Title: **Biomimetic microenvironmental preconditioning of human WJ-MSC enhance their neuroprotective properties**

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Abstract:

Tissue engineering and regenerative medicine are currently vast and rapidly growing research fields. The intrinsic qualities of stem cells, including differentiation potential and self-renewal, make them a frontline source for the cell therapy. However, protection of the transplanted cells from the host immunological attack and the modulation of their properties by in vitro preconditioning are the main issues to consider for successful treatment.

Wharton's Jelly-derived mesenchymal stem cells (WJ-MSCs) were encapsulated in 3D hydrogels derived from human fibrin (FB) or platelet lysate (PL) and the oxygen level was adjusted to physiological normoxia (5% O2). The influence of the type of the scaffold and physiological normoxia conditions was tested on the WJ-MSCs' survivability, proliferation, migratory potential, the level of expression of selected trophic factors, cytokines, and neural markers. For ex vivo studies an experimental model of oxygen glucose deprivation was used in order to mimic an ischemic injury. MSCs-induced neuroprotection was evaluated after 24 h in OHC co-cultured with WJ-MSCs in 2D or 3D conditions. WJ-MSCs from control (2D) and 3D scaffolds were characterized with qRT-PCR for the expression of growth factors and cytokines after 24 h of co-culture.

Encapsulated WJ-MSCs revealed high survivability, stable proliferation rate, and ability to migrate out of the hydrogel and the up-regulated expression of all tested factors, e.g., BDNF, GDNF, VEGF-A, bFGF as well as the increased expression of neural differentiation markers. Ex vivo studies with indirect co-culture of organotypic hippocampal slices and cell-hydrogel bio-constructs revealed strong neuroprotective effect of WJ-MSCs against neuronal death in the CA1 region of the rat hippocampus. This effect was potentiated further by FB scaffolds under 5% O2 conditions. Moreover, WJ-MSCs cultured on 3D scaffolds revealed the increased expression of several neurotrophins (BDNF, NGF), growth factors (bFGF, EGF) and decreased expression of pro-inflammatory cytokines, e.g., IL-1β, together with higher expression of anti-inflammatory TGF-β1.

The results have indicated that physiological normoxia and 3D microenvironmental conditions induce positive neuroprotective and immunomodulatory response of WJ-MSCs in injured neuronal tissue. Moreover, the analysed scaffold models, together with modulating oxygen level allow building up biomimetic conditions for in vitro stem cells culture and serving as a promising material for future use in MSCs-based therapy.

Sponsored by NCBR grant No STRATEGMED1/234261/2/NCBR/2014, statutory funds to MMRI and ESF, POWR.03.02.00-00-I028/17-00.