Exploring a Novel Spheroid 3D Cell Culture System for Tie2+ Nucleus Pulposus Cells of the Intervertebral Disc

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Introduction

Low back pain (LBP) is a major problem in our ageing society. LBP impacts the patient's quality of life and places an immense burden on the healthcare system worldwide [1]. Degeneration of the intervertebral disc (IVD) is one of the common causes of LBP. Recently, nucleus pulposus (NP) progenitor cells (NPPC) were discovered, which are positive for Angiopoietin-1 receptor (aka. Tie2/CD202b). These NPPCs are a promising cell source for IVD regeneration and may spark the development of novel cell therapies. NPPCs are pretty rare (2-10% of all IVD cells) in human IVDs and diminish in number with increasing age [2]. The low numbers of sorted primary NPPCs necessitate an expansion of the NPPCs. It has been demonstrated that 3D culture is superior to classic 2D culture to maintain the pluripotent phenotype of the NPPCs. The goal of this research is to test the expansion and culture of bovine NPPCs cells in a novel spheroid cell culture plate.

Materials and Methods

NP cells were isolated from bovine tails (aged 10-14 months). Primary NP cells were stained with a Tie2 antibody conjugated with Alexa 488 and DAPI for fluorescence activated cell sorting (FACS). Sorted NP cells were expanded for 2 weeks under hypoxia (2% O_2) and were supplemented with 2.5 ng/ml basic fibroblast growth factor (bFGF) until 80% confluency. Tie2+ and Tie2- cells were then seeded in the functionalized and in the 2D control wells of the spheroid plate SP5D of Kugelmeier Ltd (see Fig. 1). The cells were cultured for 2 weeks under hypoxia and with bFGF. Colony forming unit-assay was started on day 0. Cell activity, DNA, glycosaminoglycan (GAG) content and gene expression of selected genes were analysed on day 1, 8 and 15 using qPCR.

Results

Cell isolation from NP tissue yielded on average 15.7 million primary cells of which 10'497 cells (0.063%) were positive for Tie2. Assessing the colony-forming ability of expanded cells revealed no significant differences between Tie2+ and Tie2- cells (see Fig. 2). Cells in 2D plastic control wells proliferated significantly more in comparison to the spheroids ($p = 0.0002$). Analysing gene expression after expansion revealed a 5.48-fold increase of KRT19 and a 4.37-fold increase of SOX2 in Tie2+ cells in relation to Tie2- cells. Gene expression of KRT19 and SOX2 was found to be significantly downregulated in Tie2+ cells in spheroid and 2D culture after 15 days.

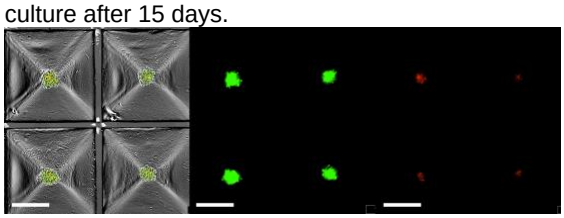

Fig.1: Tie2- NP spheroids (400 cells) in the SP5D stained for live (Calcein AM, green) and dead (ethidium homodimer, red) cells, 30h after seeding. Imaged with 10x magnification in IncuCyte S3. Scale bar = 200 µm

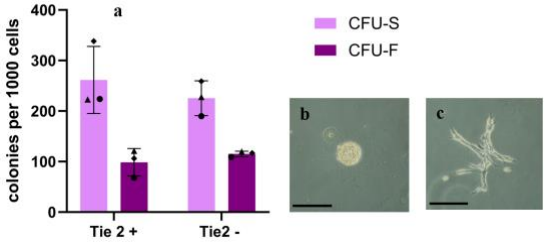

Fig.2: a) Results of colony-forming unit assay. Mean ± SD, N = 3. Spheroid (b) and fibroblastic (c) colony forming unit of bovine NP cells, scale bar = 200 µm

Discussion

The results indicate that sorted NPPCs differentiated during expansion, resulting in a low share of Tie2+ cells. Successive culture in the SP5D revealed that such expanded Tie2+ cells could not be kept in a non-differentiated stem-cell like state. The results of the study show that further research on NPPCs has to be conducted. Nevertheless, NPPCs are undeniably a promising approach for therapy of the degenerated IVD.

References

[1] GBD 2017 Disease and Injury a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Lond. Engl.* **392**, 1789–1858 (2018).
[2] Sakai, D. *et al.* Exhaustion of nucleus pulposus progenitor cells with ageing and degeneration of the intervertebral disc. *Nat. Commun.* **3**, 1264 (2012).

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