

Supplementary Materials

Selective neuronal differentiation of neural stem cells induced by nanosecond microplasma agitation

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This supplementary materials section includes:

S1. NSCs and C17.2 NSCs identification

S2. Treatment areas

S3. Optical emission spectra

S4. Results of NO measurements

S5. Additional details of qRT-PCR analysis

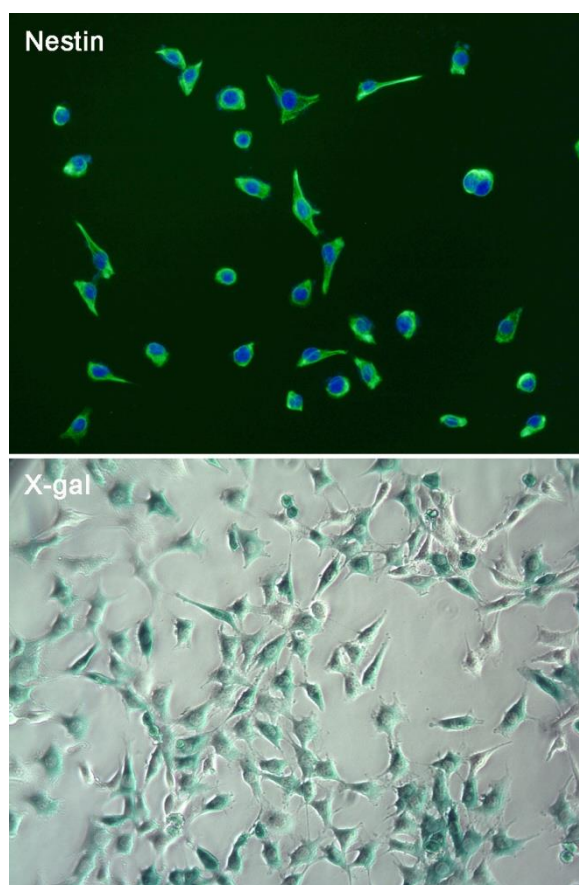
S6. Microplasma jet device

Supplementary Figures S1-S5

Supplementary Table S1

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S1. NSCs and C17.2 NSCs identification

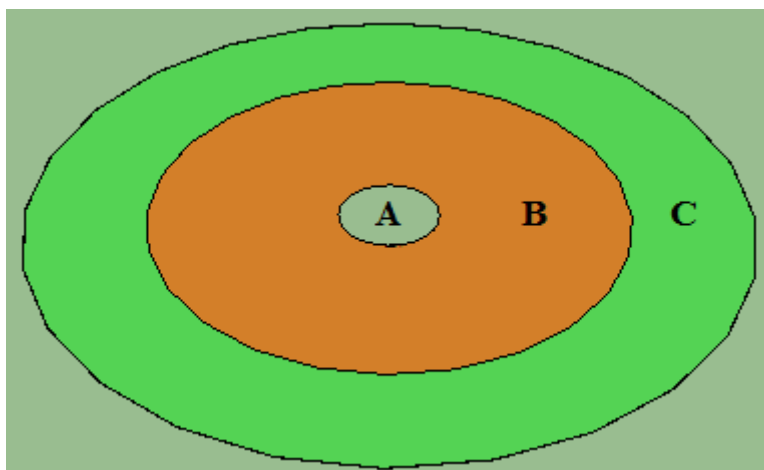


Supplementary Figure S1. Identification of NSCs and C17.2 NSCs. Nestin (upper panel); X-gal (lower panel).

Original NSCs were identified by detecting the expression of specific Nestin (method is presented in main text). We also examined the expression of the reporter gene β -gal in C17.2 using a commercial X-gal staining kit (Beyotime, Jiangsu, China). As can be seen in Supplementary Figure S1, that 95% cells were nestin positive (upper panel), and the X-gal staining shows that more than 90% C17.2 NSCs were stained blue (lower panel). Cell structure and boundary are very clear, which indicates that the β -gal gene inside the C17.2 NSCs is stable.

S2. Treatment areas

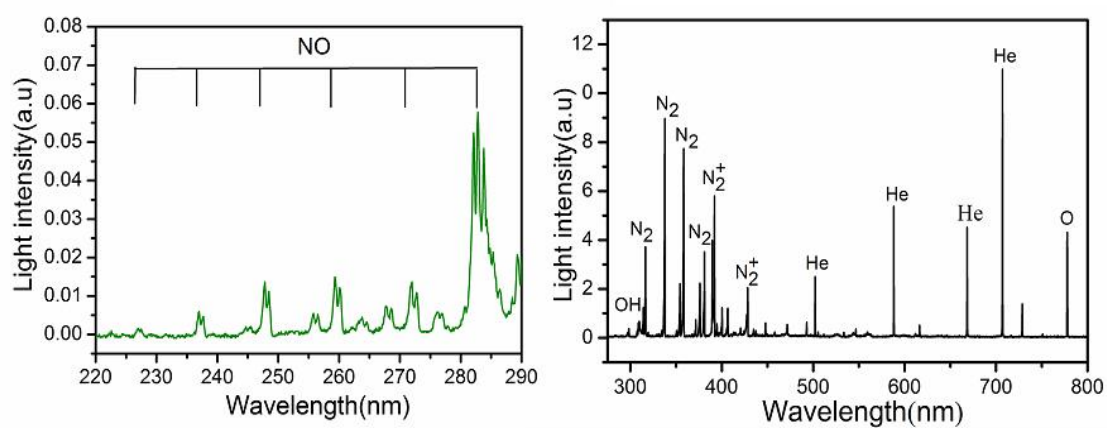
The sample areas affected by the microplasma treatment are shown in Supplementary Figure S2 below. Cell responses in different areas were different and are described in the Results and Discussion section of the main text.



Supplementary Figure S2. Schematic of different areas affected by the plasma treatment. A stands for the nearly cell-less area; B is the indirectly affected area; and C is the plate-well edge.

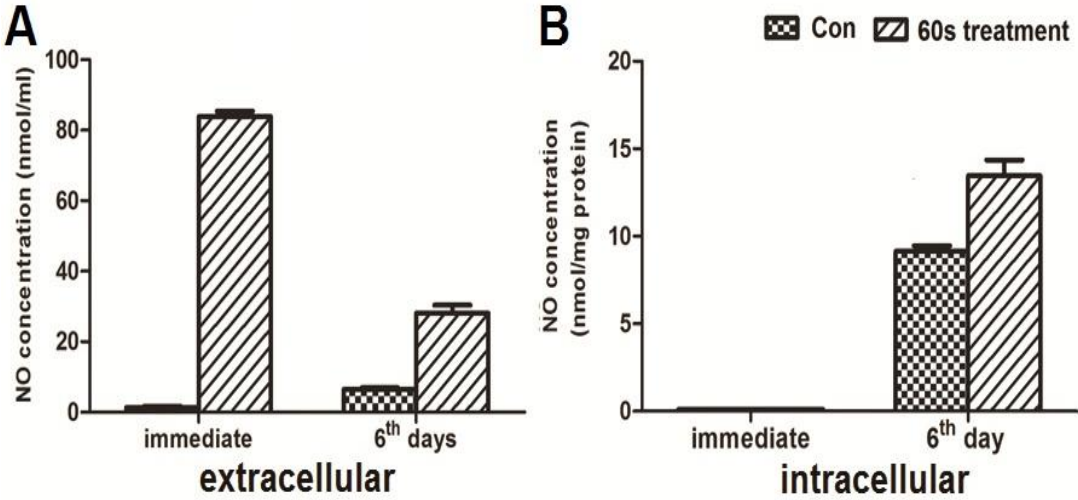
S3. Optical emission spectra

The optical emission spectra generated by the microplasma jet are shown in Supplementary Figure S3 below. The microplasma produces various biologically relevant species that affect cellular responses. Please refer to the Results and Discussion section of the main text.



Supplementary Figure S3. Optical emission spectra of the atmospheric-pressure plasma plume. 220 nm – 290 nm (a); 300 nm – 800 nm (b).

S4. Results of NO measurements



Supplementary Figure S4. NO concentration detection immediately and after 6 days culture in the control group and the 60s plasma-treated group. (a) Extracellular; (b) intracellular.

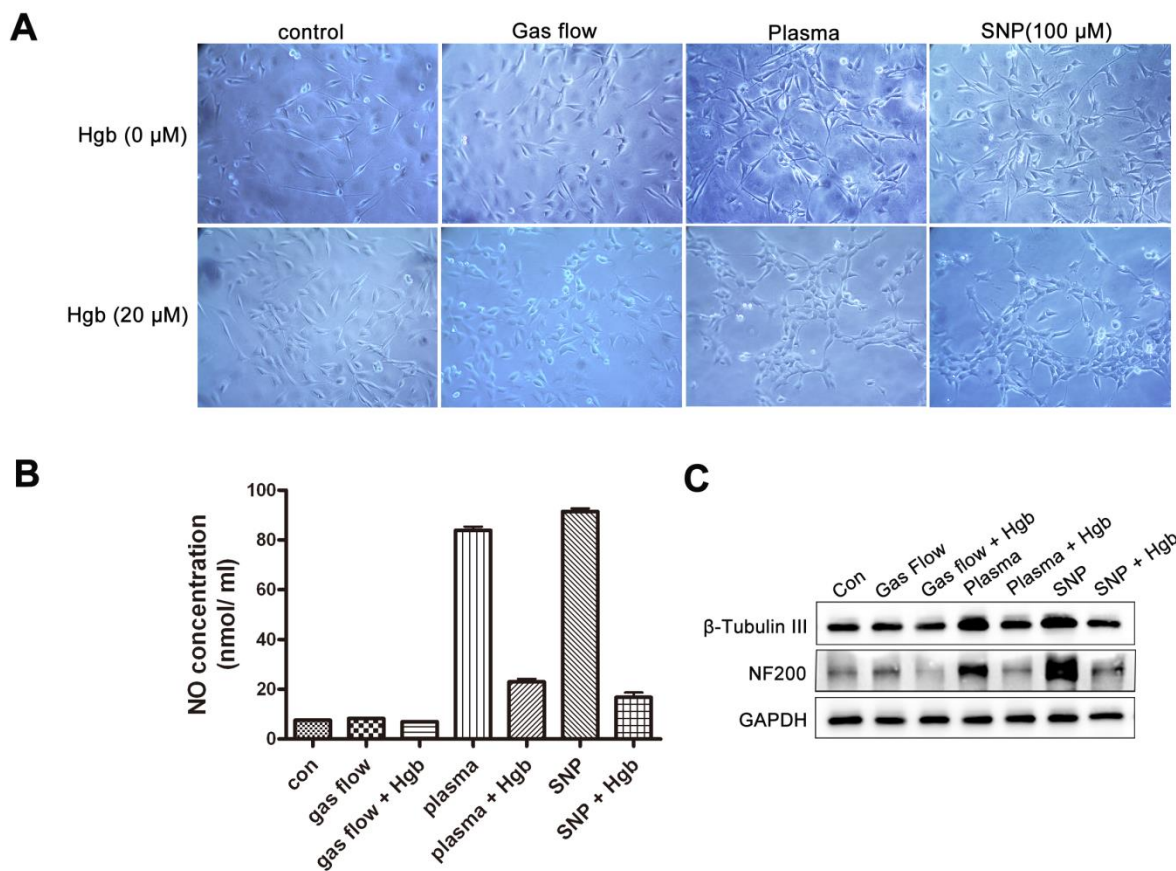


Figure S5 A: inverted phase contrast light microscope images of the C17.2 differentiation. C17.2-NSCs were divided into eight groups: non-treated control, He/O₂ gas flow (60s), plasma (60s), SNP (100 μM, NO donor), Hgb (20 μM, NO-scavenger), He/O₂ gas flow and Hgb co-treated group, plasma and Hgb co-treated group, SNP and Hgb co-treated group. As seen in panel A, C17.2-NSCs with the plasma treatment showed similar differentiation effects as the SNP-treated group. And, the NO-scavenger reduced the plasma-induced NSCs differentiation similarly in SNP treated NSCs (positive control). B: NO production in each group. To monitor the NO production by the plasma, plasma-treated groups were examined immediately after the treatment. And, SNP treated groups were detected 48h after SNP were added (SNP can release NO slowly in the medium). C: Western Blot for the expression of neuron markers. β-Tubulin III and NF200 were markedly up-regulated in the plasma and SNP treatment groups. However, these effects were attenuated by Hgb scavenger.

S5. Additional details of qRT-PCR analysis

Table S1 below shows the primers used in qRT-PCR analysis. For more description of the qRT-PCR analysis and related protocols please refer to the Materials and Methods section, Quantitative real-time PCR (qRT-PCR) section of the main text.

Supplementary Table S1. Primers for qRT-PCR

Genes	Forward primers	Reverse primers
Nestin	5'-GGGTCTACAGAGTCAGATCGCTCA-3'	5'-AGCGAGAGTTCTCAGCCTCCA-3'
β -tubulin III	5'-ACCTATTCAGGCCCGACAACCTTTA-3'	5'-GCAGGCAGTCACAATTCTCACAC-3'
GFAP	5'-GACCAGCTTACGGCCAACAG-3'	5'-TCTATACGCAGCCAGGTTGTTCTC
Olig2	5'-GATGCTTATTACAGACCGAGCCAAC-3'	5'-CAGGGATGATCTAAGCTCTCGAATG-3'
GAPDH	5'-TGTGTCCGTCGTGGATCTGA-3'	5'-TTGCTGTTGAAGTCGCAGGAG-3'

S6. Microplasma jet device

The plasma jet device is driven by a sub-microsecond pulsed DC voltage. It can generate a non-equilibrium plasma plume with a length typically up to 4 cm long in open air. The high-voltage (HV) wire electrode, which is made of a copper wire with a diameter of 2 mm, is inserted into a 4 cm long quartz tube with one end closed. The inner and outer diameters of the quartz tube are 2 mm and 4 mm, respectively. The quartz tube along with the HV electrode is inserted into a hollow barrel of a syringe. The diameter of the hollow barrel is about 6 mm and the diameter of the syringe nozzle is about 1.2 mm. The distance between the tip of the HV electrode and the nozzle is 1 cm. When the working gas is injected into the hollow barrel and the HV pulsed DC voltage is applied to the HV electrodes, a homogeneous plasma is generated in front of the end of the quartz tube, along the nozzle, and in the surrounding air. The length of the plasma plume can be adjusted by the gas flow rate and the applied voltage (amplitude, frequency, and pulse width). Because the device has only one electrode, the discharge is generated between the HV electrode and the surrounding air. High speed ICCD camera (Princeton Instruments, Model: PIMAX2, exposure time down to 2 ns) is used to capture the nanosecond dynamics of the plasma bullets. The applied voltages are measured by a P6015 Tektronix high voltage probe and the currents by a TCP202 Tektronix current probe. More information (including applications) about this and similar types of atmospheric-pressure plasma jets can be found elsewhere (Lu et al., 2008; Wu et al., 2010).

References

- Lu, X., Jiang, Z., Xiong, Q., Tang, Z., Hu, X., Pan, Y., (2008). An 11 cm long atmospheric pressure cold plasma plume for applications of plasma medicine. *Applied Physics Letters* 92, 081502.
- Wu, S. Q., Lu, X. P., Xiong, Z. L., Pan, Y. (2010). A touchable pulsed air plasma plume driven by DC power supply. *IEEE Transactions Plasma Science* 38, 3404-3408.