about this review: 1) Insight: What did you discover from your survey of literature and from

your own research? or How did you approach the problem differently (uniqueness about why YOU should be writing this review)? 2) Solution: Provide some specific detail about the solution. 3) Evidence: Summarize the evidence you have for your approach: A proof, an implementation, or quantitative results.

***Responses*:** The abstract has been modified. The insight, solution and evidence sections have been added.