# Cartilage Tissue Engineering Using Chondrocytes and Woven P(L/D)LA Scaffolds

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## Introduction

The autologous chondrocyte transplantation (ACT) technique has been used successfully to treat defined cartilage lesions. However, so far the treatment of degenerated cartilage has not been possible due to the lack of suitable scaffolds. In this study we developed P(L/D)LA-scaffolds which might enable the treatment of osteoarthritic lesions with ACT. Since the differentiation state of the transplanted chondrocytes is an important factor determining the success of this procedure is, we investigated if chondrocytes differentiation was affected by scaffold geometry.

## Methods

*Scaffold production:* Polymer used in this study was medical grade polylactide L- and D-copolymer with L, D-monomer ratio 96 to 4 (P(L/D)LA 96/4, PLA96) (Purac biochem b.v., Gorinchem, The Netherlands). Multifilament yarns were melt-spun using a Gimac microextruder $\phi$ 12 mm (Gimac, Gastronno, Italy) equipped with a spinneret with orifices each $\phi$ 0.4 mm (4 and 8 filament) or $\phi$ 0.2 mm (12 filament yearn). The yarns were oriented by drawing freely in a three-step process at elevated temperatures to the draw ratio around 4.3-4.6 depending on the batch used. All yarns were knitted to tubular single jerseys. Parallely or anti-parallely aligned layers were used to produce a total of 12 different scaffolds ($\phi$ 6 mm h 3 mm). All samples were sterilized by -irradiation.

*Chondrocyte seeding*: Scaffolds (n=4) were seeded with $1.10^5$ porcine chondrocytes and cultured for three weeks. After embedding in Technovit 8100 (Hereus-Kulzer), 4µm sections were cut using a Leica microtome. Haemotoxylin and Eosin- or Safranin O-stained sections were then analyzed using stereological methods.

## Results

Three weeks after seeding, all scaffolds were covered with a layer of shiny white cartilage-like material. Histological analysis showed that most of the cartilage formation occurred on the surface of the scaffolds, while deeper in scaffold, large areas were devoid of cartilage. The varying of the scaffold geometry had only minor effects on cell seeding and chondrocyte distribution.

Interestingly, clear differences could be observed between the distribution of round chondrocytes with matrix and flat fibroblast-like cells (these probably represent dedifferentiated chondrocytes) (fig 1).

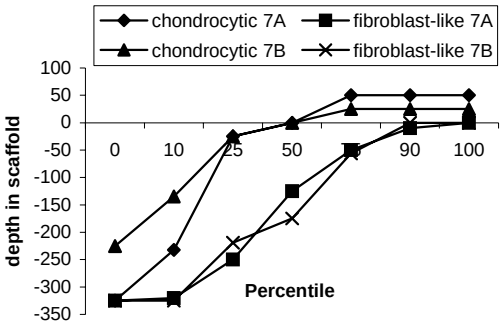


*Fig 1. Distribution of chondrocyte- and fibroblast-like cells within P(L/D)LA scaffold with parallely (7A) and anti-parallely (7B) aligned layers.*

At the surface of the scaffold, only chondrocyte-like cells could be found, while deeper in the scaffold, increasing numbers of dedifferentiated cells were present. The cells penetrating the deepest into the scaffold basically all had a fibroblastic phenotype.

**Discussion and Conclusions**

The preferential location of cartilage-like tissue at the surface of the PLDLA-scaffolds, might be caused by two factors: firstly, it appears that the woven structure of the scaffolds prevents the efficient seeding of the chondrocytes. Secondly, reduced diffusion of nutrients to the inner parts of the scaffold might prevent the optimal formation of cartilage proteins. This lack of nutrients might also induce chondrocytes in the zones farther removed from the surface to dedifferentiate. This would explain why a large part of the cells in the deeper zones have a fibroblast-like phenotype. On the other hand this distribution might simply be explained by the fact that dedifferentiated cells are more mobile than differentiated chondrocytes and therefore move deeper into the scaffolds. Changes in scaffolds structure should improve the seeding of cells within these scaffolds and improve the distribution of differentiated cells within the scaffold.

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