phenotype in isosmotic and hyperosmotic culture condition by monitoring some of their important NPC surface antigens by flow cytometry.

Methods: Human NPC cells were expanded in monolayer culture to passage 1 and then seeded at 10,000 cells in each well of the 6-well plate in duplicates and cultured in α-MEM containing 10 % FCS either in isosmotic medium (300 Osm/L), or in hyperosmotic medium (400 Osm/L) adjusted with 1 % 5 M NaCl and 0.4 M KCl. [2,3] Cells were trypsinized after 7 days culture and stained with three NPC surface markers (GD2, Tie2, CD24) for flow cytometry characterization. Cumulative population doubling and changes of NPC cell surface antigens were monitored at day 0, 7, 14, 21 and 28.

Results: Cumulative population doubling time indicated that NP cell growth was inhibited in hyperosmotic condition over the 28-day culture, which was 50 % lower in hyperosmotic condition than isosmotic condition (Fig. 1). Our data indicated that the hyperosmotic culture medium could inhibit the growth of NPC both in monolayer culture (Fig. 2). It is postulated that the hyperosmotic condition in the NP plays a role in maintaining the quiescent state of the NP cells and maintenance of their phenotype.

Conclusions: Our data indicated that the hyperosmotic culture medium could inhibit the growth of NPC both in monolayer and 3D alginate beads culture. It is hypothesized that the hyperosmotic condition in the NP plays a role in maintaining the quiescent state of the NPC and the maintenance of their phenotype. Hyperosmotic medium also tended to increase the percentage of GD2 + cells in monolayer culture.

Acknowledgments: This project was funded by two projects of the Swiss National Science Foundation grant number # IZK0Z3_154384 and #310030_153411.

References

1. Spillekorn et al (2014) Tissue Eng Part C
2. Tao et al (2013) Cell Biol Int 37:826–834

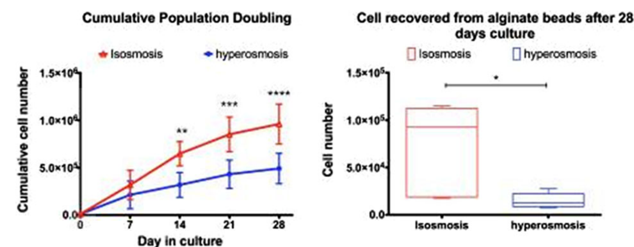

Fig. 1 NPC cumulative population doubling in isosmatic or hyperosmotic medium in monolayer (left) and 3D alginate beads (right). Cell growth was statistical significantly higher in isosmotic then hyperosmotic media starting from day 14, 21 and 28. (N = 5) Cells cultured in alginate were recovered after 28 days culture. *Indicate statistical significantly difference between isosmotic and hyperosmotic condition: *$p < 0.05$, ** <0.01, ***<0.0001, ****$p < 0.00001$)

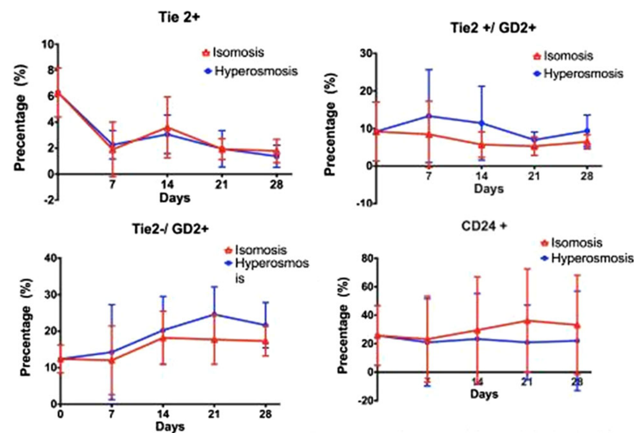

Fig. 2 Changes of nucleus pulposus cell surface antigens when expanded in isotonic or hypertonic medium. Osmolarity did not affect Tie2+ cell percentage, but hyperosmotic condition seemed to increase GD2+ cells and maintained CD24+ cell population than isosmotic condition. Plot of mean ± SD, N = 5

22

BOVINE COCCYGEAL INTERVERTEBRAL DISCS CONTAIN MULTIPOTENT TIE2+ CELLS WHICH CAN DIFFERENTIATE INTO OSTEOGENIC AND ADIPOGENIC LINEAGES

*A. Tekari[1], S. C. Chan[1,2], K. Wuertz[3,4], D. Sakai[4,5], L. M. Benneker[4,6], S. Grad[4,7], B. Gantenbein[1,4]

[1]Tissue and Organ Mechanobiology Group, Institute for Surgical Technology and Biomechanics, University of Bern, Bern, Switzerland;
[2]Bioactive materials, EMPA, Swiss Federal Laboratories for Materials Science and Technology, St Gallen, Switzerland;
[3]Institute for Biomechanics, ETH Zurich, Zurich, Switzerland;
[4]AOSpine Research Network, Duebendorf, Switzerland;
[5]Department of Orthopedic Surgery, Tokai University School of Medicine, Isehara, Kanagawa, Japan;
[6]Department of Orthopedic Surgery, Inselspital, University of Bern, Bern, Switzerland;
[7]AO Research Institute, Davos, Switzerland

Question: The intervertebral disc (IVD) has a limited regenerative potential and low back pain represents a leading cause of disability [1]. IVD repair strategies require an appropriate cell source that is able to regenerate the damaged tissue such as progenitor stem cells. Recently, progenitor cells that are positive for the angiopoietin receptor (Tie2) in the nucleus pulposus were identified [2]. Here we isolated primary cells from bovine IVD and sorted bovine nucleus pulposus progenitor cells (NPPC) for the marker Tie2. Furthermore,

source: https://doi.org/10.7892/boris.67971 | downloaded: 20.4.2024

we tested whether Tie2 expressing cells can differentiate into osteogenic and adipogenic lineages in vitro.

Methods: NP cells were obtained from 1 year old bovine tails by sequential digestion with pronase for 1 h and collagenase overnight. Sorted Tie2− and Tie2+ cells were cultured in osteogenic and adipogenic medium for 3 weeks. The formed cell layers from both subpopulations were stained for calcium deposition and fat droplets. Colony forming units were prepared for both cell suspensions in methylcellulose-based medium and formed colonies (>10 cells) were analyzed macroscopically after 8 days.

Results: After 3 weeks of culture, sorted Tie2+ cells were able to differentiate into osteocytes and adipocytes as characterized by calcium deposition and fat droplet formation. By contrast, Tie2− cells generated a weak staining for calcium and no fat droplets were obtained (Fig. 1). Sorted Tie2− and Tie2+ subpopulations of cells both formed colonies, however with different morphologies. The colonies formed from Tie2+ cells were spheroid in shape whereas those from Tie2− cells were spread and fibroblastic.

Conclusion: Our data showed that Tie2+ cells of the nucleus pulposus cells are progenitor-like cells that are able to differentiate into osteogenic and adipogenic lineages. Sorting of NPPC for Tie2 may represent a promising strategy with the potential to be used in the clinics for treatment of intervertebral disc damage.

References

1. Freemont AJ (2009) The cellular pathobiology of the degenerate intervertebral disc and discogenic back pain. Rheumatology (Oxford) 48:5–10
2. Sakai D, Nakamura Y, Nakai T et al (2012) Exhaustion of nucleus pulposus progenitor cells with ageing and degeneration of the intervertebral disc. Nat Commun 3:1264

Acknowledgments: This project was funded by two projects of the Swiss National Science Foundation grant number #IZK0Z3_154384 and #310030_153411.

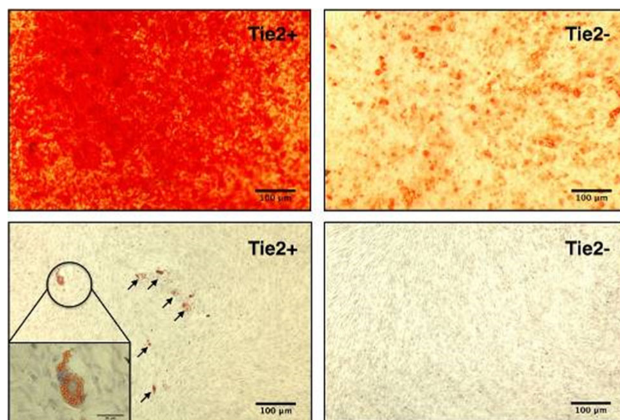

Fig. 1 .

25

INTERVERTEBRAL DISC REGENERATION: THE ROLE OF MSCS RECRUITMENT BY HYALURONAN-BASED DELIVERY OF STROMAL CELL-DERIVED FACTOR-1 IN MATRIX PRODUCTION IMPROVEMENT

*C. Leite Pereira[1,2], R. M. Gonçalves[1], D. Eglin[3], M. Alini[3], S. Grad[3], M. A. Barbosa[1,2]

[1]INEB, Instituto De Engenharia Biomédica, Porto, Portugal;
[2]ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal;
[3]AO Research Institute Davos, Davos, Switzerland

Question: Intervertebral disc (IVD) degeneration is characterized by loss of water content and some matrix components. Simulated degenerating IVDs were shown to release chemoattractors that can recruit human mesenchymal stem/stromal cells (hMSCs) [1]. Recently our team, showed that hMSCs recruitment towards IVD could be potentiated by the injection of a stromal cell derived factor-1 (SDF-1) delivery system in an ex vivo model of degenerating IVD [2]. Here the goal was to evaluate if recruited hMSCs could contribute to stop and/or revert the degenerative state of IVD, while improving the production of IVD healthy matrix.

Methods: An IVD model of nucleotomized bovine discs was used. IVDs were maintained in culture during 21 days and tissue viability was assessed. Metabolic activity was analysed by rezasurin conversion and cell proliferation was evaluated by DNA content. hMSCs recruitment was promoted by injection of SDF-1 (250 ng/disc) using a hyaluronan hydrogel carrier (HAP). After 21 days of culture, the presence of hMSCs and proliferating cells was evaluated by Immunofluorescence (IF) for anti-human nuclei and Ki67 (proliferation marker). The contribution of the recruited hMSCs to IVD regeneration was assessed by histology (H&E and Safranin-O), IF for Coll II and Coll XI and western blot for Coll II. GAGs were also quantified in the tissue.

Results: A long term culture was established, IVDs remained viable during 21d, with constant metabolic activity and DNA content. hMSCs were observed within the IVD tissue after 21d of culture, and some proliferative cells ($Ki67^{+}$, both hMSCs and disc cells) were observed (Fig. 1). Injection of HAP + SDF-1 did not promote overall GAGs production and expression of Col XI was similar for all the groups tested. Nevertheless, Col II increased from 43.84 % (cavity) to 93.09 % Cavity + (HAP + SDF-1) (Fig. 2). Moreover, higher intensity staining for chondroitin 6-sulphate was observed in the nucleolus pulposus region of groups treated with HAP + SDF-1, in comparison to the controls.

Conclusions: hMSCs recruited by HAP-SDF-1 can contribute to IVD regeneration by stimulating the production of IVD key matrix components, namely Col II. Further experiments will be performed to evaluate other matrix components, such as Col I, Col XI and aggrecan. The results presented here constitute a proof of concept for an innovative therapy for IVD regeneration aiming to enhance the tissue's endogenous repair capacity.