Spheroid-like Cultures for Cell Expansion of Angiopoietin Receptor-1 (aka. Tie2) positive Cells from the human Intervertebral Disc

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INTRODUCTION:Low back pain is the leading cause of disability worldwide (1). Nevertheless, the mechanism of the intervertebral disc (IVD) degeneration is still not clear. In this context, the nucleus pulposus (NP) and more precisely NP progenitor cells (NPPCs) present in the IVD, positive for angiopoietin-1 receptor (aka. Tie2) display multipotent and stem capacity (2,3). In this study, the first aim was to determine whether spheroid formation in suspension-culture will increase the amount/percentage of NPPCs during the expansion compared to traditional monolayer culture. The second aim of this study was to investigate if the percentage of NPPCs will be enriched even further by the resuspension of the spheroid-like cultured cells (=1st generation) and reformation of those spheroids one more time (= 2nd generation).

METHODS:Human NP tissues from trauma patients (N=3) were obtained with written ethical consent and isolated by a two-step digestion protocol (3). The NP cells were resuspended and frozen at -150°C after reaching confluence of passage 0. At passage 1, NP cells were seeded in standard or ultra-low attachment tissue culture flasks with 2.5 ng/ml FGF-2 in low glucose - DMEM (supplemented with 10 % FBS). Flow cytometry was used to analyze and quantify the percentage of NPPCs using Tie2 antibody. We defined the spheroids formed after passage 1 NPCs as 1st generation spheroid. We obtained the 2nd generation spheroids by resuspending the 1st-generation-spheroid and reassembly. The NPCs from 1st and 2nd spheroid were quantified by CFU-assay.

RESULTS:As a result, the percentage of NPPCs in monolayer culture condition was reaching 7 ± 2 % (Mean±SEM), however, in the 1st and 2nd generation spheroids culture condition, we were observing 20 ± 10 % and 28 ± 6% of Tie2+ cells, respectively. Concerning the CFU-assay, the NPCs from the 2nd generation spheroid formed 30 CFU-S per 1,000 cells, which were twice more CFU-S compared to the 1st generation spheroid. DISCUSSION & CONCLUSIONS:From these data we conclude than the spheroid-like formation of NPCs would be a more efficient method for expansion and enrichment of NPPCs than monolayer expansion in a context of future cell therapy.

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