Towards 3D bioprinting of a hypoxia-gradient for generating a heterogenous cartilage scaffold

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Background

Heterogenous, patient-specific tailored cartilage scaffolds are needed to repair large nasal septum perforations The inclusion of oxygen gradients in scaffolds, thus might offer a strategy to control chondrocytes phenotype by activating pathways that lead to the formation of hyaline or hypertrophic cartilage.

Aim: To investigate hypoxia-related activation of differentiation pathways in nasal chondrocytes (NCs) to further translate this knowledge into fabricating a zonally organized cartilage scaffold.

Methods

NCs were isolated from the nasal septum cartilage biopsies of patients undergoing septoplasty. Cells were cultured either in 2D or 3D aggregates (i.e. pellets) under normoxic conditions (21%-O_2), hypoxic conditions (2%-O_2), or in a normoxic environment supplemented with hypoxia inducing compound (21%-O_2 + 1mM DMOG) for 14 days. The effect of oxygen tension on chondrogenesis was determined with histological assessments of matrix deposition (by Alcian Blue (AB), or Safranin-O) and by RT-qPCR analysis.

Results

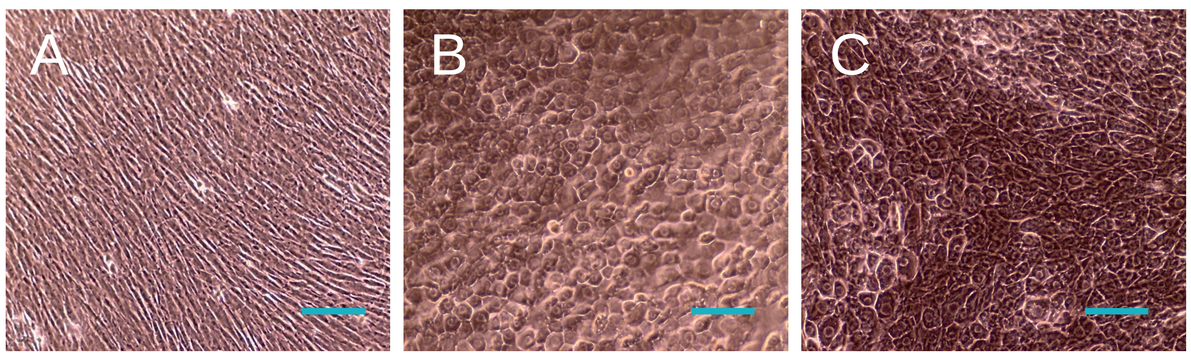

Fig. 1: Phase-constrast microscopic appearance of representative NC monolayers at monolayers under normoxia (A), normoxia + 1mM DMOG (B) or hypoxia (C). Scale bar = 100µm.

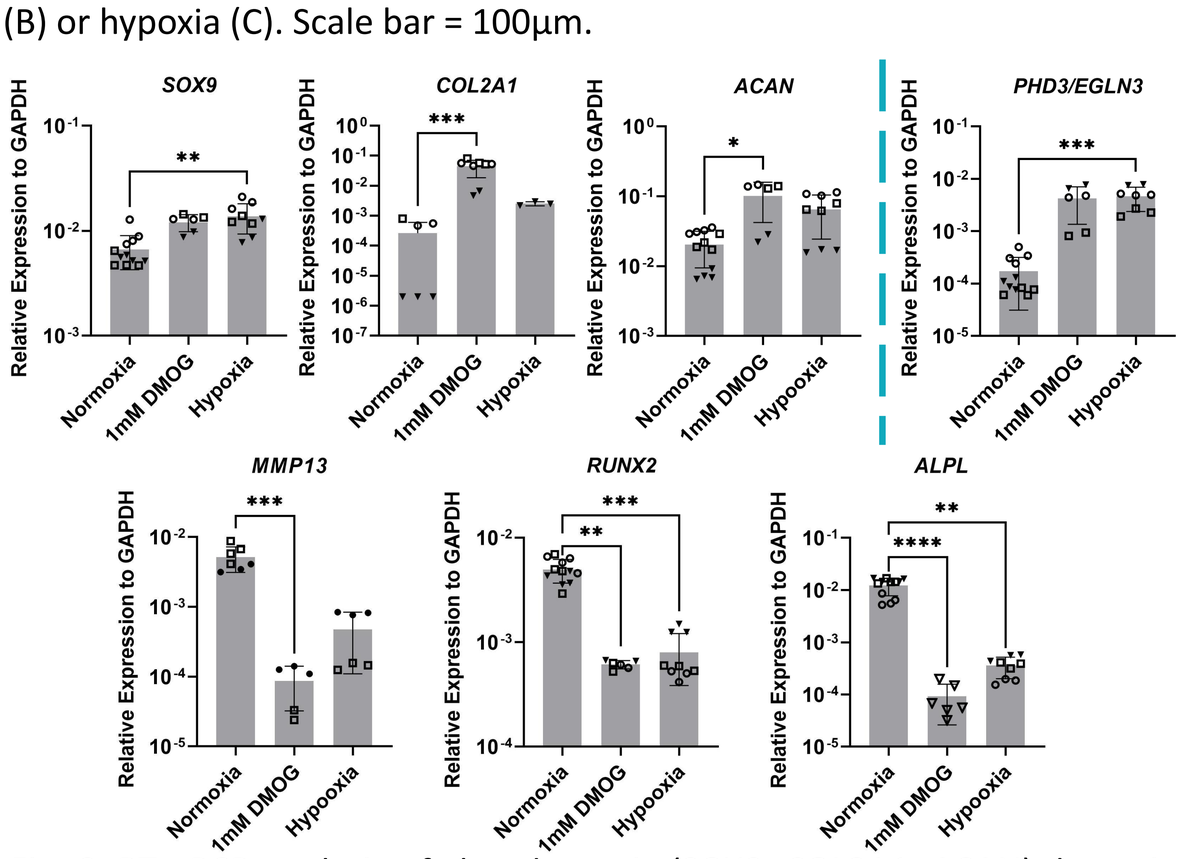

Fig. 2: RT-qPCR analysis of chondrogenic (SOX9, COL2A1, ACAN), hypoxic (PHD3), and hypertrophic (MMP13, RUNX2, ALAP) gene expression markers at day 14. Levels are expressed as mean and SD to the relative expression of GAPDH at day 0, *n*=5; **P*<0.05, ***P*<0.001, ****P*<0.0001.

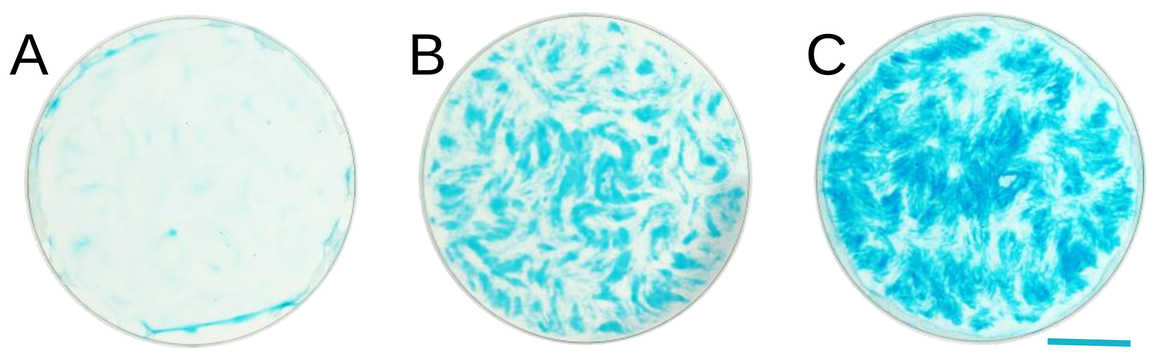

Fig. 3: Photographs of AB stained NCs monolayers at day 14 under normoxia(A), normoxia+1mM DMOG(B) or hypoxia(C). Scale bar = 3mm.

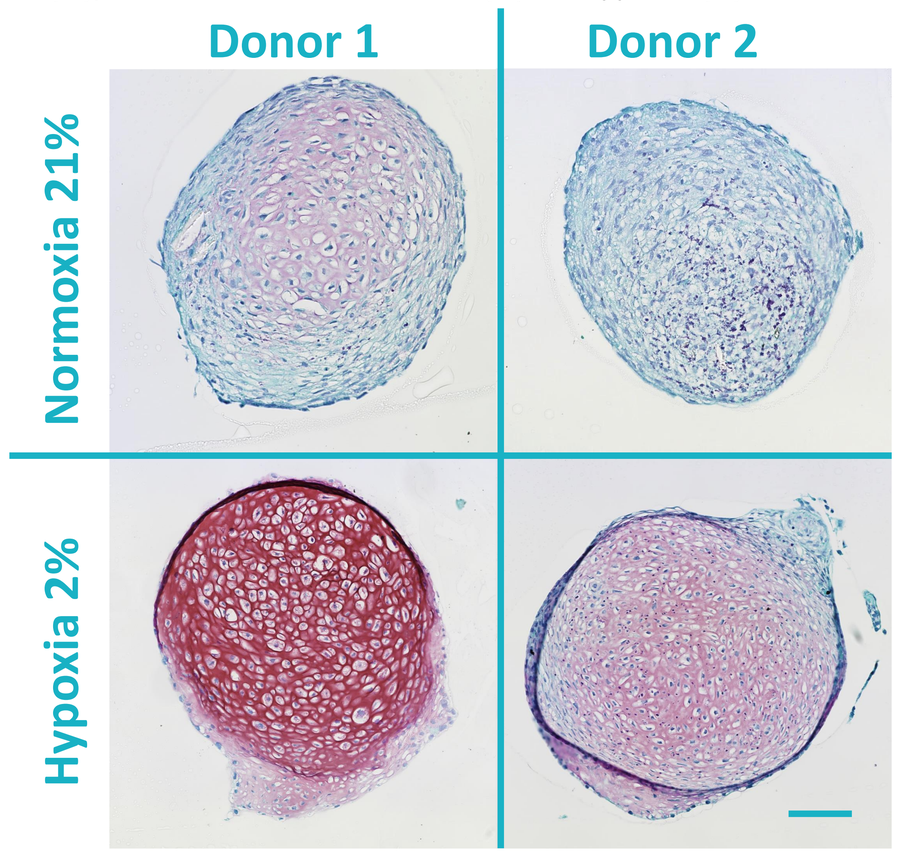

Fig. 4: Glycosaminoglycan (GAG) production in pellets. Safranin-O staining visualizes GAG production in pellets consisting of NCs expanded in 2% and 21% oxygen concentration at day 14. Scale bar = 200µm.

Cells cultured in hypoxia, and those conditioned with DMOG-, maintained their native spheroidal-shape and were positive for AB staining. Conversely, NCs cultured in normoxic condition showed an elongated-fibroblastic morphology and a limited amount of AB positive matrix (*Fig. 1* and *Fig. 3*). Hypoxia (vs normoxia) conditioned NCs expressed statistically significant higher levels of the chondrogenic and hypoxia related genes and decreased levels of the hypertrophic cartilage genes (*Fig. 2*). Results obtained in 3D pellet cultures showed that hypoxic culture induced NCs to form tissue with superior hyaline cartilaginous features (i.e.: dense and abundant GAG positive matrix and cells with a more round morphology) (*Fig. 4.*).

Conclusion/Outlook

Hypoxia positively affected NCs chondrogenic potential and supported the generation of hyaline-like cartilage tissues. Further experiments are ongoing to investigate the cross-talk between HIF-1α and BMP2 signaling pathways in hypoxia-induced chondrogenesis of NCs. Furthermore, 3D bioprinting will be used to generate scaffolds with controlled topology, to obtain intrinsic oxygen-gradients guiding the formation of different cartilage phenotypes.

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