Therapeutic effect of neural progenitor cells expanded in the 3D nano-engineered Nichoid substrate in a Parkinson's disease preclinical model

S. Carelli[1,2], T. Giallongo[1], F. Rey[1], B. Barzaghini[1,4], G. Al Haj[1], T. Zandrini[3], G. Cerullo[3], R. Osellame[3], A.M. Di Giulio[1,2], M.T. Raimondi[4]

[1]*Department of Health Sciences, University of Milan, Milan, Italy,*
[2]*PediatricClinicalResearch Center Fondazione "Romeo ed Enrica Invernizzi", University of Milano, Milano, Italy.*
[3] *Istituto di Fotonica e Nanotecnologie (IFN)-CNR and Department of Physics, Politecnico di Milano, Milano, Italy.*
[4]*Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milano, Italy*

***Abstract* — 3D microscaffolds are becoming more and more relevant in regenerative medicine, as they lead to the creation of a structure similar to a physiological niche. An example is the nano-engineered Nichoid, a 3D structure in which the cells are able to proliferate. In this work, we investigated the proliferation and stemness properties of Er-NPCs when grown inside the Nichoid, and their potential therapeutic application in the treatment of Parkinson's Disease.**

***Keywords* — Parkinson's disease, Nichoid, biomechanical manipulation, regenerative medicine.**

I. Introduction

Stem cells therapy represents a promising strategy for replacement therapy in neurodegenerative diseases, such as Parkinson's disease (PD), which is the most common neurodegenerative diseases after Alzheimer's disease [1]. In 2011, we isolated a subclass of murine sub ventricular zone-derived neural progenitors, named Erythropoietin releasing post mortem Neural Precursors Cells (Er-NPCs), which show high neuronal differentiation capabilities [2]. Er-NPCs exert high neuroprotective and regenerative effects when transplanted in both traumatic spinal cord injury and Parkinson's disease mice preclinical models [3]-[7]. The use of biomaterials allows the generation of active biophysical signals for directing stem cell fate through 3D microscaffolds, recreating the physiological niche in which stem cells are usually present. In 2013 Raimondi's group applied femtosecond laser two-photon polymerization (2PP) to fabricate a three-dimensional microscaffold, named Nichoid [8]. The Nichoid shows a good ability to maintain and preserve the stemness of primary rodent and human stem cells [9], [10]. The aim of this study was to investigate: i) the proliferation, differentiation and stemness properties of Er-NPCs following their cultivation in the Nichoid substrate; ii) the therapeutic effect and safety of Er-NPCs cultivated in the Nichoid in preclinical experimental model of PD.

II. Materials and Method

Nichoids were fabricated by femtosecond laser two-photon polymerization (2PP) onto circular glass coverslips using a hybrid organic-inorganic SZ2080 photoresist. The laser used for 2PP was a Yb:KYW system producing pulses of 300 fs duration and 1 MHz repetition rate at a wavelength of 1030 nm [10]. Er-NPCs where isolated from SVZ of CD1 mice and characterized for neuronal markers expression as described in Marfia et al. 2011 [2]. Er-NPCs were grown inside the Nichoid for 7 days ($1x10^4$ cells/cm^2), counted and characterized by means of immunofluorescence, western blot and Real Time PCR analysis. To investigate the Er-NPCs growth features inside the Nichoid, cells were plated in standard growth medium for 7 days, then counted and analysed by immunofluorescence analysis, western blot and Real Time PCR analysis. Er-NPCs were transplanted in a murine experimental model of PD after a 7 days' growth inside the Nichoid. Dopaminergic degeneration in C57BL/6 mice was obtained through the administration of MPTP [5]-[7]. $7x10^4$ GFP-positive cells were infused in the mouse left striatum by stereotaxic injection. The effects of transplanted cells were determined by means of performance tests aimed at detecting specific behavioural improvements. The in vivo grafted Er-NPCs' fate and mechanism of action were investigated by immunohistochemistry (Fig.1) [5]-[7].

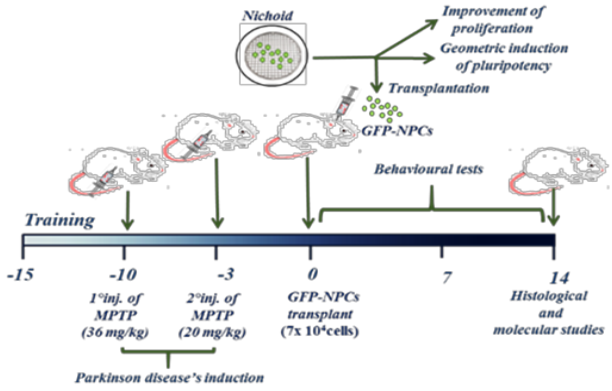

Figure 1: Experimental plan applied for the therapeutic effect evaluation of Nichoid-grown Er-NPCs in a preclinical model of PD.

III. Results

Seven days after plating, Er-NPCs grown inside the Nichoid show a significantly higher cell viability and proliferation than in normal floating culture conditions (Fig. 2).

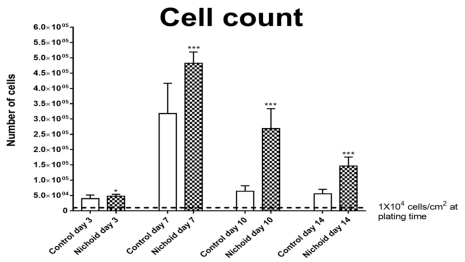

*Figure 2: Er-NPCs were plated in NSC medium at the density of $1X10^4$ cells/cm^2 at the plating. The analysis was performed three time for each condition. Data are expressed as a mean ± SD. The statistical significance of the count performed respect to the control is expressed by an * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ vs control standard floating conditions.*

After the detachment, cells were analysed by Real Time PCR and Western Blot analysis, in order to evaluate the influence of the Nichoid on the stemness capabilities of Er-NPCs. All the three kind of analysis demonstrated that when Er-NPCs are grown inside the Nichoid the expression of stemness markers SOX2, OCT4 and NANOG is increased, suggesting that the Nichoid induces Er-NPCs pluripotency (Fig. 3).

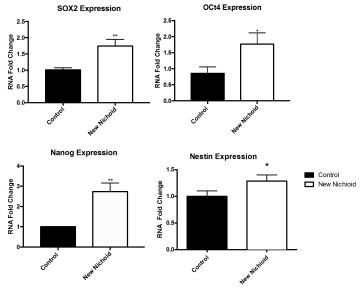

*Figure 3: mRNA levels of staminal marker in Er-NPCs grown in the Nichoid respect to control floating conditions. Data are expressed as a mean ± SD. The statistical significance of the count performed respect to the control is expressed by an * p<0.05 and ** p<0.01 vs control standard floating conditions*

Furthermore, after being re-plated in floating conditions for 7 more days, Er-NPCs formed smaller but more abundant neurospheres with respect to control, suggesting that they have the ability to retain a "memory" of the niche in which they were previously grown. The replated cells, analysed by immunofluorescence, Real Time-PCR and Western blot, also present an increase in pluripotency markers SOX2, OCT4 and NANOG (Fig. 4).

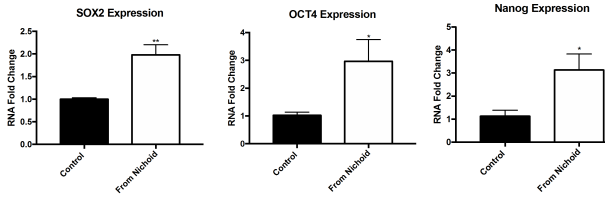

*Figure 4: mRNA levels of staminal marker in Er-NPCs grown both on Nichoid and in standard conditions from 7 days and replated in floating standard conditions for 7 more days. Data are expressed as a mean ± SD. The statistical significance of the count performed respect to the control standard floating conditions is expressed by an * p<0.05 and ** p<0.01.*

The therapeutic potential and safety of Nichoid-grown NPCs was evaluated by their intrastriatal infusion ($7x10^4$ cells) in the brain of PD affected mice. Behavioural performances were evaluated with two different tests showing that Nichoid-grown NPCs promoted the recovery of PD symptoms better than NPCs maintained in normal floating conditions (Fig. 5).

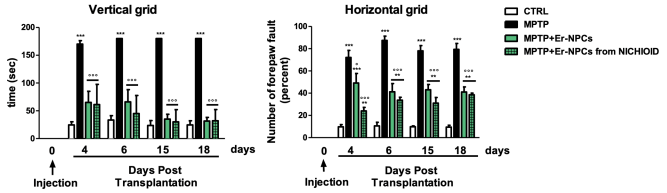

*Figure 5: Horizontal grid test and Vertical grid test show the recovery of function in MPTP animals treated or not with Er-NPCs. A group (n=3) of MPTP animals were infused with both Er-NPCs detached from Nichoid and from normal floating conditions. Data are expressed as a mean ± SD. The statistical significance of the count performed respect to the control is expressed by an ** p<0.01 and *** p<0.001 vs control; ° p<0,05 and °°° p<0.01 vs MPTP.*

IV. DISCUSSION AND CONCLUSION

Er-NPCs demonstrate an increase in pluripotency features when grown inside the Nichoid, and they maintain this increase when they are grown outside the Nichoid. When transplanted in a preclinical model of PD, Er-NPCs from the Nichoid are safe and lead to a recovery of function, which is more efficient than with Er-NPCs grown in standard floating conditions. These results together highlight that the Nichoid strongly improve the therapeutic potential of stem cells and represent a great promise in the field of regenerative medicine applied to neurodegenerative disease.

ACKNOWLEDGEMENTS

Financial support was received from "Neurogel-en-Marche" Foundation (France) to SC; Fondazione "Romeo and Enrica Invernizzi" to AMDG. ERC grant NICHOIDS, G.A. 754467.

REFERENCES

[1] A. Elbaz, L. Carcaillon, S. Kab, F. Moisan. "Epidemiology of Parkinson's disease". *Rev Neurol* (Paris). 172(1):14-26. doi: 10.1016/j.neurol.2015.09.012, 2016.

[2] G. Marfia, L. Madaschi, F. Marra, M. Menarini , D. Bottai et al. "Adult neural precursors isolated from post mortem brain yield mostly neurons: an erythropoietin-dependent process". *Neurobiol Dis*. 43(1):86-98, 2011.

[3] S. Carelli, T. Giallongo, G. Marfia, D. Merli, L. Ottobrini et al. "Exogenous adult postmortem neural precursors attenuate secondary degeneration and promote myelin sparing and functional recovery following experimental spinal cord injury". *Cell Transplant*. 24(4):703-19. doi: 10.3727/096368914X685140, 2015.

[4] S. Carelli, T. Giallongo, Z. Gombalova, D. Merli, A. M. Di Giulio et al. "EPO-releasing neural precursor cells promote axonal regeneration and recovery of function in spinal cord traumatic injury". *Restor Neurol Neurosci*. 35(6):583-599. doi: 10.3233/RNN-170750, 2017.

[5] S. Carelli, T. Giallongo, C. Viaggi, Z. Gombalova, E. Latorre et al. "Grafted Neural Precursors Integrate Into Mouse Striatum, Differentiate and Promote Recovery of Function Through Release of Erythropoietin in MPTP-Treated Mice". *ASN Neuro*. 8(5). pii: 1759091416676147, 2016.

[6] S. Carelli, T. Giallongo, C. Viaggi, E. Latorre, Z. Gombalova et al. "Recovery from experimental parkinsonism by intrastriatal application of erythropoietin or EPO-releasing neural precursors". *Neuropharmacology*. 119:76-90.doi: 10.1016/j.neuropharm. 03.035, 2017.

[7] S. Carelli, T. Giallongo, Z. Gombalova, F. Rey, M. C. F. Gorio et al. "Counteracting neuroinflammation in experimental Parkinson's disease favors recovery of function: effects of Er-NPCs administration". *J Neuroinflammation*.15(1):333. doi: 10.1186/s12974-018-1375-2, 2018

[8] M.T. Raimondi, S.M. Eaton, M. Laganà, V.Aprile, M.M. Nava, et al. "Three-dimensional structural niches engineered via two-photon laser polymerization promote stem cell homing". *Acta Biomater*. 9(1):4579-84, 2013.

[9] M. T. Raimondi, M. M. Nava, S. Eaton, A. Bernasconi, K. Vishnubhatla et al. "Optimization of femtosecond laser polymerized structural niches to control mesenchymal stromal cell fate in culture". *Micromachines*, 5(2), 341-358, 2014.

[10] M.M. Nava, N. Di Maggio, T. Zandrini, G. Cerullo, R. Osellame et al. "Synthetic niche substrates engineered via two-photon laser polymerization for the expansion of human mesenchymal stromal cells". *Journal of tissue engineering and regenerative medicine*, 11(10), 2836-2845, 2017.