Notochordal cell-based treatment strategies and their potential in intervertebral disc regeneration

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Supplementary Material

Supplementary Table 2. Studies using NC-rich NP tissue for IVD regeneration purposes (2011-2020)

Study	Source (animal)	Decellularization	Generation	Cells/tissue where the effect is determined on	Effect
Mercuri, 2011 (1)	* Porcine (*n*=?, age=?)	* 72 h incubation in decellularization solution (50 mM Tris-aminomethane buffer containing 0.1% (w/v) ethylenediamine tetra acetic acid and 0.02% (w/v) sodium azide). Four options were tested: - 1: 0.15% (v/v) Triton X-100 with 0.25% (w/v) deoxycholic acid - 2: 0.1% Triton X-100 with 0.15% deoxycholic acid - 3: 0.05% Triton X-100 with 0.05% deoxycholic acid - 4: 0.6% Triton X-100 with 1% deoxycholic acid * Samples incubated in 720 mU/mL DNase + 720 mU/mL RNase (48 h) * Effect of ultrasonication was tested (10 min periods every 24 h) with option 1 and 4	N/A	* Human ASCs (*n*=?, age=?), monolayer culture on scaffold, $2.5*10^4$ cells/cm^2	* Decellularization was best achieved in combination with ultrasonication (lowest DNA content) * *in vitro* - 7 days: ASC viability and proliferative capacity were maintained
Mercuri, 2013 (2)	* Porcine (*n*=?, age=?)	* Decellularization solution (72 h): 50 mM Tris-aminomethane buffer (pH 7.5) containing 1% (w/v) deoxycholic acid, 0.6% Triton X-100 (v/v), 0.1% (w/v) ethylenediamine tetra acetic acid, and 0.02% (w/v) sodium azide * 10 min periods of ultrasonication (every 24 h) followed by 720 mU/mL DNase + 720 mU/mL RNase treatment (48 h) prior to 0.1% peracetic acid sterilization (4 h)	N/A	* Human ASCs (*n*=?, age=?), monolayer culture on scaffold, $2*10^3$ cells/cm^2 * *In vivo* biocompatibility tested in male juvenile Sprague-Dawley rats (*n*=20), scaffold was implanted in subdermal pockets	* *in vitro* - 14 days: ASCs expressed NP-cell markers. GAG and collagen content of ASC-seeded hydrogels increased vs. non-seeded controls **in vivo* - 28 days: presence of mononuclear cells (e.g. macrophages and fibroblasts), blood vessel infiltration, collagen deposition
Pei, 2012 (3)	* Porcine (*n*=2, 3 months of age)	* After NC monolayer culture reached 90% confluence, 50 μM ascorbic acid was added (8 days) * Deposited ECM was incubated with 0.5% Triton X-100 + 20 mM ammonium hydroxide (37°C, 5 min)	N/A	* Porcine SDSCs (*n*=2, 3 months of age, 3000/cm^2) were expanded on ECM deposited by NCs, SDSCs, or NC:SDSC (50:50); thereafter, the expanded SDSCs were cultured	* 14 days: ECM deposited by NC:SDSCs increased SDSC viability and differentiation toward the NP lineage; this effect is comparable

				in pellets (300,000 cells) in chondrogenic medium (incl. TGF-β_3)	with ECM deposited by SDSCs but higher than that deposited by NCs alone
Liu, 2014 (4)	* Porcine (n=?, age=?)	N/A	* Lyophilization, crushed and resuspended in culture medium	* human induced pluripotent stem (iPSC) cell line HDFa-YK26 * Cells were differentiated for 10 days * Thereafter pellets were generated and chondrogenically differentiated for 28 days	* 10 days differentiated: NP matrix induced iPSCs differentiation towards NC-like cells with expression of NC marker genes (T, CK8, CK18). * 28 day chondrogenically differentiated: NC-like cells generated NP-like tissue rich in aggrecan and collagen type II
Liu, 2015 (5)	*Porcine (n=?, 2 years of age)	N/A	* Lyophilization, crushed and resuspended in culture medium	* human induced pluripotent stem (iPSC) cell line HDFa-YK26 * Cells were differentiated for 10 days * Thereafter pellets were generated and chondrogenically differentiated for 28 days	* 10 days differentiated: NP matrix induced iPSCs differentiation towards NC-like cells with expression of NC marker genes (T, CK8, CK18). * 28 day chondrogenically differentiated: NC-like cells generated NP-like tissue rich in aggrecan and collagen type II
Wachs, 2017 (6)	* Porcine (n=?, age=?)	* NP tissue was immersed in water (7 h), followed by 100 mM sodium and 50 mM phosphate buffer (10 h), SB-10 detergent (4 h), 100 mM sodium and 50 mM phosphate buffer (15 min), Triton X-200/SB-16 (3 h), 100 mM sodium and 50 mM phosphate buffer (3 × 15 min), SB-10 detergent (1.75 h), 100 mM sodium and 50 mM phosphate buffer (15 min), Triton X-200/SB-16 (3 h), 100 mM sodium and 50 mM phosphate buffer (3 × 15 min), and DNase/RNase (75 U/mL/100ug/mL; 34 h)	N/A	* Human NPCs (*n*=?, age=?) were mixed with solubilized decellularized NP samples. The mixture was pipetted in 30 µL droplets (40,000 cells/gel) and incubated (45 min, 37°C) for thermal gelation. Thereafter, the gels were cultured in well-plates	* 21 days: NPC viability was maintained, with morphology similar to native NPCs, and increased GAG deposition
Bai, 2017 (7)	* Rabbit (*n*=18, 3 months of age)	N/A	* Two hour digestion with 0.025% collagenase type II	* Human NPCs (*n*=5, 44-53 years of age) cocultured with partially digested NC-rich NP tissue (transwell, $1 \cdot 10^4$ NPCs/well)	* 14 days: increased proliferation, *T* and *KRT18* expression and chondrogenic ECM production

Zhou, 2018 (8)	* Porcine (*n*=?, age=?)	* NP tissue was immersed in SB-10 detergent (4 h), Triton X-200/SB-16 (3 h), SB-10 detergent (1.75 h), Triton X-200/SB-16 (3 h), and DNase/RNase (75 U/mL/100 lg/mL; 36 h)	* Lyophilization, crushed and mixed with CS (1.7 mg/mL), PBS, ASCs ($2.0*10^6$/mL) * Concentration: 3.5 mg/mL (based on GAG content before and after decellularization) * Genipin (0.02% w/v) for subsequent cross-linking	* *In vitro*: Human ASCs (*n*=?, age=?) * *In vivo*: rabbit ASCs (*n*=5, 4 month old) in rabbit IVD degeneration (puncture model: IVDs were stabbed with 16G needle, depth 5 mm)	* *In vitro* - 7, 14 days: NP-like differentiation of human ASCs * *In vivo* - 16 weeks: partly regenerated the degenerated rabbit NP
De Vries, 2018 (9)	* Porcine (*n*=12, 3 months of age)	N/A	* Lyophilization, resuspended in plain medium * Concentration was adjusted to a similar protein concentration as NCCM (~0.4 mg/mL)	* Bovine NPCs in alginate beads (*n*=6, 2-2.5 years of age vs. 4-6 years of age, $3*10^6$ cells/mL alginate) * Adolescent bovine NPCs were also cultured with 5 ng/mL interleukin-1β	* 28 days: increased DNA and GAG content in adolescent and adult bovine NPCs * 28 days: NC-rich NP tissue anabolic effect was stronger compared with NCCM derived from the same porcine spines * 28 days: porcine NC-rich NP tissue exerted stronger effect than bovine NPC-rich NP tissue * 28 days: anabolic response was observed in an inflammatory environment
De Vries, 2018 (10)	* Porcine (*n*=5, 3 months of age)	N/A	* Lyophilization, resuspended in plain medium (2 mg/mL)	* HUVEC monolayer culture (pool of 10 donors, *n*=5 biological replicates) * Human neuroblastoma SH-SY5Y monolayer culture (poly-D-lysine coated well plate versus polystyrene culture surface; *n*=5 biological replicates)	* 24 hours: no anti-angiogenic and anti-neurogenic effects observed; on a polystyrene surface, it even induced a higher number of neurite-expressing cells
Bach, 2018 (11)	* Porcine (*n*=6, 3 months of age)	N/A	* Lyophilization, resuspended in plain medium (10 mg/mL)	* Canine (*n*=6, 2–7 years of age) and human (*n*=6, 47–72 years of age) NPC micro-aggregates *in vitro*	* *In vitro* - 28 days: increased chondrogenic ECM production of NPCs and MSCs *in vitro* * *In vivo* - 6 months: beneficial effects at macroscopic and MRI level, induced collagen type II-rich

				* Canine MSC micro-aggregates *in vitro (n*=3, 4 months - 3 years of age*)* * Degenerated canine IVDs *in vivo* (*n*=6, 14 months of age)	ECM production, improved the disc height, and ameliorated local inflammation *in vivo*
Xu, 2019 (12)	* Porcine (*n*=20, age =?)	* After 5 freeze-thaw cycles, NP tissue was immersed in 0.5% SDS for 8 h followed by 2 h 200 U/mL DNAse treatment and flushing in PBS for 12 h to eliminate residual chemicals	N/A	* *in vitro* - Human MSCs (n=?, age=?) * *in vivo* – Rabbit IVDs (n=?, 6 weeks of age, degeneration induced 4 weeks before treatment injection)	* *in vitro*- 14 days: MSCs seeded in the NP-ECM scaffold differentiated into NP-like cells with aggrecan and collagen type 2 expression due to increased TGF/Smad signaling * *in vivo* - 8 weeks: decelerated the degeneration of the IVD on MRI

ASC: adipose-derived mesenchymal stromal cells, CK: cytokeratin, ECM: extracellular matrix, GAG: glycosaminoglycans, h: hours, HUVEC: human umbilical vein endothelial cells, IVD: intervertebral disc, KRT: cytokeratin, min: minutes, MRI: magnetic resonance imaging, MSC: mesenchymal stromal cell, N/A: not applicable, NC: notochordal cell, NCCM: NC conditioned medium, NP: nucleus pulposus, NPC: nucleus pulposus cell, SDSC: synovium-derived stem cell, T: brachyury, TGF: transforming growth factor

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