

## Visualization of Chondrocytes on Collagen Scaffolds by Two Photon Laser Scanning Microscopy (Tplsm)

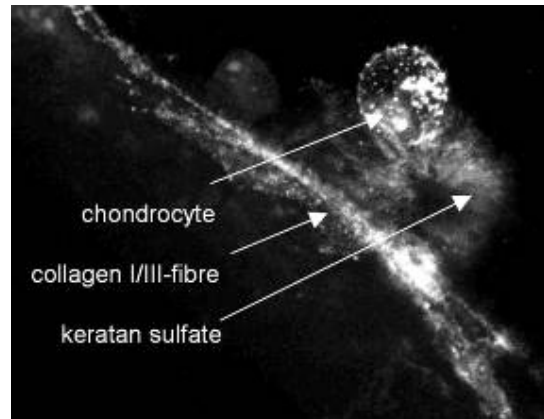
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**INTRODUCTION:** The successful application of tissue engineered cartilage constructs depends on the redifferentiation of chondrocytes after the in vitro cultivation expressed by the synthesis of cartilage specific ECM components. Several approaches are investigated to enhance the cell response and cell differentiation including the choice of appropriate scaffold materials as well as biochemical and biomechanical stimulations. The valuation of preimplantative in vitro cultivated scaffolds including cell morphology, cell distribution, cell number and metabolic activity by non-invasive techniques enables a sample specific controlling of the required cultivation time and the type of stimulation. We introduce methodical aspects of the visualization of chondrocytes on collagen I/III scaffolds by two photon laser scanning microscopy (TPLSM).

**METHODS:** Bovine chondrocytes were isolated from knee biopsies, expanded in monolayer cultures and cultivated on collagen I/III scaffolds with two different structures (fleece and sponge-like). The TPLSM analysis was performed using a TriMScope of LaVision BioTec (Bielefeld, Germany). The excitation wavelength was in the range of 790-820 nm (Ti:Sa). The autofluorescence of samples (Z-stacks) was detected by CCD camera and/or photomultiplier. The selective visualization of autofluorescent signals of cells and scaffolds was achieved by a) analysis of Second Harmonic Generation (SHG), b) the spectral analysis of autofluorescence and c) Fluorescence Lifetime Imaging (FLIM).

**RESULTS:** The results demonstrate that cells and collagen scaffolds can be visualized selectively using autofluorescent signals. Spectral differences of collagen signals and cells provide a powerful tool to distinguish cells from scaffold materials. SHG signals of collagen fibres in fleece structures as well as



*Fig. 1: Chondrocyte cultured on a collagen based scaffold material (fleece structure) synthesizing cartilage like extracellular matrix molecules (keratansulfate carrying proteoglycans).*

FLIM analysis increase the possibilities of a selective visualization.

Furthermore the results indicate the stimulation of cell differentiation processes of chondrocytes growing on sponge-like collagen scaffolds.

**DISCUSSION & CONCLUSIONS:** The results provide the basis for targeted online investigations on the influence of biochemical and biomechanical stimulations on the redifferentiation of in vitro cultivated chondrocytes. The quantitative analysis of cell number, distribution and morphology depending on the scaffold structure and cell stimulating cultivation conditions can be achieved by powerful 3D rendering software tools.

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