Jump to step: Preview

Please ensure that the information below is correct and is displayed correctly.

Conditioned medium of intervertebral disc cells inhibits osteogenesis on autologous bone-marrow-derived mesenchymal stromal cells and osteoblasts (99933)

SHUIMU CHEN [1 2], Andreas S. Croft [1 2], Sebastian Bigdon [3], Zhen Li [4], Christoph E. Albers [3], Katherine B. Crump [1 2], Paola Bermudez [1 2], Benjamin Gantenbein [1 3]

1. *Tissue Engineering for Orthopedics & Mechanobiology (TOM), Bone & Joint Program, Department for Bi-oMedical Research (DBMR), Faculty of Medicine, University of Bern, Bern, Switzerland*
2. *Graduate School for Cellular and Biomedical Sciences (GCB), University of Bern, Bern, Switzerland*
3. *Department of Orthopaedic Surgery & Traumatology, Inselspital, Bern, Switzerland*
4. *AO , Research Institute Davos, Davos, Switzerland*

INTRODUCTION: Low back pain (LBP) is a significant global burden and is associated with the degeneration of the spine and human intervertebral discs (hIVD). Current surgical treatment for hIVD degeneration is the removal of the affected tissue with a cage to promote spinal fusion and relieve discomfort. Despite progress in the treatment of LBP, however, in ~30% of all cases this procedure ends in non-fusion and painful pseudo-athrosis after operation. Previous research from our laboratory showed morphogenetic protein (BMP) antagonists secreted by the intervertebral disc have been potentially associated with inhibiting the process of osteogenesis. However, the co-cultured cells did not come from the same donor. In this study, we investigated the hypothesis that IVD cells secrete BMP inhibitors that inhibit osteogenesis in autologous osteoblast (hOB) and bone marrow mesenchymal stem cell (hMSC).

METHODS: Conditioned Medium (CM) collected from primary hIVD cells in 3D alginate culture was co-cultured with seven donor-matched hOB and hMSC in 2D culture. Osteogenesis after 10 days was then quantified at the transcript level using qPCR to measure the expression of bone makers and BMP antagonists, and at the protein level by alkaline phosphatase (ALP) activity. Additionally, they were evaluated histologically by alizarin red (ALZR) staining on Day 21. The relationship between ALP activity, osteogenesis and Noggin expression in hOB or hMSC or hIVD was investigated to uncover the potential causes.

RESULTS: ALP activity significantly decreased and the formation of calcium deposits in alizarin red staining was inhibited after culture with CM derived from hIVD. Interestingly, less changes of bone makers and BMP inhibitors' expression were found in hOB or hMSC on Day 10. Noggin was relatively higher expressed (Average fold change: AF, 6.9; CEP, 10.0; NP, 6.3; relative to autologous hOB. AF, 2.3; CEP, 3.4; NP, 3.2; relative to autologous hMSC.) in hIVD compared to hOB or hMSC.

DISCUSSION: The up-regulation of Noggin mRNA (and possibly other BMP inhibitors) in residual nIVD tissue after spinal fusion surgery is potentially a potent reason for the prevention of successful osteogenesis. Similar results were found previously with allogeneic co-cultures. However, in the previous study, there were merely trends of inhibition. Here we show a significant decrease with autologous donor-matched samples.

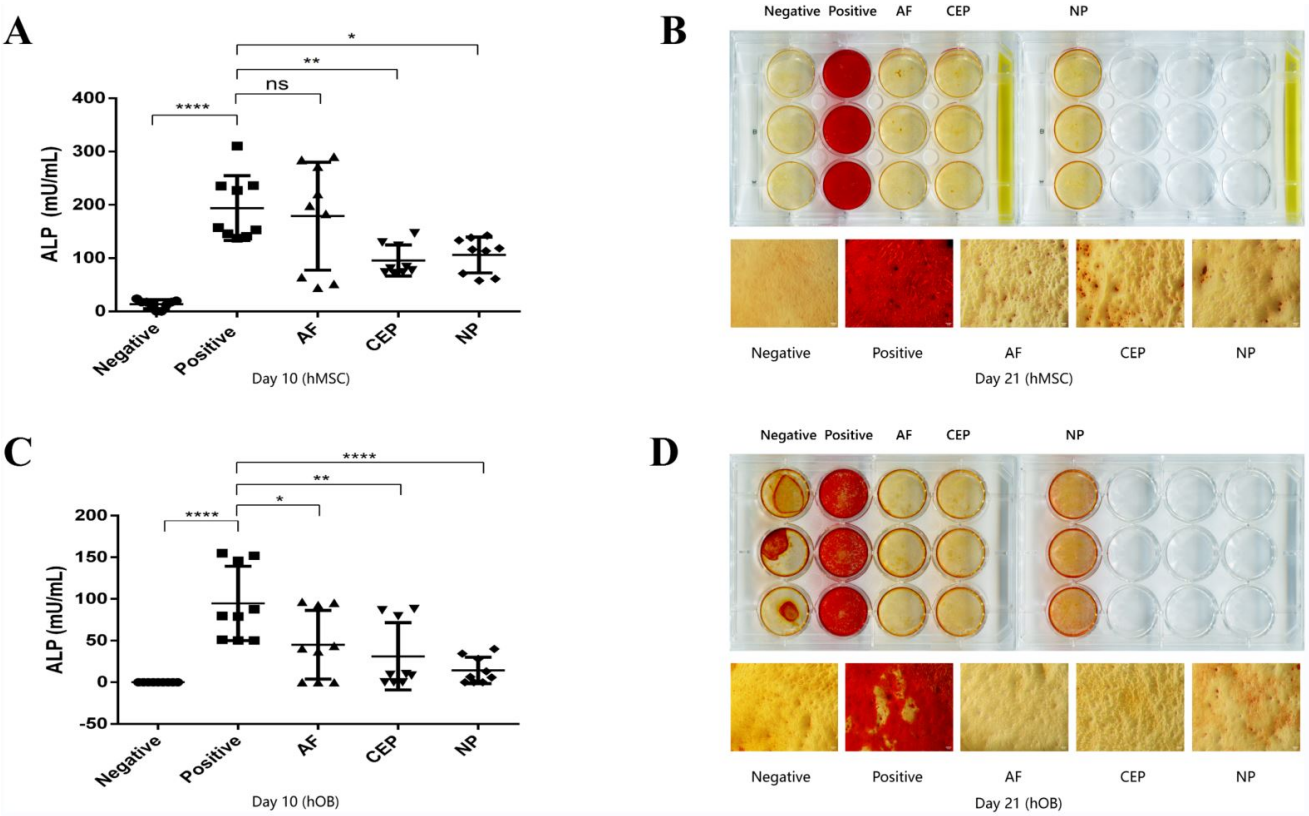

Figure: The osteogenesis of human OBs/MSCs was inhibited after culture with CM from autologous IVD cells. (A, C) ALP assay showed ALP activity significantly decreased when adding CM from IVD cells into autologous OBs and MSCs to culture for 10 days. (B, D) A trend of lower calcium deposit could be observed on Day 21 using alizarin red staining after OBs and MSCs cultured with CM from autologous different IVD cells. (Calcium deposits: Bright orange red; Scale bar, 100um. Mean ± SD. p-value, * < 0.05; * * < 0.01; * * * < 0.001; * * * * < 0.0001; N =3-5)

1. Ferreira ML, et al. Global, regional, and national burden of low back pain, 1990–2020, its attributable risk factors, and projections to 2050: a systematic analysis of the Global Burden of Disease Study 2021. Lancet Rheumatol 5(6):e316-e329.
2. May RD, et al. Inhibitory Effects of Human Primary Intervertebral Disc Cells on Human Primary Osteoblasts in a Co-Culture System. Int J Mol Sci. 2018 Apr 13;19(4):1195.
3. Tekari A, et al. The BMP2 variant L51P restores the osteogenic differentiation of human mesenchymal stromal cells in the presence of intervertebral disc cells. Eur Cell Mater 33: 197-210