Investigating the Physiological Relevance of *Ex Vivo* Disc Organ Culture Systems for the Development of Cell Therapies based on Nutritional Demands

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INTRODUCTION:There has been significant interest in developing cell-based therapies aiming to repopulate the nucleus pulposus (NP) and augment tissue repair. *Ex vivo* disc culture systems have become a valuable tool during development and preclinical testing, providing a platform between cell culture and *in vivo* studies. It is critical to assess these therapies under the hostile biochemical niche typically experienced *in vivo*, ensuring normal cellular function and regeneration[1]. However, it remains to be elucidated whether the *ex vivo* culture microenvironments are comparable to human degeneration. This work aims to create a validated computational model which can be used to predict the metabolite gradients generated in *ex vivo* culture systems.

METHODS:Finite element models of cultured disc were created using COMSOL Multiphysics®. These models were governed by coupled reaction-diffusion equations, taking into account geometrical differences, cell viability, cellular metabolism and nutrient diffusion through the different tissue domains. Metabolic rates were dependent on local oxygen and pH levels by employing equations derived previously[2] [3]. Experimental verification of these models was performed by measuring the metabolite concentrations in discs cultured for 7 days, in a custom-made bioreactor.

RESULTS:The diffusion distance across the NP was significantly different between bovine caudal disc locations (n=6, $P<0.05$). The viable cell density employed computationally was 5,578±801 cells/mm³ and 14,465±3,937 cells/mm³ for the NP and AF (n=3), respectively. Cell density remained constant as there was no significant difference in NP viability at day 7. The predicted central glucose concentration of a disc cultured in 25mM media was 6.22mM, which was within the standard deviation (SD) of the experimental value 5.35±1.47 mM (n=3). The lactate concentration predicted under the same conditions, was 10.44mM, which also lay within the SD of the experimental measurement, 9.64±1.49 mM (n=3). The experimental pH level decreased from 7.3±0.1 in the media to 6.4±0.1 in the disc centre (n=8). While the predicted pH in the disc centre ranged from 6.3 to 6.6 depending on disc size. DISCUSSION & CONCLUSIONS:This work presents the first experimentally verified predictive model, advancing the knowledge of the microenvironment within *ex vivo* disc cultures. Ultimately, it is imperative that the critical metabolite values (minimum glucose, oxygen & pH values) are matched to those at a stage of human IVD degeneration, where regenerative cell-therapy is an appropriate strategy, to realise successful clinical translation based on nutritional demands.

References:[1] Buckley (et al.) JOR Spine. 1:e1029;2018 [2] Bibby (et al.) Spine. 30:487-496;2005 [3] Huang & Gu J. Biomech. 41:1184-1196;2008

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BMP Antagonists – A Possible Cause for Spinal Non-Fusion?

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INTRODUCTION:Spinal fusion is a procedure where the intervertebral disc (IVD) is removed and two adjacent vertebrae are forced to fuse by compression. This procedure is the most commonly applied procedure to achieve spinal stability and relief of back pain. However, non-successful fusion leads to pseudo-athrosis and ongoing pain. There is increasing evidence that supraphysiological doses of BMP2 and burst-release of this cytokine did not generate satisfying results in clinical studies. Current hypothesis was raised that IVD cells and/or tissue seem to inhibit the action of BMP2. In this overview we summarize the current evidence that BMPs might be inhibited by the secretome of human IVD cells, i.e., nucleus pulposus cells (NPC), annulus fibrosus cells (AFC) and cartilaginous endplate (CEPC) cells.

METHODS:We stimulated low-passage (2-3) human bonemarrow-derived mesenchymal stromal cells (MSCs) and femoral hip-derived osteoblasts (OBs) and co-cultured these with allogeneic IVD cells obtained from spinal surgery. We then stimulated MSCs and the OBs in monolayer and osteogenic medium, whereas IVD cells were kept in 3D alginate bead culture and separated by high density pore culture inserts (0.4 µm pore size). We quantified relative gene expression at bone-relevant genes, alkaline phosphatase (ALP) activity and Alizarin red (ALZR) staining after 21 days. Furthermore, to test the effect of a previously investigated BMP2 analog to block the inhibitors, cells were further stimulated with 100 ng/mL BMP2 and/or L51P.

RESULTS:We found significant inhibitory effects of IVD cells onto MSCs undergoing differentiation in presence of NPC, AFC and CEPC as shown in reduced osteogenic gene expression, ALZR staining and ALP activity (N = 11 donors paired on each side). In the case of allogeneic human OBs only a trend towards inhibition could be demonstrated (N = 7 donors on each side). The addition of L51P to the co-culture recovered ossification. On the side of the IVD cells BMP2 and/or L51P had a strong chondrogenic effect.

DISCUSSION & CONCLUSIONS:Our data suggested evidence for inhibition for MSCs. However, OBs did not show the same inhibitory effects but showed a trend in presence of IVD's secretome. This warrants for animal models where the donor variance can be better controlled.

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Keywords: Intervertebral disc / spine and their disorders, Biologics and growth factors

Non-Viral Transfection of Nucleus Pulposus Cells with FOXF1 induces reprogramming to a Healthy Phenotype in-vitro and in-vivo