The authors would like to thank the Reviewers for the valuable comments. In response to Reviewers comments, please find below the list of changes according to reviewers' suggestions, point by point. All changes are highlighted by yellow color in the revised version of manuscript.

Reviewer's code: 03773730

Question 1. The aim and conclusion in the Abstract of this manuscript is too big to demonstrate, here, only gene expression and cytokines were detected, so, these descriptions should be narrow down to the examination you done in this manuscript.

Answer: In response to Reviewer comments: in this study not only detection of gene expression and cytokines were performed, we also analyzed stability of MSC phenotype, differentiation potential and ability of MSC of different tissue origin to form fusion. We agree with Reviewer that our studies do not assess all biological properties of MSC. As suggested by the Reviewer, we changed term "biological properties" for "biological activity" in the aim and conclusion of the Abstract in the revised version of manuscript.

Question 2. Is it possible to characterize MSC with different phenotype by double immunostaining and flow cytometry analysis, such as CD73/CD146, CD90/PDGFR, or CD105/PW1, so that we can clearly know the percentage of MSC with different phenotype.

Answer: We performed double immunostaining for CD73/CD146 and additional pictures illustrated expression of CD73/CD146 on MSC of different tissue–origin are implemented in the Figure 1, and proper information is also added in the Results section in the revised version of manuscript (page 13). We are also considering to complete other analyzes suggested by the Reviewer in future studies.

Question 3. Was it possible of the MSC phenotype changed during the passage, or after some passages. Can you examine the percentage of MSC with different phenotype during the passage?

Answer: The percentage of MSC with basic phenotype was examined after each passage. As suggested by the Reviewer, additional data are introduced on the new Figure 2 illustrated changes of the percentage of MSC with analyzed phenotype in passages P1, P5 and P10, corresponding to other analyzes introduced in this study,

and proper information is added in Results section (page 13) in the revised version of manuscript. Phenotype changes are also summarized in the new Table 2 implemented in the revised version of manuscript.

Question 4. Quantification of osteogenic, adipogenic and chondrogenic potentials changes during passage. So that you can give us enough information that MSC still have differentiation ability after some passages.

Answer: Differentiation ability of MSCs isolated from examined tissues was analyzed during follow-up period at P1, P5 and P10. However, differentiation potential changed during subsequent passages and varied between MSCs originated from different tissues. Results are illustrated on Figure 4 and summarized in the new Table 2.

Question 5. How do you know the spontaneous fusion happened, two dye merged with yellow color is not enough to demonstrate, CLSM should be used to check the colocalization of these two dyes in one cell, moreover, you also can us flow cytometry analysis to check the DNA content after coculture, and then based on the quantification, you can know how many cells infusion happened spontaneously.

Answer: The observation that during co-culture two populations of MSCs, labelled with PKH26 (red) or PKH67 (green), were able to form fused cells was suggested by co-localization PKH26 (red) and PKH67 (green) dye into yellow color. We did not performed analysis using confocal microscopy. In our studies the presence of fused cells was confirmed by flow cytometry analysis. Cells were detached from the culture plate at the end of observation, after 120 h, and single cells were analyzed using flow cytometry to assess the presence of cells that displayed merged fluorescence signals. Figure 7 (Figure 6 in the original version of manuscript) documented the presence of a population of cells with fluorescence emission within the 480–560 nm range of the spectrum (Channel 2), characteristic for PKH67, the 595–642 nm range (Channel 4), characteristic for PKH26, and the 560–695 nm range (Channel 3), which confirmed the immersion of two dyes with each other. These data analysis method are described in Results section in paragraph entitled "*Co-cultures of MSCs isolated from different tissues*" in the original version of manuscript (page 19 in the current version of manuscript) and are illustrated on Figure 7. Single cell was captured and analyzed for positive staining for PKH26, PKH67 and for co-localization of both colors. Pictures of single cells were taken directly during cytometric analysis, and cell number what was analyzed is introduced on the picture (Figure 7).

Question 6. From the discussion, biological properties evaluation should be clearly described, or can you clearly answer how long we can culture the MSC which still keep the differentiation potential.

Answer: All examined MSCs kept basic phenotype of naïve MSCs up to P10. Differentiation potential of examined MSCs from different tissue was maintained up to P10, however, was varied. In BM-MSCs and AT-MSCs osteo- and chondrogenic potential increased, whereas adipogenesis was diminished. In SM-MSCs differentiation potential was stable during follow-up period, but in SK-MSCs adipogenic activity also decreased.
Based on the results obtained in this study there is difficult to clearly answer how long we can culture the MSC which still keep the differentiation potential because it depends on the tissue niche of which MSCs are originated. The most stable biological activity for BM-MSCs and AT-MSCs was observed up to 5 passages, however, proangiogenic markers such as CD146, PDGFRα, decreased in AT-MSC but are compensated by an increased activity of IL-8 and VEGF.
As suggested by the Reviewer this explanation is implemented in the Summary in the Discussion section (page 29), and summarized in the new Table 2.

Question: Minor concern: 1. The figure 5 have 8 histograms, you'd better label them using different letter. 2. Y axis in figure 5 should be amending to fit the value of all groups in the graph shown, such as BM-MSC (IL-5, IL-6 and MIP1) AT-MSC (MCP-1, IL8 and VEGF), no need to keep all the maximum same in different groups.

Answer: (1) In the Results section, results of cytokine activity are described as a comparison between MSCs, and Figure 5 (Figure 6 in the revised version of manuscript) without specific label with different letter is handier for citation in the manuscript body. (2) As suggested by the Reviewer, Y axis on Figure 6 (Figure 5 in the original version of manuscript) is corrected in the revised version of manuscript.

Reviewer's code: 02492656

Question 1: This reviewer is having a problem getting my head around all of the data which are extensive. I was looking for a table that allows cross referencing among all (or at least many) of the variables. In other words if one could run down a column that includes a stem cell from adipose tissue and wants to know if that cell type releases IL-8 or expresses P53, the reader could go directly to the IL column or the gene expression column and quickly know what the AT-Stem cells can do.

Answer: In response to Reviewer suggestion we prepared new Table 2 which summarized biological activity of examined MSCs. Table 2 is implemented in the revised version of manuscript.

Question 2: Much time and effort was spent on cell fusion, but it is not clear what the significance of "fusion" may be when stem cells are used experimentally or in therapeutic trials. A brief introduction to this topic before the data are presented would be helpful.

Answer: As suggested by the Reviewer a brief introduction on cell fusion is implemented in the revised version of manuscript in the Introduction section (page 7), and proper references No. 39-42 are added in the revised version of manuscript.

Reviewer's code: 00504800

Specific comments:

Question: 1. The manuscript is very long/wordy, and could be shortened considerably without altering the conclusions.

Answer: In response to Reviewer suggestion an Introduction section and Discussion section are shortened from original manuscript. However, we have added new paragraphs according to Reviewers comments and suggestions.

Question: 2. Introduction, page 5: The authors should at least briefly mention teeth (dental pulp, periodontal ligament) as another source of MSC, as there is an extensive literature on dental-derived MSC. Furthermore, there are several papers comparing dental MSC to MSC from other tissues (e.g., Alge DL, et al., J Tissue Eng Regen Med. 2010; Yamada Y et al., Tissue Eng Part A 2010; Kunimatsu R, et al., Biochem Biophys Res Commun. 2018) which should be referenced and considered in the Discussion.

Answer: As suggested by the Reviewer proper information on dental pulp and deciduous teeth as a source of MSC is added in the Introduction section (page 5) and new referenced No 10-12 are implemented in the References section in the revised

version of manuscript.

Question: 3. Methods, page 7: What were the ages and age range of the donors for each type of tissue? Do the authors think donor age could have impacted some of their findings/caused variability? Same with the timing of death to when tissues were obtained for culture.

Answer: As suggested by the Reviewer, donors age is implemented in the Materials and Methods section in *Tissue collection* paragraph in the revised version of manuscript (page 8).
BM-MSCs were isolated from deceased donors with age ranged from 23-49 years, collected 24 -48 h after death. We do not observed significant changes in biological activity of MSCs between samples obtained from these donors. Other examined MSCs from adipose tissue, skeletal muscle and skin were isolated from tissues obtained from limbs amputated due to critical limb ischemia. Age of these patients was ranged from 60-69 years. In these samples proangiogenic activity and mRNA for Sox2 and Oct4 decreased with age of culture. Differences between age of BM-MSCs donors and age of patients from whom tissue-specific MSCs were obtained do not allow to give clear answer whether the variability between MSC depends on the age of the donor or is related to their natural tissue niche. However, given into consideration that age affects the biological activity of the whole organism, we can suppose that the reduction of proangiogenic activity of MSCs obtained from other tissues than the bone marrow may be related to the age of the donor. This observation needs further studies.

Question: 4. Methods, page 8: Why was immunohistochemistry chosen to analyze expression of CD146 and PDGFRa instead of flow? This would allow easier quantitation for the reader in Figure 1. I understand this may not be feasible for PW1.
Answer: CD146 was analyzed by both methods by flow cytomerty and by immunohistochemistry, whereas PDGFRα by immunohistochemistry only. The percentage of CD146 after P1, P5 and P10 is introduced on the new Figure 2 which summarizes the changes in the percentage of MSCs during follow-up period and this information is implemented in the Results section (page 13) in the revised version of manuscript.

Question: 5. Results, page 11: More explanation is needed on HOW expression of

CD146 and PDGFRa is different between the cell types, and why the authors consider this to be significant.

Answer: Expression of PDGFRα was seen in early passages, and around passage P5, expression of PDGFRα decreased in MSC from all examined sources as confirmed by immunofluorescence staining. Considering proangiogenic activity of MSC for therapeutic use cells from early passages should be applied. Dynamic of CD146 changes is illustrated on the new Figure 2 implemented in the revised version of manuscript. Changes in CD146 and PDGFRα expression are summarized in the new Table 2. The role of CD146 and PDGFRα in regeneration processes is discussed in the Discussion section and new explanation is implemented in the current version of manuscript (pages 21-22)

Question: 6. Results, page 12, paragraph 4: The potential role of naive MSC markers in myogenesis needs to be explained more in the Discussion.

Answer: As suggested by the Reviewer proper explanation is added in the Discussion in the revised version of manuscript (page 26).

Question: 7. Results, page 13 and 14: The presentation of the data on mRNA is expression is very lengthy and somewhat confusing. More importantly, the authors need to better explain (here or in the Discussion) whether these statistically significant increases and decreases in mRNA expression are thought to be real or artifact; how they compare to other MSC studies; and if true, what the biological significance might be. For example, c-Myc expression in AT-MSCs declined in P5, but went up again in P10 - why? What does this mean - something significant biologically, or simply variability in the samples studied?

Answer: We assume that changes in the mRNA expression during subsequent passages may reflect variability in the samples. These changes are widely discussed in the context of other analyzed parameters such as differentiation potential and their role on MSC activity associated with the niche of origin (pages 23-24).

It is worth to mention here, that some MSC in passage P10 showed a noticeably high value of SD (standard deviation). This observation suggests that gene expression in late passages varies between patients which leads to conclusion that a

comprehensive assessment of the gene expression background is required before cells from late passages can be transplanted – even if the graft is autologous. There are many factors that can influence the biological behavior of cells in patients, including individual genetic differences.

In case of AT-MSC the high level of c-Myc expression can't be ignored and the risk of oncogenesis should be studied further.

As suggested by the Reviewer this explanation is added in the Discussion section in the revised version of manuscript (page 25)

Question: 8. Discussion, page 17: Same comment for the lengthy discussion of CD146 variability - what does this mean? The finding that CD146 expression is most stable in BM-MSC, and that they may be the most useful cells for impacting angiogenesis is notable; what does the low and/or variable expression in the other MSC types mean, if anything?

Answer: CD146 is a proangiogenic marker. In tissue regeneration not only direct tissue restoration is necessary, also proper angiogenesis which allow to restore vasculature of injured tissue is very important for blood supply and tissue oxygenation and nutrition. The subpopulation of MSCs with proangiogenic potential facilitates angiogenesis in the regenerating tissue. That's why for clinical application it is important to consider the source of MSC with preferred biological activity to achieve desired effect. For example when we need to use MSC to treat systemic disease (e.g. graft-versus-host disease) proangiogenic activity of MSC are less important than anti-inflammatory ability, whereas when we expected to regenerate injured tissue (e.g. muscular dystrophy, myocardial infarction) MSC with anti-inflammatory activity and proangiogenic potential should be considered.

As suggested by the Reviewer this explanation is added in the Discussion section in the revised version of manuscript (pages 21-22).

Question: 9. Discussion, pages 18-20: The background on Sox2 and Oct4 could be shortened considerably by just citing others' work, allowing for better discussion of the authors' findings. In particular, I am still unclear as to the potential role of PW1 in the MSC from these tissue types. Its expression seems very variable as well; can a

conclusion be drawn?

Answer: As suggested by the Reviewer the background on Sox2 and Oct4 is shortened in the current version of manuscript.

In regenerative processes PW1 is well recognized in skeletal muscle regeneration as a one of non-satellite stem cells, different from satellite cells which are specific muscle precursor cells, and actively cooperate in muscle regeneration. This topic is discussed in the context of p53 expression (page 25, first paragraph, of the current version of manuscript). The role of PW1 is not well recognized in regeneration processes in other tissues. MSC expressing PW1 which reside in different tissues are on focus in our studies, and we assume that PW1 play supportive role in tissue homeostasis and regeneration, however, their role in tissue regeneration is variable and depend on the activity of stem/progenitor cells residing in a given tissue.

This explanation is implemented in the Discussion section in the revised version of manuscript (page 23)

Question: 10. Discussion, pages 20-21: Again, the cytokine expression data and discussion is lengthy and could be improved by more concisely summarizing which families of cytokines are produced by which MSC types, why these differences exist biologically, and how this may be exploited clinically.

Answer: Cytokines play important role in immunoregulatory capability of MSC. MSCs by secretion of immunomodulatory factors are characterized by ability to switch pro-inflammatory M1 macrophage phenotype to anti-inflammatory M2 phenotype. This is an important mechanism in tissue regeneration because reduction of inflammatory microenvironment in the injured tissue facilitates effective tissue regeneration. Moreover, MSCs secrete proangiogenic factors IL-8 and VEGF which support angiogenesis in the injured tissues. Biological differences between MSCs for their potential clinical use are introduced in the Discussion section (pages 26-27). Similarities and differences between biological activity of MSCs from examined sources are summarized in the new Table 2.

Question: 11. Discussion, page 21: The fusion data is interesting, and its potential application in DMD therapy is intriguing. Of all the items in the Discussion, this subject would benefit from a more extensive discussion.

Answer: As suggested by the Reviewer, discussion on the potential application of MSCs and fusion with damaged muscle in DMD patients is extended in the Discussion section (page 28).

Question 12. Discussion, page 22: It is really true that BM is more difficult to access than adipose for a given patient? It's simply a different procedure for procuring marrow than adipose.

Answer: We are agreeing with Reviewer that obtaining BM or adipose tissue is a different procedure. We removed the statement that BM has limited accessibility and changed for "Moreover, similar observations of the fusion of the SM-MSC fraction with the BM-MSC or AT-MSC fraction provide hope for an alternative source of MSCs for this purpose" in the Discussion section (page 28).

Question 13: Discussion, page 22: Were cells from later passages (P6-P8) used for any fusion experiments, especially P6-P8 SK-MSC when they express higher levels of CD146?

Answer: Fusion experiments were performed at P2 only. We chose P2 for this experiment because for clinical application usually MSCs from early passages between P0 to P3 are applied. This was preliminary experiments and we considering to perform fusion in later passages in future studies.

Question 14: Summary, page 22, first paragraph: The authors use the term "different" or "different role" for stemness marker expression in several places, including the Summary, when I think a statement that expression is variable is more appropriate.

Answer: As suggested by the Reviewer the term "different" or "different role" for stemness marker expression was changed for a statement that expression "varied" in Summary section in the revised version of manuscript (page 29).

Reviewer's code: 02446319

Reviewer comment: Thank you for your great manuscript about Similarities and differences between mesenchymal stem/progenitor cells derived from various human tissues. It's very valuable to readers.

Answer: Authors are grateful for very nice comment. Thank you very much.