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NON-VIRAL GENE DELIVERY OF GROWTH AND DIFFERENTIATION FACTOR 6 (GDF6) TO WHOLE BOVINE INTERVERTEBRAL DISC

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Question: Low back pain is an increasing global health problem, which is associated with intervertebral disc (IVD) damage and degeneration. Major changes occur in the nucleus pulposus (NP), with the degradation of the extracellular matrix (ECM) [1]. Further studies showed that growth factors from the transforming growth factor (TGF) and bone morphogenic proteins (BMP) family may induce chondrogenic differentiation of mesenchymal stem cells (MSC) [2]. Focusing on non-viral gene therapies and their possible translation into the clinics, we investigated if GDF6 (syn. BMP13 or CDMP2) can induce regeneration of degraded NP. We hypothesized that IVD transfected with plasmid over-expressing GDF6 also up-regulates other NP- and chondrogenic cell markers and enhances ECM deposition.
Methods: Bovine IVD cells were isolated by pronase/collagenase II overnight digestion. After monolayer expansion up to passage 3, cells were transfected with the plasmid pGDF6 (RG211366, Origene, SF) or with green fluorescence protein (GFP) control using the Neon® transfection system (Invitrogen, Basel), both equipped with a Cytomegalovirus (CMV) promotor to induce over-expression. We tested a range of yet unpublished parameters for each of the primary disc cells to optimize efficiency. To test a non-viral gene therapy applied directly to 3D whole organ culture, bovine IVDs were harvested from fresh tails obtained from the abattoir within 5 h post-mortem [3]. Discs were then pre-incubated for 24 h in high glucose Dulbecco's Modified Eagle Medium and 5 % fetal calf serum. Each disc was transfected by injection of 5 µg of plasmid GDF6 (Origene, RG211366) into the center by 25G needle and using Hamilton syringe. Electroporation was performed using 2-needle array electrode or tweezertrodes; 8 pulses at 200mv/cm with an interval of 10 ms were applied using ECM830 Square Wave Electroporation System (Harvard Apparatus, MA) (Fig. 1). After transfection discs were cultured for 72 h to allow expression of GFP or GDF6. Discs were then fixed, cryosectioned and analysed by immunofluorescence against GDF6.
Results: We successfully transfected bovine NP and AF cells in monolayer culture with the two plasmids using a 1,400 V, 20 ms and 2 pulses with a ~25 % efficiency using 0.15 M cells and 3 µg DNA (Fig. 1). Organ IVD culture transfection revealed GFP6 positive staining in the centre of the disc using 2-needle array electrode. Results from tweezertrodes did not show any GFP positive cells.
Conclusions: We identified novel parameters to successfully transfect primary bovine IVD cells. For transfection of whole IVD explants electroporation parameters need to be further optimized.
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References

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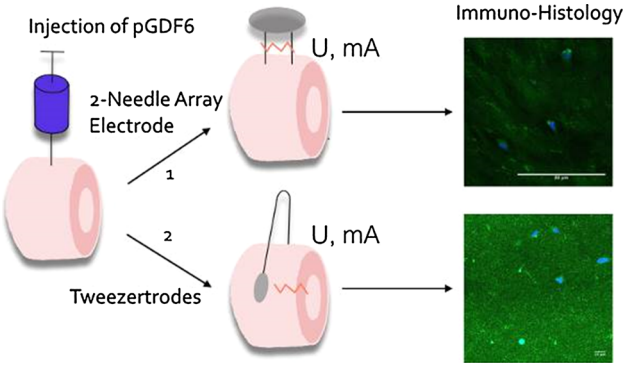

Fig. 1

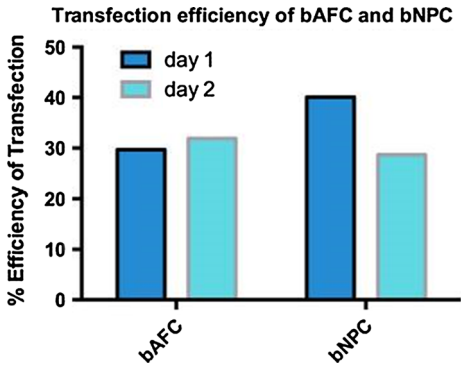

Fig. 2 .

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IS HYPEROSMOSIS A BETTER ENVIRONMENT TO MAINTAIN NUCLEUS PULPOSUS PROGENITOR CELLS (NPPC)?

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Question: The high glycosaminoglycans side-chains of the aggrecan in the intervertebral disc (IVD) raise the osmolarity of the nucleus pulposus, therefore, nucleus pulposus cells (NPC) are under hyperosmotic condition inside the disc. Recent studies raised contradictory results in the NPC responses to isosmotic and hyperosmotic culture conditions, while one proposed hyperosmotic culture condition could preserve the matrix production activity of NPC [1], but another study suggested that a hyperosmotic condition reduced NPC viability and matrix production [2]. This study aims to monitor cell growth and changes of the NPC