Revolutionizing Osteochondral Repair Through 3D - Bioprinting

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Introduction

Osteochondral defects due to trauma or osteoarthritis affect the cartilage layer and the underlying subchondral bone. Cartilage is a heterogenous tissue divided into the articular and hypertrophic zones. The phenotype of chondrocytes can be regulated by the oxygen concentration.
This study investigates the role of hypoxia in cartilage formation using human nasal chondrocytes (hNCs) in monolayer culture and embedded in a collagen/tyramine hyaluronic-acid-based (Col/THA) hydrogel.

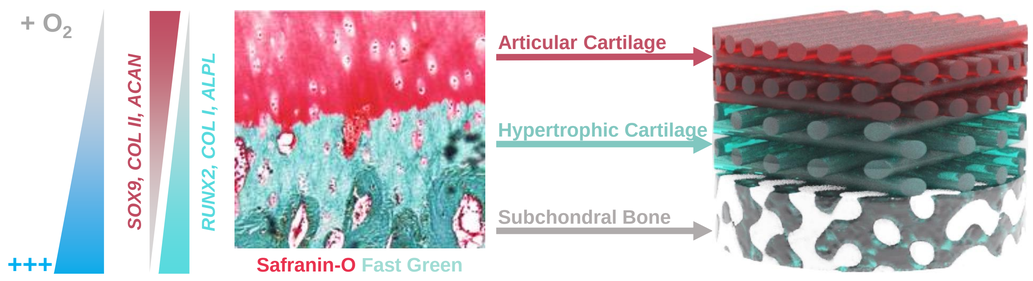

Figure 1. *From left to right: oxygen concentrations are important for the expression specific gene markers to maintain the zonal architecture of the cartilage tissue which is composed of articular and hypertrophic cartilage. Histological image of the osteochondral tissue representing the zonal architecture. 3D – Bioprinting will be applied to replicate the heterogeneous cartilage structure.*

Methods

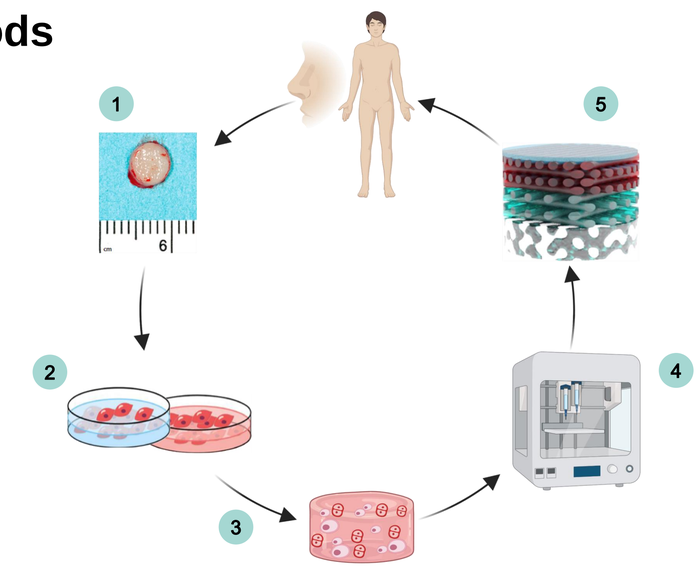

Figure 2. *Workflow of the project: the project aims to treat osteochondral defects in patients by extracting hNCs from the nose and utilizing them to generate a cultured and printed osteochondral construct. This innovative approach offers a surgical treatment option for joint defects.*

1 Isolation of hNCs from the nasal septum cartilage of patients.

2 To evaluate the effect of hypoxia hNCs were cultured as monolayer under normoxia (21%-O_2), hypoxia (2%-O_2), or supplemented with hypoxia inducing compound (21%-O2+1mM-DMOG).

3 hNCs were embedded into the Col/THA (2.5%/15%) hydrogel with Ruthenium/Sodium Persulfate (0.2mM/2mM) as a photoinitiator and crosslinked using visible light for 10min.

hNCs were assessed for quality using Alcian Blue (AB) and Safranin-O and immunofluorescent (IF) staining. Gene expression analysis for chondrogenic/hypertrophic markers was evaluated at day 14.

Results

2 Monolayer experiment to evaluate the effect of oxygen concentrations

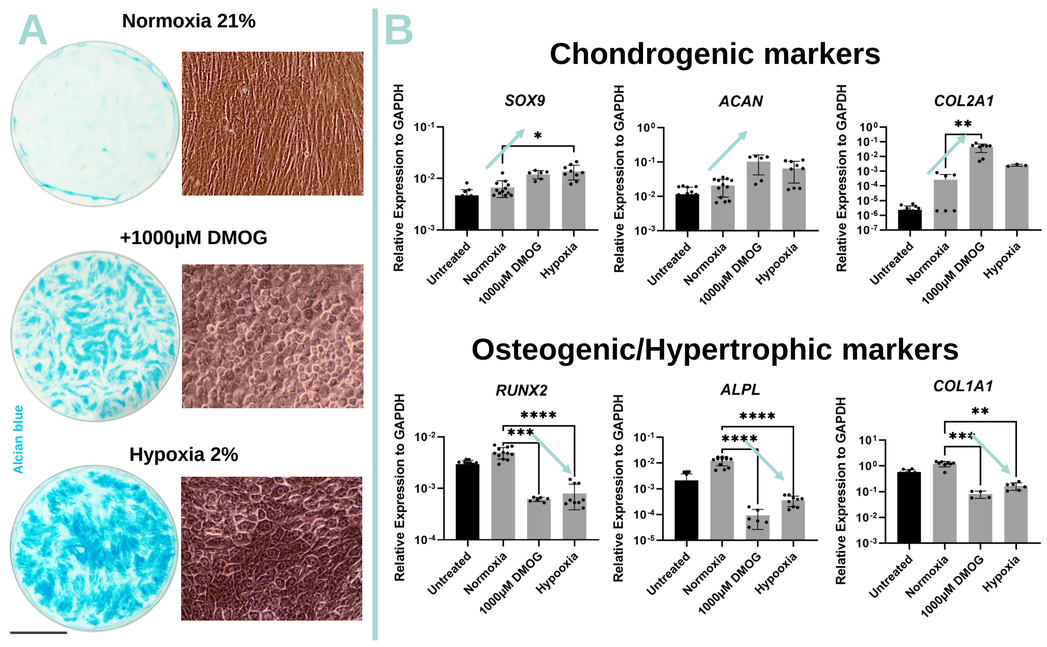

Figure 3. *A) Representative images of AB-stained hNCs monolayer at day 14. B) Analysis of chondrogenic (SOX9/ACAN/COL2A1) and osteogenic/hypertrophic (RUNX2/ALAP/COL1A1) gene expression markers at day 14. Expression of each gene was normalized to untreated samples. GAPDH was used as a housekeeping gene. Scale bar = 3mm.*

3 Evaluation of Col/THA based hydrogel composition

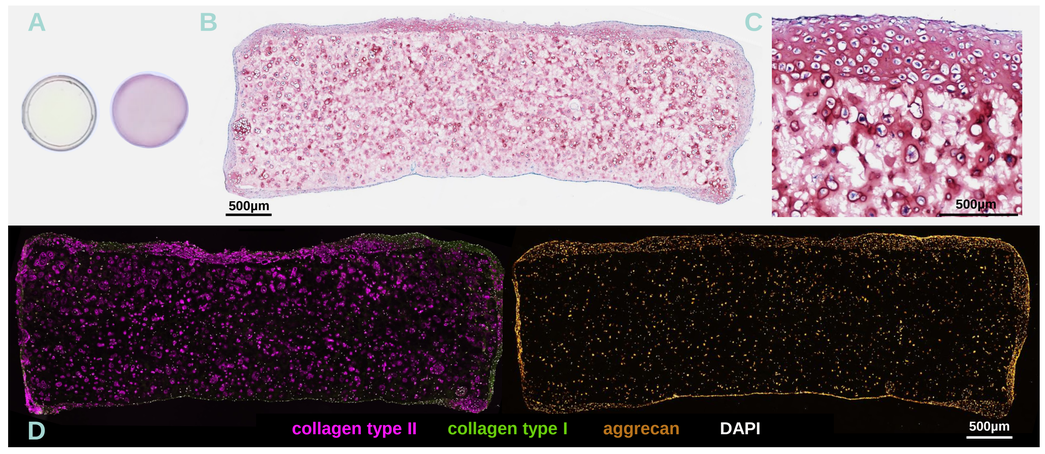

Figure 4. *A) Crosslinked hydrogel construct without cells and after 28 days of culture with hNCs embedded into the hydrogel. B) Safranin-O staining of the cross-section of the construct after 28 days. C) Close up image of the cell morphology of the hNCs. D) IF staining to characterize the distribution of new extra cellular matrix deposition.*

Conclusion/Outlook

Hypoxia promotes articular type cartilage generation with hNCs, while Col/THA hydrogels support cartilaginous matrix deposition.

4 Furthermore, topological studies on the role of hydrogel architecture and oxygen gradients are undergoing using 3D-bioprinting in the direction of stable osteochrondral construct formation.

5 The integration of the cartilage layer with a subchondral bone scaffold will be the next challenge addressed in this project.

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