**Authors’ Response**

We sincerely appreciate the time and careful reading by the editor and reviewers. We addressed all the comments listed and have made corresponding changes to our revised manuscript. Details of our replies to the comments are the following:

**To editor:**

Thank you. We have made changes to 1-7, 22 according to your kind reminder.

* 8. 1.1.2: What is the temperature for culturing?

The culturing temperature is 37 ℃. We have added to the manuscript.

* 9. 1.2.2: What volume of PBS is used to wash? What is the concentration of trypsin used?

The volume of PBS is 1mL. The concentration of trypsin is 0.25%. We have added to the manuscript.

* 10. 1.2.3: How to count the density of cells?

We used hemocytometer to count the cell density. Added.

* 11. 1.2.5, 1.3.4: What is the incubation temperature? Please specify throughout.

The incubation temperature is 37 ℃. We have already specified throughout.

* 12. 1.3.1: Unclear sentence. Please revise.

We have revised into ‘Culture the primary rat NSCs suspension in Rat NSCs Growth Medium on uncoated T25- flasks at a density of 5×105 cells.’

* 13. 2.2: Please specify the size of the syringe.

The size of the syringe is 5mL. We have added to the manuscript.

* 14. 2.3: Please specify the length, dimeter, and type of the soft pipe.

The length of the silicon rubber pipe is ~1m, and the inner diameter is 12mm.

* 15. 6.1: What volume of PBS is used to wash? Please specify through out.

The volume of PBS is 1mL. We have already specified throughout.

* For 16~19, we have changed the format and made the proper highlights.
* Discussion: Please also discuss any limitations of the technique.

We have added some discussion about the limitations in the discussion.

* References: Please do not abbreviate journal titles. Please include volume and issue numbers for all references.

We have modified the reference information.

**To reviewer 1:**

Thank you for your comments.

Major Concerns:

* This study demonstrated that plasma can promote the transition process from NSCs to neurons, but did not show the type of neurons. Thus, it is still far from being clinically applicable for directional neuronal differentiation to achieve tissue transportation.

In fact, in our previous study, we did specify the neuronal fate. And we added this in Fig.5. After plasma treatment, large numbers of mature neurons, cholinergic and motor neurons appear in the plasma-treated group. And yes, for clinical transportation, it is still a long way to go.

Minor Concerns:

* The authors should set the conventional methods of inducing NSC differentiation as another control besides the gas flow group.

Thank you for your construction suggest. Set conventional methods as another controls are necessary for the scientific study and it is within in our next step.

**To reviewer 2:**

Thank you!

**To reviewer 3:**

Thank you for your comments.

Minor concerns:

* Line 47: "invited " has to be changed to "inverted"

Thanks. We already made a change.

* Line 55: Please, explain abbreviation "DA".

DA is short for ‘dopaminergic’.

* Lines 61-62: one more sentence should be added explaining what physical plasma is.

We have added and changed the explanation of plasma in page 2, paragraph 4.

* Lines 62-63: It is not clear what this sentence means. ("In the last two decades, plasm a medicine has attracted huge attention worldwide as 63 the development of cold atmospheric pressure plasma (CAP) technology.")

‘Plasma medicine’ is a term for plasma application in biomedicine. We have changed the sentence.

* Line 90 and following chapter 1.2: Please, give some more information about the cell line.

The information of C17.2 NSCs is given in the discussion part, page 7, paragraph 2.

* Line 105 and following chapter 1.3: Please, give some basic information how to obtain the primary rat neural stem cells.

The obtain of the primary rat neural stem cells is following reference 8. We have added in the protocol.

* Line 124 and following chapters 2. and 3.: If you give such detailed information how to construct such kind of self-made CAP jet, you have to give some more information about the power supply, because it is known that the power supply device can be critical for plasma generation. The same is true for any specific requirements for the oscilloscope. Otherwise, you should give a more basic information how CAP jets are to be used in this experimental setup independent on the individual kind of jet applied.

The information of the power supply, the oscilloscope and the high voltage probe is listed in ‘materials’ supplementary. And the general preparation of the whole system has been modified in chapter 3.

* Line 142 and following chapter 142: You should give some information if there is any liquid loss by evaporation that can be intensified by the gas flow of the CAP jet. This and possible drying effects are known to be very critical if plasma jets are used for cell treatment.

I agree with you that drying effects are very critical in the plasma jet treatment. However, in order to avoid this, we had pretest the effect of gas flow rate, the treatment time and the distance to make sure there is no obvious liquid loss. We have added in the protocol.

**To reviewer 4:**

Thank you for your comments.

Major concerns:

* 1. How do they determine the parameters of CAPs, including the distance between the nozzle of the quartz tube and the platform, the treatment time, etc.?

We did pretest to make sure the plasma dose is proper for the NSCs differentiation. Large plasma dose and intense plasma treatment will induce cell apoptosis and necrosis. We tested the cell survival and differentiation rate as a function of treatment time, distance to find a proper condition and also gas flow rate to avoid liquid loss.

* 2. In the "DISCUSSION" p art, authors should discuss the advantages of CAP application for NSCs differentiation over alternative techniques, rather than detailed "Immunofluorescence staining" and "Good photography technique".

Thanks for the suggestion. We have made some changes. Add plasma advantages and also the limitations.

* 3. Line 94: Concentration of trypsin? Trypsin incubation time? What is "relevant medium"?

The concentration of trypsin is 0.25%, incubation time is 1min. "relevant medium" is the mentioned culture medium.

**To reviewer 5:**

Thank you for your comments.

Major Concerns:

* line 225, Fig3 an d Fig4 have no statistics data or graph, they should add it. In the abstract, 75% NSCs selectively differentiated into neurons, no data supported it, and no prove said that these are mature, cholinergic and motor neurons. should change the saying or add more prove.

We have added statistics data to Fig.3 and Fig.4. And we also added a new figure 5 to show the neuronal fate specification.

* Minor Concerns:
* more details should be mentioned in plasma jet treatment. How did the plasma jet cover the whole 12 well plate. did you rotate the 12 well plate when treating with plasma jet or just fixed in the center of the plate? what about the evaporation of medium during plasma treatment?

We just fixed in the center of the plate. There is no obvious evaporation of medium during the treatment. We have already added this in the manuscript.