Point 1.1.1) comment A1.

Is this correct

Answer: yes, it is correct

Point 3.5) comment A2.

Why only these two time points and not other? It will be nice if you provide the passage number as well.

Answer: we propagated the cells up to 21 or 28 days (approximately 6-8 passages) to avoid the presence of not stem cells in the culture.

Point 3.7) comment A3, A4, A5

Please provide the step number for both instead

Answer: we provided the step number

Point 4.1.1) comment A6

Is there a specific reason to have 444.7 and not 445 ml?

Answer: Yes, it is a standard preparation to have 500 mL of final neuronal culture medium (now point 5.1.1.)

Point 4.2)

A7. So the neuronal induction is performed only after 28 days of culture?

Answer: Yes. The neuronal induction is performed only after 28 days of dental pulp separation (now point 5.2)

A8. Do you remove the medium and then add the new one? Do you perform a wash in between?

Volume?

Answer: We improved the point 4 (now point 5)

Point 4.3) A9

Is there a specific reason to work only after 7 and 14 days? Do you check for neuronal morphology/biomarker at this stage? Please include a step stating the same.

Answer: yes, generally after 14 days is possible to observe morphological and biochemical changes. Moreover, we decided to investigate at 7 days to check a middle step.

Yes, we checked the neuronal morphology and neuronal biomarkers at 14 days (Figure 3 and 4). Moreover we tested the presence of prion protein at 7 and 14 days with neuronal culture medium.

Point 5.1) A10, A11

Is this after neuronal induction? Please provide the step number. If this is induced, then please refer to as induced hDPSCs. If not then please provide the step number which comes before this step. Volume?

Answer. No, it is before the neuronal induction. So, we moved the step before neuronal induction process of hDPSCs. We improved the point with missed things.

Point 5.2) A12

If 5.2. is highlighted, the steps describing the procedure should also be highlighted. Please either highlight the substeps or convert this to a table and reference the table in step 5.2.

The table should be uploaded separately as a .xls/.xlsx file in your editorial manager account.

Answer: we unmarked the point 5.2 (now 4.2)

Point 5.2.4) A13

Do you perform the vortexing for 10 min or after vortexing you incubate for 10 min. please check.

Answer: we improved the point 5.2.4 (now 4.2.4)

Point 5.4) A14

Please provide the volume as well. Do you remove the transfection mix before adding the complete medium?

Answer: we improved the point 5.4 (now 4.4)

Point 5.5.1) A15

Needs clarity. Please reword.

Answer: we reworded the point 5 (now 4)

Point 6.2) A16

Is there a specific reason to use these time points? Do you add medium to stop the trypsin action?

Answer: Yes. We propagated the cells up to 28 days (approximately 8-10 passages) to avoid the presence of not stem cells in the culture. Yes, we added the trypsin stop solution.

Point 6.3) A17

Volume and concentration?

Before this step, there are few missing steps. Do you stop the trypsin action, do you plate the cells for treatment with growth factors, do you leave it for incubation or 14 days? Please include all specific details with respect to your protocol.

Are these treatment performed on coverslips coated with cells, slide, plate? Please specify.

Answer: we improved the point 6

Point 7) A18, A19

Is this cytofluorometric analysis or flow cytometry? Please highlight the difference between step 6 and 7 apart from the antibody.

These detail needs to be provided earlier.

Answer: It is a flow cytometry. The most important differences are: the time of induction with neuronal culture medium, the time of culture of hDPSCs (21 and 28 from the dental pulp separation). The experiment showed in point 7 (Fig. 5A) was modified from ““Role of Prion protein-EGFR multimolecular complex during neuronal differentiation of human dental pulp-derived stem cells”. Prions

Point 7.2) A20

Please include the step number or include all necessary information.

Answer. We included the step number.

Point 7.4) A21

The cells in this case are fixed in the

Answer: PBS

Point 7.5) A22

0.1% in PBS?

Answer: yes