







Title: Effect of long-term 3D neurosphere culture on WJ-MSC

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## **Abstract:**

2D culture conditions are typical and characteristic for mesenchymal stromal/stem cells (MSC). Under special treatment MSC can be also cultured in 3D conditions for a short time. We attempted to develop a protocol enabling a derivation from Wharton Jelly derived MSC (WJ-MSC) subpopulation with increased expression of pluripotent and neural genes. We decided to apply 3D conditions for longer time than standardly in hope to select cells with primitive features. We examined the effects of prolonged sphere culture on WJ-MSC – influence on proliferation, senescence, clonogenicity, pluripotent and neural gene expression. WJ-MSC were cultured in 3D neurosphere induction medium for about 20 days in vitro. Then, cells were transported to 2D conditions and confronted to the initial population and population constantly cultured in 2D. Longtermed sphere culture of WJ-MSC resulted in increased senescence, decreased stemness and proliferation. We observed reduced viability of cells during 3D culture. However spheroid culture allowed for selection of cells exhibiting increased expression of early neural and SSEA4 markers what might indicate the survival of cell subpopulation with unique features. Nevertheless, we continue searching for isolation method of primitive subpopulation in MSC that could be applied in therapies.

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