V CONGRESSO
GRUPPO NAZIONALE DI BIOINGEGNERIA
(GNB 2016)

20-22 Giugno 2016

Università degli Studi di Napoli "Federico II"

ABSTRACT eBOOK

A miniaturized *in vitro* 3D model to assess the neuroprotective effect of mesenchymal stromal cell secretome on neuroblastoma cells exposed to oxidative stress

L. Boeri[1,2], M. Tunesi[1,3], C. Giordano[1,3], D. Albani[2], and M.T. Raimondi[1]
[1] *Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, Milan, Italy*
[2] *IRCCS-Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy*
[3] *Unità di Ricerca Consorzio INSTM, Politecnico di Milano, Milan, Italy*

***Abstract*—To develop reliable and effective *in vitro* models for basic neuropathological research purposes, we have optimized a miniaturized microfluidic bioreactor. In this work we have adapted this 3D cell culturing concept to assess the effect of mesenchymal stromal cell (MSC) secretome on neuronal-like cells (SH-SY5Y) subjected to oxidative stress. MSC secretome has shown a significant neuroprotective effect, laying the groundwork for molecular studies searching for protective effect mediators.**

***Keywords*—Bioreactor, 3D cell culture, neuroprotection, mesenchymal stromal cell.**

I. Introduction

BECAUSE of the biological complexity of the mammalian brain, conventional 2D culture systems fail to provide topographical cues, enable cell differentiation into specific phenotype and fully reproduce *in vivo* cell behaviour [1]. Mimicking the 3D physical microenvironment of the multi-cellular cerebral architecture is thus crucial to study both neuropathological features and innovative therapeutic approaches.

We have recently developed a miniaturized bioreactor for the dynamic culturing of 3D cell constructs under perfused condition (Fig. 1A). It is also optically accessible, allowing the direct observation of cell constructs by viable staining and a standard fluorescence microscope (Fig. 2) [2].

In this work, we have optimized this 3D culture system to develop a reliable and effective *in vitro* model of Parkinson's disease to study the neuroprotective effect of mesenchymal stromal cell (MSC) secretome on SH-SY5Y neuroblastoma cells subjected to oxidative stress.

II. Materials and Methods

A. The miniaturized and optically accessible bioreactor

The dynamic culture system is characterized by three independent culture chambers, two of which may be eventually connected by a hydraulic bypass. Each chamber contains a 3D scaffold (6x3x0.4 mm, 3D Biotek, Fig. 1B). The scaffolds are made of poly(styrene) and they are obtained by fuse deposition modelling. They are composed of four layers of fibres (100 μm diameter, with a pore size of 300 μm) shifted of 150 μm with respect to the adjacent. The miniaturized bioreactor is optically accessible thanks to the optical transparency and low thickness of the components, allowing the direct observation of cell constructs while in culture, without the need of stopping the flow of culture medium [2].

B. Cell culture

SH-SY5Y human neuroblastoma cells (ATCC® CRL-2266™) were used as a model of dopaminergic neurons, while MSCs were extracted from rat bone marrow.

SH-SY5Y cells were cultured in Dulbecco's Modified Eagle medium, while MSCs were kept in alpha-Minimum Essential medium, both supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin sulphate (Euroclone®).

Cell seeding in 3D scaffolds was optimized from a previous work [2]. The scaffolds were placed in an ultra-low attachment 24-well microplate (Corning® Costar®) and incubated overnight with 400 μl medium (under 5% CO_2 at 37 °C). The following day, they were covered with cell suspension (0.5 or $1 \cdot 10^6$ SH-SY5Y cells/400 μl; $0.25 \cdot 10^6$ MSCs/400 μl) and shaken by a 3D multifunction rotator (PS-M3D, VWR) for 3 h. The following day the samples were moved into clean wells and covered with 400 μl fresh medium.

To evaluate the number of SH-SY5Y cells in 3D scaffolds, cells were detached and lysed by repeated cycles of freezing (-80 °C) and thawing (37 °C on a orbital shaker). 2 μl lysate was assayed on a NanoQuat Plate™ (Infinite M200 PRO, Tecan) and DNA concentration was calculated by measuring the absorbance at 260 nm. Finally, the total number of cells in 3D scaffolds was estimated considering 6.4 pg DNA as the total amount of DNA in a human diploid cell [3]. Results were compared to those obtained after plating $0.5 \cdot 10^6$ SH-SY5Y cells in a standard 24-well microplate.

C. Oxidative damage

To produce oxidative and mitochondrial damage, SH-SY5Y cells were exposed to different concentrations (125 and 250 μM) of the neurotoxin 6-hydroxydopamine (6-OHDA, Sigma-Aldrich) in static or shaken conditions (by placing the samples on a multifunctional rotator). To assess the stability of the induced oxidative damage with time, cell metabolic activity was evaluated by Alamar Blue® assay (resazurin sodium salt, Sigma-Aldrich), at subsequent time points.

D. Conditioning assay

To assess the neuroprotective effect of MSC secretome on damaged SH-SY5Y cells, 3D scaffolds were assembled in the microfluidic bioreactor (Fig. 1A). A scaffold seeded with MSCs was placed in chamber 1, while chamber 2 hosted a scaffold seeded with SH-SY5Y cells previously damaged with 6-OHDA. We have connected chamber 1 and 2 by a bypass, so that MSC secretome could flow through the scaffold seeded with damaged SH-SY5Y cells. As a control, chamber 3 hosted a 3D scaffold seeded with damaged SH-SY5Y cells not conditioned by MSC secretome. After 3 days of conditioning, the bioreactor was disassembled and cell metabolic activity was evaluated by Alamar Blue® assay.

E. Alamar Blue® assay in dynamic conditions

Taking into consideration the possibility of assessing cell metabolic activity while in perfused culture, we have determined the conditions in which the results from Alamar Blue® assay in static conditions (incubation with 400 µl working solution for 3 h) are comparable to Alamar Blue® assay in dynamic conditions (perfusion with working solution at a flow rate of 5.0 µl/min for 3 h).

F. Western Blotting

To search for biochemical mediators of the neuroprotective effect of MSC secretome, 20 µg of the total protein extracts were assessed by Western Blotting and stained with antibodies specific to target proteins involved in neuroprotection against oxidative stress, such as Hsp70 and SIRT3 [4, 5]. We have quantified protein signals by densitometric analysis using ImageJ software.

G. Statistical analysis

Two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used for comparisons among the groups and time frames, while one-way ANOVA followed by Tukey's multiple comparison test was used for comparisons among the groups. The significance level was set at *p-value* < 0.05. All the results were reported as mean ± standard deviation (SD).

III. Results

A. Assessment of oxidative damage on SH-SY5Y cells

DNA measurements have indicated that the scaffolds seeded with $1 \cdot 10^{-6}$ SH-SY5Y cells contained a greater number of cells with respect to those seeded with $0.5 \cdot 10^{-6}$ SH-SY5Y cells and the 2D controls. However, morphological analysis has shown that scaffolds seeded with a greater cell density revealed pore occlusion due to cell clusters. On the contrary, scaffolds seeded with $0.5 \cdot 10^{-6}$ SH-SY5Y cells have shown a more homogeneous cell distribution in the fibres. For this reason, $0.5 \cdot 10^{-6}$ SH-SY5Y cells/scaffold was selected as seeding density.

The incubation with 6-OHDA reduced cell metabolic activity. In particular, we observed a reduction by 50% and 80% (mean value) for samples treated with 125 µM 6-OHDA or 250 µM 6-OHDA, respectively (*p-value* < 0.0001). Since a stable damage may be induced with a lower toxin concentrations, the incubation with 125 µM 6-OHDA may be preferred.

B. Neuroprotective effect of MSC secretome on SH-SY5Y cells

In the conditioning assay, SH-SY5Y cells conditioned with MSC secretome have shown a significant recovery from damage by up to 30% with respect to the untreated control. The protocol for Alamar Blue® assay adapted to perfused culture resulted in a reliable method to assess cell metabolic activity while in culture, avoiding the need to remove cell constructs from the bioreactor.

Currently we are searching for molecular basis of this neuroprotective effect. So far, we have identified Hsp70 and SIRT3 as possible candidates.

IV. Conclusion

Coherently with previous observations in static *in vitro* models and *in vivo* [6, 7], our advanced *in vitro* model was able to reproduce the neuroprotective effect of MSC secretome on damaged neuronal cells. In addition, thanks to the perfusion and optical accessibility of the bioreactor, our system offers the advantage of monitoring the effect of MSC conditioning non-destructively and for a longer culture period in comparison to standard 2D cultures, thus mimicking features of chronic neurodegeneration more realistically.

Acknowledgement

We acknowledge funding from European Commission (ERC grant No. 646990-NICHOID).

Disclosure

The authors have nothing to disclose.

References

[1] Y. Lai, K. Cheng, W. Kisaalita. *Three dimensional neuronal cell cultures more accurately model voltage gated calcium channel functionality in freshly dissected nerve tissue.* PLoS One. 2012; 7:e45074.

[2] M. Laganà, M. T. Raimondi. *A miniaturized, optically accessible bioreactor for systematic 3D tissue engineering research.* Biomed Microdevices. 2012 Feb; 14:225-34.

[3] J. Dolezel, J. Bartos, H. Voglmayr, J. Greilhuber. *Nuclear DNA content and genome size of trout and human.* Cytometry A. 2003 Feb; 51:127-8.

[4] M. Amadio, G. Scapagnini, U. Laforenza, M. Intrieri, L. Romeo, et al. *Post-transcriptional regulation of HSP70 expression following oxidative stress in SH-SY5Y cells: the potential involvement of the RNA-binding protein HuR.* Curr Pharm Des. 2008; 14:2651-8.

[5] S. H. Dai, T. Chen, Y. H. Wang, J. Zhu, P. Luo, et al. *Sirt3 protects cortical neurons against oxidative stress via regulating mitochondrial Ca2+ and mitochondrial biogenesis.* Int J Mol Sci. 2014 Aug 21;15:14591-609.

[6] Y. Yan, T. Ma, K. Gong, Q. Ao, X. Zhang, et al. *Adipose-derived mesenchymal stem cell transplantation promotes adult neurogenesis in the brains of Alzheimer's disease mice.* Neural Regen Res. 2014 Apr 15; 9:798-805.

[7] P. Hao, Z. Liang, H. Piao, X. Ji, Y. Wang, et al. *Conditioned medium of human adipose-derived mesenchymal stem cells mediates protection in neurons following glutamate excitotoxicity by regulating energy metabolism and GAP-43 expression.* Metab Brain Dis. 2014 Mar; 29:193-205.

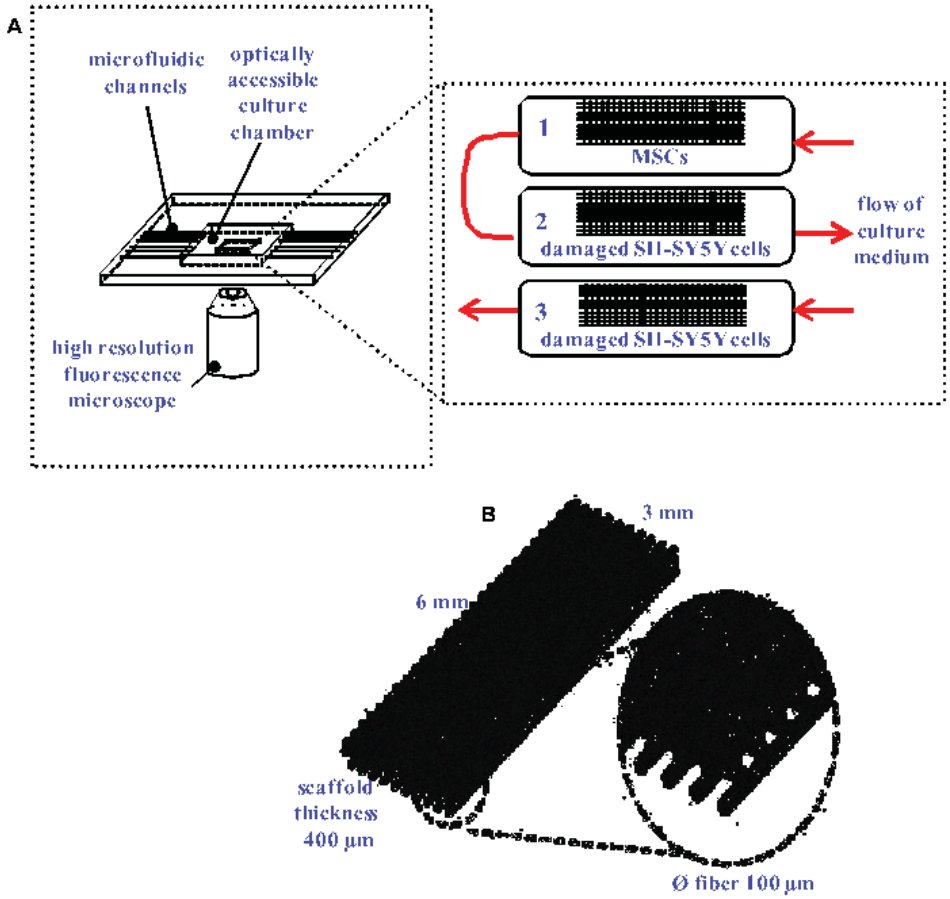

Fig. 1. (A) Scheme of experimental set-up. Culture medium is conveyed to the chambers by a syringe pump and microfluidic channels. The optical transparency and low thickness of the components allow the direct observation of cell constructs by a standard fluorescence microscope. Chambers 1 and 2 are connected by a hydraulic bypass. Chamber 1 contains a scaffold seeded with MSCs, chamber 2 hosts a scaffold seeded with damaged SH-SY5Y cells, while chamber 3 is independent and hosts a scaffold seeded with damaged SH-SY5Y cells. (B) 3D scaffold (6 mm×3 mm×0.4 mm).

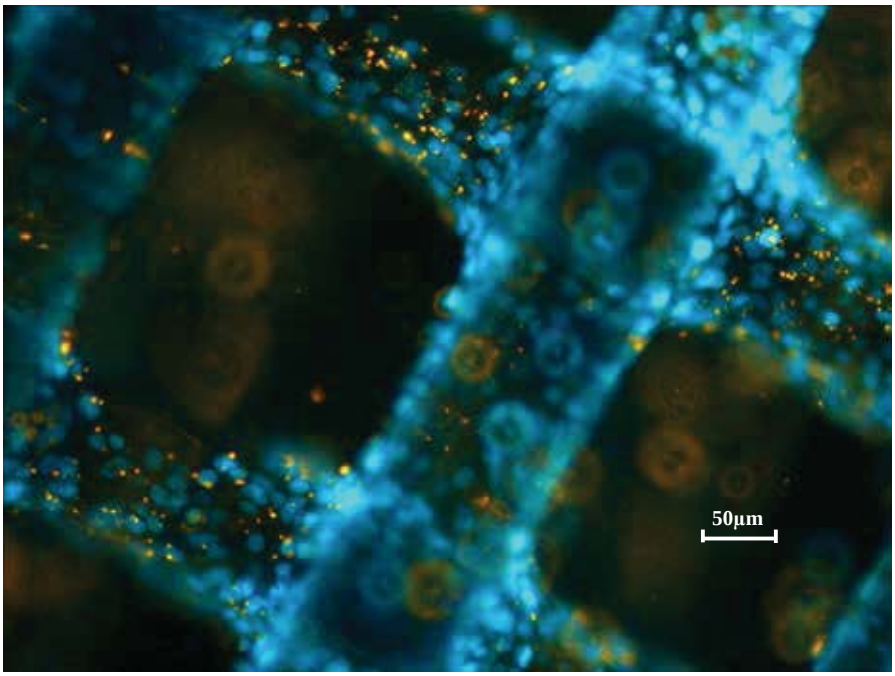

Fig. 2. Live imaging of a cell-seeded 3D scaffold under perfusion in the bioreactor. Cells are double-labelled with DAPI (nucleus, in blue) and quantum dots (cytoplasm, in orange).

ORGANIZING SECRETARIAT
YES MEET
Via S. Nicola, 4
80067 – Sorrento (NA), Italy
Tel. +39 0818770604
Fax + 39 0818770258
info@yesmeet.it

PUBLISHER
EDIZIONI ZIINO – Italy
www.stampacongressi.com
info@stampacongressi.com

ISBN
978-88-941906-0-1