Nose to back: spheroids derived from human nasal chondrocytes for nucleus pulposus repair

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I have nothing to disclose.
Introduction:

Cell therapy for IVD repair shows limited success. Recently, NCs have been identified as a possible cell source for the treatment of IVD degeneration. Benefits of NCs are:

- Autologous cell source with high self-renewal and plasticity [1]
- Safe and feasible in human clinical trials for cartilage repair [2,3]
- More resilient in in vitro conditions mimicking the physiological environment of a degenerative IVD than commonly administered cells of IVD clinical trials [4]

Aims:

Assessing the therapeutic potential of NCS for IVD cell therapy: their capacity for injectability, NP-like ECM accumulation, and integration in conditions simulating DDD.

IVD = intervertebral disc; NCs = human nasal chondrocytes; NCS= NC spheroids; ECM = extracellular matrix; DDD = degenerative disc disease
Methods:

Experimental design. (A) Isolation of nasal chondrocytes (NC) from human nasal septal cartilage tissue and fabrication of NC chondrospheres (NCS), (B) Assessing the potential of NCS for injectability and their characterisation in in vitro and ex vivo settings.

NP = nucleus pulposus cells, FBS = fetal bovine serum, HSA = human serum albumin, TDA = TGFβ3 (T), dexamethasone (D), ascorbic acid (A).
Results: NCS injectable through spinal needle were reproducibly generated

Injectability of NCS generated in GM and CHM. (A) Size (diameter) and (B) shape (roundness) of NCS. 600 μm and 0.8 were set as size and shape thresholds, respectively. NCS morphology on day 1, 3, and 7 in (C) GM and (D) CHM. Scale bar = 200 μm. (E) Relative shear stress related gene expression in NCS 24h after being passed through the spinal needle. (F-G) Images of NCS passed through the spinal needle. (n = 3-5 donors, mean ± SD, **p < 0.01, ANOVA).

NCS = nasal chondrocytes spheroids; GM = growth medium, CHM = chondrogenic medium, ne = not expressed.
Biomechanical properties of NCS is tuneable depending on the culture supplements.

**Biomechanical and biochemical characterization of NCS cultured in GM and CHM.** (A) $E$ of NCS measured by Microsquisher ($E$ of healthy NP tissue: 5kPa). (B-C) Correlation (Pearson’s $r$) between $E$ of NCS and other tested parameters for (B) GM and (C) CHM. (D-E) Relative gene expression of (D) aggrecan, and (E) collagen type II. (F-H) Biochemical content of NCS, (F) GAG/DNA ratio, (G) HYP/DNA ratio, (H) GAG/HYP ratio ($n = 3-5$ donors, mean ± SD, *$p < 0.05$, **$p < 0.01$, ANOVA).
NCS are viable and rapidly fuse with NP in DDD-mimicking conditions

Fusion kinetics of NCS with NPS in control and DDD-mimicking conditions. (A) Schematic representation of the fusion process. Fusion parameters as (B) intersphere angle, (C) contact length, and (D) doublet length, were assessed. (E) Viability of NCS-NPS during the fusion process (mean ± SD, *p < 0.05, 2-way ANOVA). (F) Catabolic shift measured by IL-8 expression within NP micro-tissue model containing either NCS or NC suspension (mean ± SD, *p < 0.05, unpaired t test). (NP: n=1-2, NC: n=3).

NCS = nasal chondrocytes spheroids; NPS = nucleus pulposus spheroids; DDD = degenerative disc disease
Injected NCS reside within bovine disc without visible damage or leakage

Preparation of intact bovine tail IVD for organ culture & injection of NCS into it. (A) Bovine tails were received max. 24h after slaughter. (B) The muscles were removed and the discs were isolated by cutting through the proximal and distal disc-cartilage endplate boundaries. After degenerating the discs with papain, NCS were injected into the NP tissue and cultured for 24h in GM statically. (C) safranin o staining visualizing proteoglycan content of NCS within the fixed degenerated IVD (4µm sections) containing and (D) alu staining detecting human cells within the bovine disc (violet coloured cells = human cells).

NCS = nasal chondrocytes spheroids; IVD = intervertebral disc; GM = growth medium

Discussion:

Our study provides \textit{in vitro} evidence of the applicability of NCS for scaffold-free NP repair:

- Size and shape of all NCS, except day 1 NCS cultured in CHM, satisfied the requirements.

- NCS injection through the spinal needle did not affect the NCS morphology nor change the expression of shear stress related genes hence intradiscal NCS delivery is feasible.

- Biomechanical properties of NCS correlate with its collagen content. Depending on which culture supplements are used, the elastic modulus is tuneable.

- NCS (vs. NC suspension) experience delayed catabolic shift that might prolong their survival within the degenerating IVD.

- NCS are capable to fuse with NP even under DDD-mimicking conditions.

- Long-term effects of the DDD condition on the NCS will be investigated ex vivo under dynamic loading and in a large animal model.

\textit{CHM} = chondrogenic medium; \textit{NCS} = nasal chondrocytes spheroids; \textit{NPS} = nucleus pulposus cells; \textit{DDD} = degenerative disc disease
Summary points:

- NCS are compatible with a spinal needle.
- Biomechanical properties of NCS are tuneable by culture supplements.
- In DDD mimicking conditions, NCS performed superior to NC single-cell suspension.
- NCS fusion with NP did not impair within DDD mimicking conditions *in vitro* supporting fast adhesion *in vivo* that might possibly prevent cell leakage.
- DDD mimicking conditions did not impair NCS viability *in vitro*.
- Injected NCS resided in the NP of *ex vivo* cultured bovine IVD with no visible signs of damage or leakage.

*NCS = nasal chondrocytes spheroids; DDD = degenerative disc disease; NP = nucleus pulposus cells*