Human chondrocytes show higher viability and maturity after 3D-bioprinting and 30 days *in vitro* when cultured in microcarriers (3D) compared to monolayer (2D)

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Background

The overall goal is to develop a new minimal invasive surgical treatment for children born without an external ear, i.e. microtia. The conventional surgical method is an invasive surgical approach associated with high donor site morbidity, as well as risk for severe complications and variable esthetic outcomes. We will use chondrocytes isolated from the patient's contralateral ear and 3D-bioprint a mirrored replica of the same.

We have previously shown long-term survival of 3D-bioprinted constructs with human chondrocytes in animal models. However, culturing chondrocytes in monolayer is associated with dedifferentiation.

Purpose

This study aims to compare the viability and functionality of chondrocytes cultured in porous gelatin microcarriers (PGMs) and monolayer, as well as how they survive in 3D-bioprinted constructs *in vitro*.

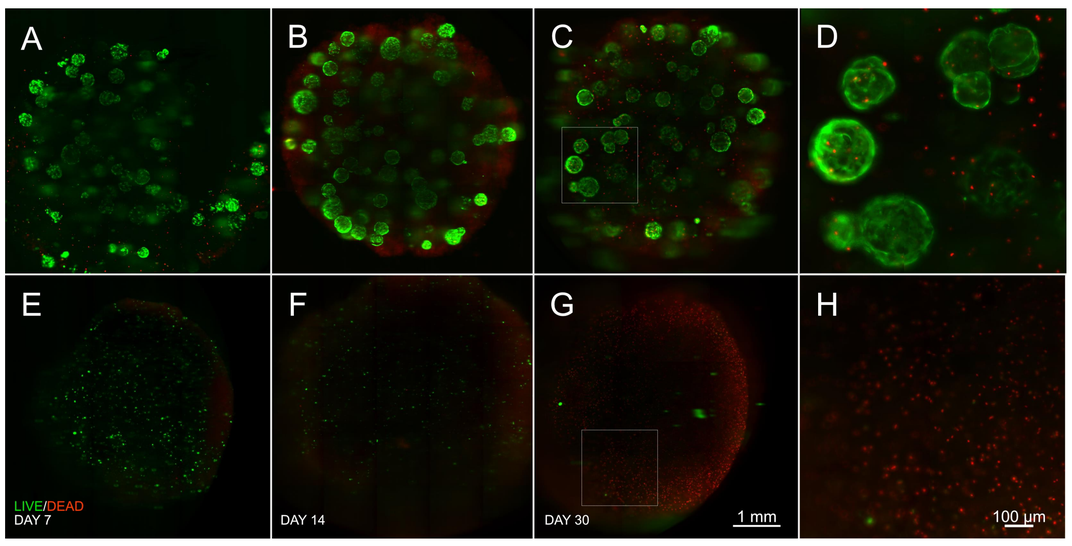


Figure 1. Chondrocyte viability in 3D-bioprinted scaffolds in vitro. Primary chondrocytes were expanded in monolayer and porous gelatin microcarriers (PGM) under normal cell culture conditions. Cell viability was assessed through calcein-AM (green) and PI (red)staining at day 7 (A, E), 14 (B, F) and 30 (C-D, G-H). Chondrocytes cultured in monolayer (E-H) and in PGMs (A-D) showed high viability at day 7, but a decline was noted in in the monolayer group thereafter. In contrary, the chondrocytes in the PGM group still showed high viability at day 30 (C-D).

Method

We isolated human chondrocytes from auricular cartilage. The chondrocytes were expanded in monolayer with DMEM/F12 medium and cultured in a stirring flask with PGMs for 72h. Single cell suspension and chondrocyte-containing PGM was 3D-bioprinted into circular scaffolds (5x1mm) using a 22 G conical nozzle at 5 mm/s and 40 respectively 22kPa. The bioink consisting of 5% enzymatically pretreated nanocellulose hydrogel and 3% alginate solution at an 80:20 ratio (v/v). All scaffolds were crosslinked with 100 mM CaCl2 and cultured *in vitro* for 30 days.

The viability was assessed after 7, 14 and 30 days by calcein-AM/propidium iodide staining. Samples (n=6) were taken at each time point and fixated, paraffin embedded, sectioned (5 μm) and stained with Alcian blue-van Gieson for histology. RNA was extracted for RT-PCR analysis.

Results

The cell viability was 98% in both groups prior to 3D-bioprinting. After 30 days *in vitro* the live/dead staining showed a considerably higher cell viability in the PGM group compared to the monolayer group (Figure 1). The histology confirms the presence of functional chondrocytes inside the PGMs producing extracellular matrix at day 30 (Figure 2).

The PCR analysis of the chondrocytes showed significantly increased expression of Col2A, COL2B and ACAN in the cells cultured in PGMs compared to monolayer indicating a more mature and healthy state.

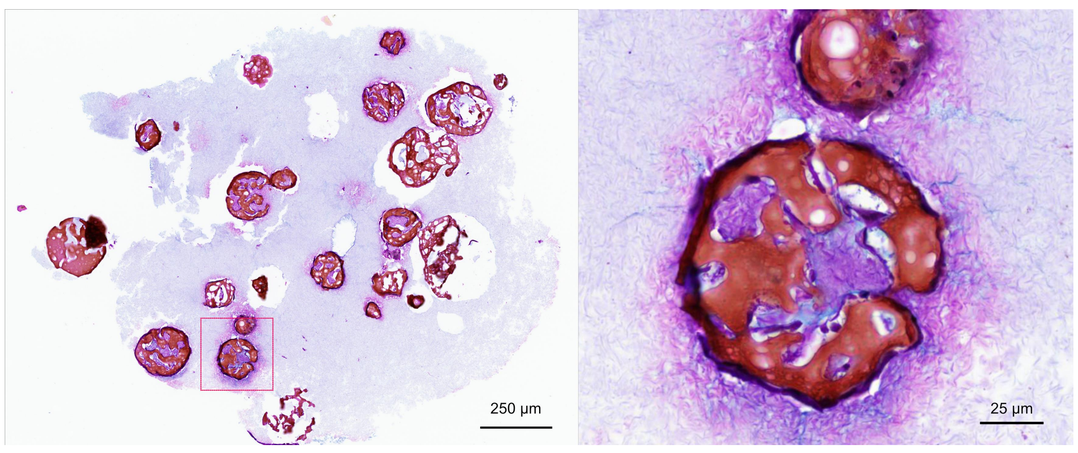


Figure 2. Glucosaminoglycan content in 3D-bioprinted constructs containing chondrocytes cultured in PGMs after 30 days *in vitro*. The ABvG stained sections showed that the PGMs were stained red, with a purple halo spreading from the inside and into the light blue ink. Magnification (right) showed H&E stained cell nuclei surrounded by GAGs (blue) inside the PGMs.

Conclusion

Chondrocytes cultured in PGMs show less signs of dedifferentiation than in monolayer and higher survival in vitro following 3D-bioprinting. Hence, 3D-bioprinted constructs with chondrocyte containing PGMs can be of interest for future clinical applications such as ear reconstructions.

Take home message

Human chondrocytes cultured in microcarriers (3D) show high viability and maturity after 3D-bioprinting and 30 days in vitro

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