Dear Editor,

Thank you for carefully reviewing our manuscript previously titled "**Allogeneic Mesenchymal Stem Cells May Be a Viable Treatment Modality in Cerebral Palsy**" for possible publication in the ''World Journal of Clinical Case Series". We are grateful to you and your reviewers for their constructive critique. We have revised the manuscript, highlighting our revisions in yellow. And have attached point-by-point responses detailing how we have revised the manuscript in response to the reviewers' comments below. Now we hope the revised paper will provide a more readable description on the study.

Thank you for your consideration and further review of our manuscript. Please do not hesitate to contact us with any further questions or recommendations.

Yours Sincerely,

Reviewer 1 (ID: 06229302)

Thank you very much for the opportunity to review this interesting article about SCT in CP. Please find below the main issues:

1. Introduction: please provide some mechansism of action for this therapy for CP, in accordance with the literature.

Response: We added some mechansism of action for MSCT for CP. In the light of relevant literature, we focused on the anti-inflammatory, neuroprotection and neuroregenerative effects of MCT, especially through paracrine mechanisms:

The possible mechanisms of action of the therapeutic effectiveness of mesenchymal stem cell therapy (MSCT) in cerebral palsy patients are evaluated on the basis of its anti-inflammatory effects, neuroregeneration and neural protection. Early studies explained the mechanism of action in terms of stem cells migrating to the stroke area, differentiating into functional cells,

and interacting with the cells in penumbra, providing regeneration of the damaged area. However, more recent studies focus on the contributions of paracrine interactions, mitochondrial transfer and extracellular vesicle secretion to the therapeutic effect of mesenchymal stem cells, in addition to these effects[1]. The paracrine effects of mesenchymal stem cells (MSCs) are realized through their cytoprotective effect, provasculogenic effect, anti-inflammatory effect, endogenous regeneration-enhancing effect, anti-fibrotic effect, and effects on metabolism[2].

In a study on the neuroprotective activity of MSCs, it was shown that the number of apoptotic neurons decreased compared to the control group after intravenous treatment in a stroke model created in female rats. In the group receiving MSCT, it was reported that stem cells migrated to the injury site, as well as showing anti-apoptotic activity by increasing the expression of basic fibroblast growth factor (bFGF) and providing functional recovery by promoting endogenous proliferation[3]. In another rat study, a reduction in the infarct area and functional improvement were detected after human-derived MSC application in a stroke model. Numerous neurotrophic factors have been identified in the MSC transplanted brain. While only insulin-like growth factor 1 (IGF-1) is of human origin, it has been reported that much more rat-derived neurotrophic factors such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and bFGF are expressed compared to the control group[4].

There are many studies reporting the anti-inflammatory activity of MSCs through paracrine mechanisms. In an in vitro study by Huang et al., they reported that IL-6 and VEGF have an important role in the anti-inflammatory activity of mesenchymal stem cells. The anti-inflammatory activity of IL-6 is due to its inhibitory effect on TNFα and IL-1[5]. It is also supported by other studies that IL-6 has many roles in this mechanism. MSCS implantation reduces apoptosis by increasing IL-6 production through resident NSC NFkB activation, independent of the PI3/Akt pathway, and thus provides neuroprotection[6]. In the study conducted by Jung et al., it was observed that IL-6 injection protected cells from oxidative stress by causing an increase in the levels of phosphorylated STAT-3 and Mn-SOD[7].

1. Zhuang WZ, Lin YH, Su LJ, *et al.* Mesenchymal stem/stromal cell-based therapy: mechanism, systemic safety and biodistribution for precision clinical applications. *J Biomed Sci* 2021; 28: 28. [PMID: 33849537 DOI: 10.1186/s12929-021-00725-7]

2. Gnecchi M, Danieli P, Malpasso G, Ciuffreda MC. Paracrine Mechanisms of Mesenchymal Stem Cells in Tissue Repair. *Methods in Molecular Biology*, 2016; 1416: 123–146. [PMID: 27236669 DOI:10.1007/978-1-4939-3584-0_7]

3. Chen J, Li Y, Katakowski M, Chen X, Wang L, Lu D, Lu M, Gautam SC, Chopp M. Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat. *J Neurosci Res.* 2003; 73(6): 778-86. [PMID: 12949903 DOI:10.1002/jnr.10691]

4. Wakabayashi K, Nagai A, Sheikh AM, Shiota Y, Narantuya D, Watanabe T, Masuda J, Kobayashi S, Kim SU, Yamaguchi S. Transplantation of human mesenchymal stem cells promotes functional improvement and increased expression of neurotrophic factors in a rat focal cerebral ischemia model. *J Neurosci Res.* 2010; 88(5): 1017-1025. [PMID: 19885863 DOI: 10.1002/jnr.22279]

5. Huang P, Gebhart N, Richelson E, Brott TG, Meschia JF, Zubair AC. Mechanism of mesenchymal stem cell-induced neuron recovery and anti-inflammation. *Cytotherapy*. 2014; 16(10): 1336-1344. [PMID: 24927715 DOI: 10.1016/j.jcyt.2014.05.007]

6. Rose-John S. IL-6 Trans-Signaling via the Soluble IL-6 Receptor: Importance for the Pro-Inflammatory Activities of IL-6. *Int J Biol Sci.* 2012; 8(9): 1237-1247. [PMID: 23136552 DOI:10.7150/ijbs.4989]

7. Jung JE, Kim GS, Chan PH. Neuroprotection by interleukin-6 is mediated by signal transducer and activator of transcription 3 and antioxidative signaling in ischemic stroke. *Stroke*. 2011; 42(12): 3574-3579. [PMID: 2194095 DOI: 10.1161/STROKEAHA.111.626648]

2. Introduction: Please mention, if other cerebral pathologies could benefit from SCT

Response: We added literature about MSCT in other Central Nervous System pathologies:

Mesenchymal stem cell therapy offers a new treatment perspective not only in cerebral palsy, but also in many central nervous system diseases such as hypoxic ischemic encephalopathy, Alzheimer's disease, stroke, Parkinson's disease, Multiple Sclerosis, and spinal cord injury, especially stroke, whose mechanisms of action we have given examples above[8,9].

In the animal study published by Lee et al. in mice, which they used as an Alzheimer's disease model, it was shown that human umbilical cord-derived MSCs inhibit the release of pro-inflammatory cytokines from microglia with regenerative and paracrine effects, and contribute to functional recovery by reducing apoptosis and amyloid plaques[10]. In the phase I clinical study, MSCs were administered by stereotactic brain infusion at two different doses in a single round (3x106 cells/60 µL and 6 3x106 cells/60 µL). No serious side effects were observed for either dose and were well tolerated by bugs. One of the most important limitations of the study is that it cannot provide sufficient information about its effectiveness due to restrictions such as not containing a sham group and being open label[11].

In a study conducted with the "Experimental autoimmune encephalitis" model, which is the most commonly used model in animal studies on multiple sclerosis, it was shown that it reduces inflammatory infiltrates and demyelination[12]. Clinical studies have been conducted on this treatment method, which has been shown to be safe and effective in pre-clinical studies, and some clinical improvements have been reported to be observed[9].

8. Martínez-Morales PL, Revilla A, Ocaña I, González C, Sainz P, McGuire D, Liste I. Progress in stem cell therapy for major human neurological disorders. *Stem Cell Rev Rep*. 2013; 9(5): 685-699. [PMID: 23681704 DOI: 10.1007/s12015-013-9443-6]

9. Sherman LS, Romagano MP, Williams SF, Rameshwar P. Mesenchymal stem cell therapies in brain disease. *Semin Cell Dev Biol.* 2019; 95: 111-119. [PMID: 30922957 DOI: 10.1016/j.semcdb.2019.03.003].

10. Lee HJ, Lee JK, Lee H, Carter JE, Chang JW, Oh W, Yang YS, Suh JG, Lee BH, Jin HK, Bae JS. Human umbilical cord blood-derived mesenchymal stem cells improve neuropathology and cognitive impairment in an Alzheimer's disease mouse model through modulation of neuroinflammation. *Neurobiol Aging*. 2012; 33(3): 588-602. [PMID: 20471717 DOI: 10.1016/j.neurobiolaging.2010.03.024]

11. Kim HJ, Seo SW, Chang JW, Lee JI, Kim CH, Chin J, Choi SJ, Kwon H, Yun HJ, Lee JM, Kim ST, Choe YS, Lee KH, Na DL. Stereotactic brain injection of human umbilical cord blood mesenchymal stem cells in patients with Alzheimer's disease dementia: A phase 1 clinical trial. Alzheimers Dement (N Y). 2015; 1(2): 95-102. [PMID: 29854930 DOI: 10.1016/j.trci.2015.06.007]

12. Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, Gerdoni E, Giunti D, Ceravolo A, Cazzanti F, Frassoni F, Mancardi G, Uccelli A. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. *Blood*. 2005; 106(5): 1755-1761. [PMID: 15905186 DOI: 10.1182/blood-2005-04-1496]

3. Introduction: Please mention the risk factors for SCT failure

Response: We also added a new passage about MSCT risk factors, in line with the data of two meta-analyses conducted in different years and discussed new data added in the intervening years.

In the meta-analysis published by Lau et al. in 2012 regarding the possible risks of MSCT no statistically significant side effects were detected other than transient fever[13] In another meta-analysis published in 2021, it was similarly revealed that the only side effect that can be associated with MSCT is transient fever, despite the expanding patient population with the studies published in the intervening years. Transient fever is more common in women, and less common in the North American population. Despite the discussed tumorigenic potential of meschemimal stem cells, only one malignancy was detected in the entire population included in the study and was not associated with stem cell application. In the same meta-analysis, studies reporting side effects such as vascular disorders, urticaria/dermatitis, dizziness/headache, diarrhea, infection, death, anaemia, metabolism and nutritional disorders, nausea, seizure and vomiting were also evaluated, but no significant relationship was found with MSCT[14].

13. Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, Granton J, Stewart DJ; Canadian Critical Care Trials Group. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PLoS One*. 2012; 7(10): e47559. [PMID: 23133515 DOI: 10.1371/journal.pone.0047559]

14. Wang Y, Yi H, Song Y. The safety of MSC therapy over the past 15 years: a meta-analysis. *Stem Cell Res Ther*. 2021 Oct 18;12(1):545. [PMID: 34663461 DOI: 10.1186/s13287-021-02609-x]

4. Material and methods: You mentioned that ˝Four patients, aged between 1 to 4 diagnosed as CP with severe disability which is required wheelchair...˝, which for a 1-year old child is not surprising... Please reconsider a validated clinical evaluation scale for motor impairaiments in small children.

Response: Although scales such as Gross Motor Function Classification System (GMFCS), Manual Ability Classification System (MACS) and Wee-FIM appropriate to the age group were used in the course of our study, the expression was changed to "unable to mobilize without assistance".

5. Rehabilitation procedures: Which kind of procedures could be applied for an 1-year and 4-years old patient for an improvement in fine motor skills? Furthermore, could you perform exercises for postural control/stabilisation in an 1-year old child, which physiologically does not have this capacity? Please describe the rehabilitation procedures in detail with regard to the age of your patients and specific goals.

Response: We added a new reference about rehabilitation.

6. Statistical analysis: A statistical analysis in a goup of 4 patients could not be appropriate, even by using a non-parametric test. Please consider to present your results from the light of a case series presentation

Response: We added case series presentation in results section.

7. Discussion: Please consider the results from the perspective of one by one case-report presentation

Response: We added case series presentation in results section.

8. General consideration: please consider the article as a case-report rather than a cohort prospective study

Response: Our study is a open label phase I study, also we added case series presentation according to your suggestions.

9. English Issues: there are some minor issues that should be adressed (i.e. sedoanalgesia, etc

Scientific Quality: Grade C (Good)

Novelty of This Manuscript: Grade B (Good)

Creativity or Innovation of This Manuscript: Grade C (Fair)

Scientific Significance of the Conclusion in This Manuscript: Grade C (Fair)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Reviewer 2 (ID: 06221767)

This is an interesting and novel study where, 4 patients with cerebral palsy were and treated with mesenchymal stem cells (MSCs) and found to have an improvement after 12 months. See below for comments in each section. In general the article is very interesting but would benefit from re-organization and improvement in grammar to clarify the results and significance of findings.

- Good review of the disease, current state of treatment, and gapas in the literature
  Response: Thanks for your kind comments

- Multiple runs on sentences/ listing sentence structure that coud be improved for readability
  Response: We performed some corrections about this.

Methods:

- This sentence is confusing to me – "Four patients, aged between 1 to 4 diagnosed as CP with severe disability which is required wheelchair, to have home nursing care and assistance with activities of daily living were included

in this longitudinal study. All children evaluated were not able to walk independently and cannot stand up without support" Is "required wheelchair, to have home nursing care.." a standard definition of severe disability or is this the author's definition ? Are these just inclusion critiera, and if so why are these features not listed there in the inclusion criteria? H the patients were recruited, and how many were enrolled

Response: Depending on the severity of the disease, mild clinical findings may be seen in cerebral palsy patients, as well as very severe clinical conditions accompanied by severe spasticity. At this point, we changed this expression, which we used to emphasize that the patients included in our study had more severe clinical conditions, to not being able to mobilize independently, in line with your criticism.

- Why are there / in the following sentence? "conservative treatment (e.g., physical rehabilitation and botulinum toxin injection). Exclusion criteria were recently diagnosed severe infection (meningitis, etc.)/development of liver, kidney/heart failure/sepsis or skin infection at the i.v. infusion site or positive for hepatitis B, C/HIV, history" seems like an auto corrector was used an replaced commas with back slashes

Response: "The inclusion criteria of the study including as age <18, without any chronic disease (i.e., cancer, kidney, heart/hepatic failure), estimated life expectancy >12-month, no significant change in neurological and functional status despite 3 months of conservative treatment (e.g., physical rehabilitation and botulinum toxin injection)."
In order to exclude spontaneous recovery, we included patients who did not benefit from conservative treatments in our study.

"Exclusion criteria were recently diagnosed severe infection (meningitis, etc.)/development of liver, kidney/heart failure/sepsis or skin infection at the i.v. infusion site or positive for hepatitis B, C/HIV, history of uncontrolled seizure disorder, presenting clinic symptoms that formation of white sphere number ≥ 15000/μL or platelet count ≤ 100.000/μL, serum aspartate aminotransferase and serum alanine aminotransferase > 3× upper limit of normal/creatinine > 1.5× upper limit of normal and to participate in an another investigational stem cell study before treatment."

One of the possible side effects of intrathecal applications is meningitis. Therefore, we excluded patients with a previous history of meningitis from our study. We excluded cases with a history of serious systemic disease, as organ failures have been previously reported in the literature, in order to more accurately evaluate whether SCT is a safe treatment[1].

[1] Wang, Y., Yi, H. & Song, Y. The safety of MSC therapy over the past 15 years: a meta-analysis. Stem Cell Res Ther 12, 545 (2021). https://doi.org/10.1186/s13287-021-02609-x

- What doe this mean? “Before starting the treatment, a multidisciplinary approach was performed with a team of pediatricians, pediatric neurologists, neurosurgeons, anesthesia and reanimation and physical medicine and rehabilitation specialists.”

  Response: The patients included in the study were evaluated by a council consisting of pediatricians, pediatric neurologists, neurosurgeons, anesthesia and reanimation, and physical medicine and rehabilitation experts and then treatment was started. In this way, the medical needs of the patients and approaches to possible complications were determined in a multidisciplinary manner.

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Results

- Grammar and spelling throughout
  Response: We made some corrections regarding grammar and spelling errors.

- The organization of the results could be changed to improve clarity, it is difficult to follow what is being reported in each moment. Consider breaking each paragraph down with a sub title and and use direct comparisons, for example: “Spasticity: Mean MAS scores at naseline were x. After intervention, mean scores were X. Treatment was associated with XX change in right sided spasticity, amd xx change in left sided spasticity”

  Response: In line with your suggestion, we rearranged the results heading to include subheadings.

Discussion

- Again having trouble with this section due to both organization and grammar.
  Response: We made some corrections regarding grammar and spelling errors.

- Typically the discussion may start with a summar of the studies findings, and then moves into discussing these findings in the context of the current literature. It is extremely unclear to me what the authors are discussing in the first half of the section as written. Further there are multiple grammar errors that interfere with the readability of the manuscript.
  Response: We performed some corrections about this.

- I think it would be helpful to discuss the differences in the 1 year old patient and the 9 year old patients – this age discrepancy is interesting because based on the methods, the 9 year old patients would have diagnosed for at least 5

years. What other treatments had the had, etc. This should be discussed or listed as a limitation,
We added this limitation in discussion section

Scientific Quality: Grade C (Good)

Novelty of This Manuscript: Grade B (Good)

Creativity or Innovation of This Manuscript: Grade B (Good)

Scientific Significance of the Conclusion in This Manuscript: Grade C (Fair)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Major revision

Reviewer 3 (ID: 06352857)

It is encouraging that stem cell therapy can be applied to clinical treatment, especially to cerebral palsy, a difficult-to-heal disease. I strongly agree that the purpose of this experiment is to apply stem cell therapy to clinical treatment. Although the results of this experiment seem to be pleasing, the presentation of the results of this study is poor due to the fact that this is an academic paper.

First, the identification of UC-MSCs by flow cytometry was not presented. Cell differentiation data and results were also not presented.

Response: We added new data about this:

In vitro adipogenic differentiation and Oil Red O staining

To induce adipogenic differentiation, cells from passage three (3,000 cells/cm2) were seeded onto coated type I collagen coverslips (BD Biosciences) in 6-well plates. The adipogenic medium Dulbecco's Modified Eagle's Medium, Low Glucose (DMEM-LG, Invitrogen) was supplemented with 10% FBS (Invitrogen/GIBCO), 0.5 mM isobutyl-methylxanthine (IBMX-Sigma-Aldich), 10^{-6} M dexamethasone (Sigma-Aldich, Fluka Chemie AG, Buchs, Switzerland), %0,02 insulin (Invitrogen/GIBCO), 200 µM indomethacin (Sigma-Aldich), and

1% penicillin-streptomycin (Invitrogen/GIBCO) for 3 weeks. The medium was replaced twice a week. Intracellular lipid droplets indicating adipogenic differentiation confirmed by Oil Red O (Sigma-Aldich) staining with 0.5% oil red O in methanol. Cells allowed to dry completely and mounted in a mounting medium.

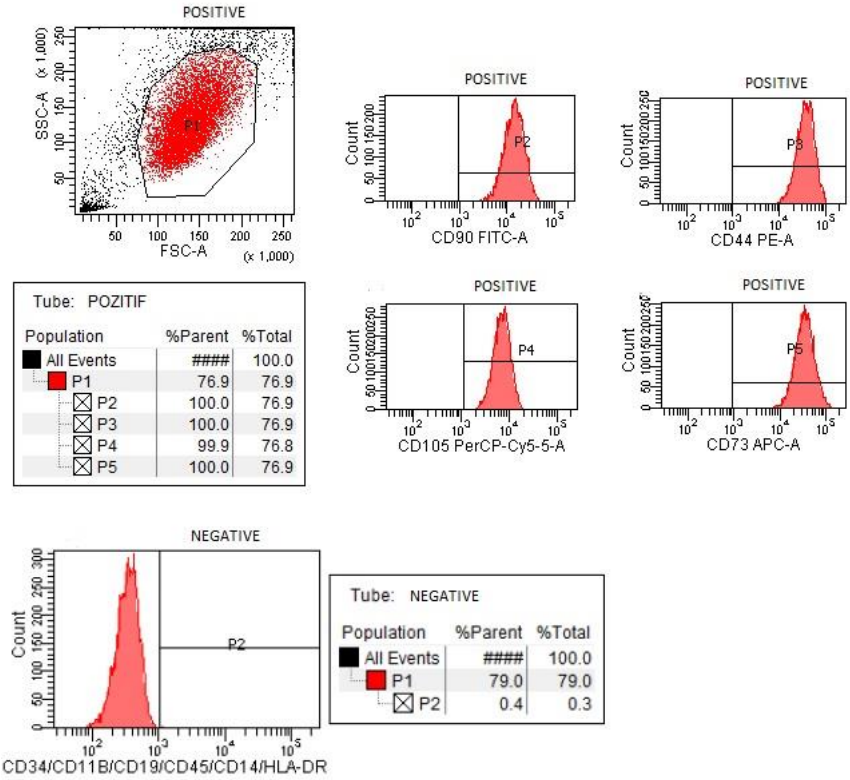
In vitro osteogenic differentiation and Alizarin red S staining

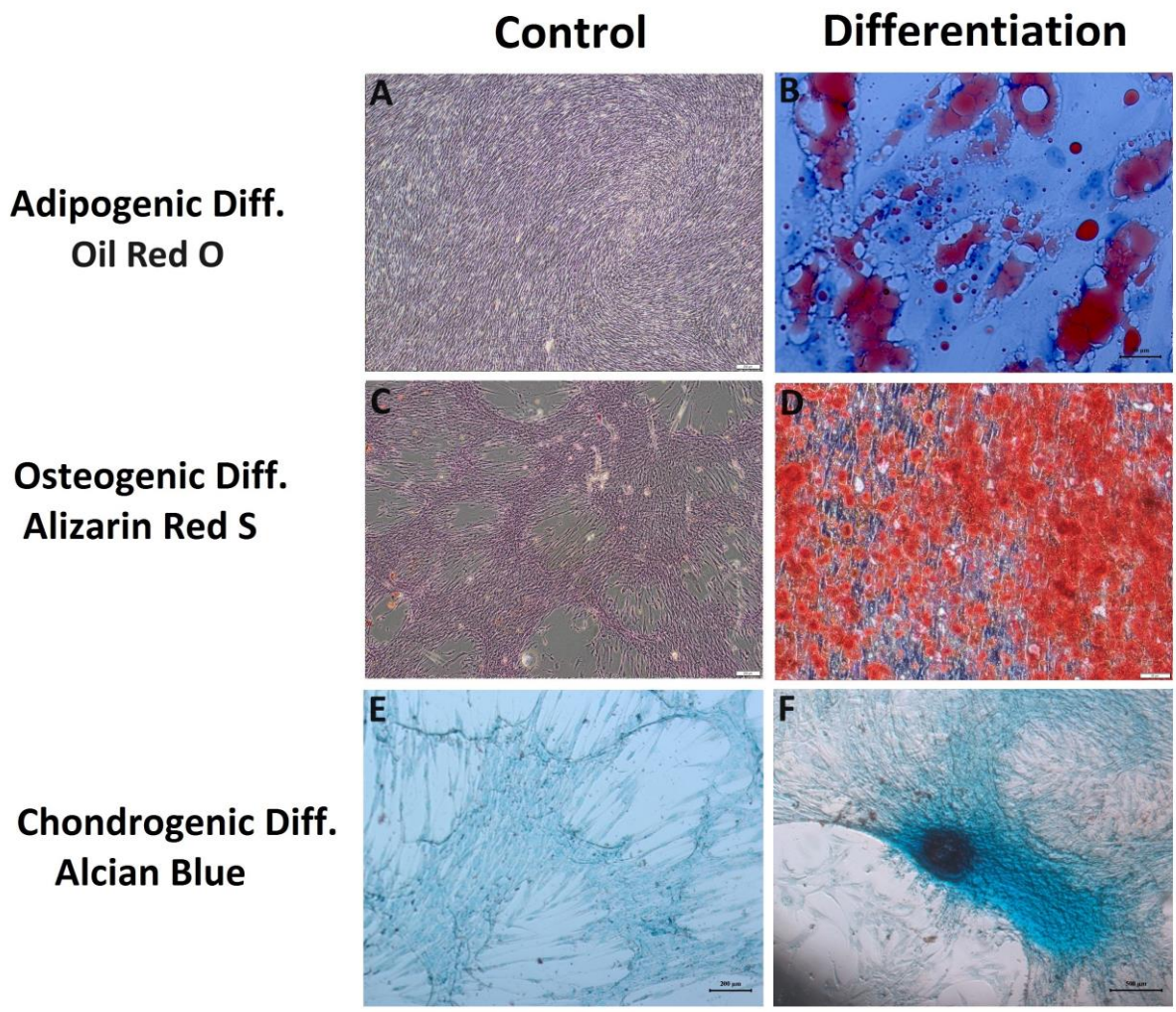
Cells from passage three (3,000 cells/cm2) were seeded onto 6-well plates in which type I collagen-coated coverslips (BD Biosciences) were included. The osteogenic medium Dulbecco's Modified Eagle's Medium, Low Glucose (DMEM-LG, Invitrogen) was supplemented with 10^{-8} M dexamethasone (Sigma-Aldich), 50 µg/ml ascorbate-2- phosphate (Wako Chemicals, Richmond, VA, USA), 10 mM b-glycerophosphate (Sigma-Aldich), 1% penicillin-streptomycin and 10% FBS (Invitrogen/GIBCO) for 3 weeks. The medium was replaced twice a week. At the end of the third week, osteogenic differentiation was assessed by staining with Alizarin Red (Sigma-Aldich, Fluka Chemie AG, Buchs, Switzerland) staining. The medium was discarded and the cells were washed with PBS. Cells were incubated with ethanol for 5 minutes at room temperature and then allowed to dry completely. Cells were stained with alizarin red solution comprising two percent alizarin red S for 1 min, after washing distilled water. Stained cells were dehydrated in acetone (20 dips), fixed in acetone–xylene (1:1) solution (20 dips), and cleared with xylene (20 dips), then allowed to dry completely and mounted in a mounting medium.

In vitro chondrogenic differentiation and Alcian Blue staining

In the chondrogenesis mechanism, high cell density and cell-cell interaction play an important role. For this reason, for chondrogenic differentiation, cells were seeded as droplets onto 6-well plates in which type I collagen-coated coverslips (BD Biosciences) were included. After the cells adhere to the coverslips, medium is added. The chondrogenic medium Dulbecco's Modified Eagle's Medium, High (4.5 g/l) Glucose (DMEM-HG, Invitrogen) supplemented with 100 nM dexamethasone (Sigma-Aldich), 50 µg/ml ascorbate-2- phosphate (Wako Chemicals, Richmond, VA, USA), 10 ng/ml transforming growth factor-beta 1 (TGF-β1, Peprotech, Rocky Hill, NJ), 1% sodium pyruvate (Invitrogen), 50 mg/ml ITS (Sigma-Aldich), 40 µg/ml proline (Sigma-Aldich), 1% penicillin-streptomycin and 10% FBS (Invitrogen/GIBCO) for 3 weeks. The medium was replaced twice a week. Cells were fixed with formaldehyde for 10 minutes at room temperature and washed with distilled water and then allowed to dry completely. Chondrogenic differentiation confirmed by Alcian Blue

(Abcam) staining. Cells allowed to dry completely and mounted in a mounting medium (Figure2).





Second, the age difference between these four patients is relatively large.

Response: We added case series presentation in results section.

Generally speaking, the older a child with cerebral palsy is, the more difficult it is for him or her to undergo rehabilitation. Older children may cause the treatment to be less effective.

I think that the four children in this experiment are more suitable for case report to be reported separately, due to the fact that the phenotypic heterogeneity of children with cerebral palsy is too large. Finally, I did not see stem cell therapy show a very convincing therapeutic effect in this experiment, due to the lack of a control group or valid statistics. The mere fact that the post-intervention assessment found that the patient's status had improved is not a sufficient indication that the stem cell therapy was effective, due to the fact that the patients were also receiving other rehabilitative treatments at the same time. It is therefore unfortunate that this article in its current state is not suitable for publication unless the author makes significant changes.

Scientific Quality: Grade D (Fair)

Novelty of This Manuscript: Grade A (Excellent)

Creativity or Innovation of This Manuscript: Grade B (Good)

Scientific Significance of the Conclusion in This Manuscript: Grade B (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision